Diabetologia (1997) 40: A 1–A 673

Diabetologia

16th International Diabetes Federation Congress

Helsinki, Finland 20–25 July 1997

Abstracts

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OP 1
Pathogenesis of NIDDM

1

PATHWAYS OF WHOLE BODY GLUCOSE DISPOSAL AFTER FASTING

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In order to identify the effects of prior fasting on the pathways of insulin-stimulated glucose disposal, 6 normal volunteers underwent in random order and at 3 weeks interval two 4-h euglycemic hyperinsulinemic (100 μU/ml) clamps, one after an overnight fast, the other after 4d of fast. Each test included the measurments of (1) total glucose turnover (3-^{3}H-glucose infusion); (2) glycolytic rate (^3H_2O production); (3) circulating glucose oxidation (U-^{14}C-glucose infusion with measurement of $^{14}CO_2$ output); (4) total carbohydrate oxidation and net carbohydrate balance (indirect calorimetry). Fasting was associated with a significant reduction in glucose disposal rate (- 48 ± 6 %; P < 0.001), glycolytic flux (- 49 ± 2 %; P < 0.001), glycogen synthesis via the direct pathway (- 44 ± 13 %; P < 0.02), glucose oxidation (- 57 ± 3 %; P < 0.001), nonoxidative glycolysis (- 36 ± 4 %; P < 0.001) and total carbohydrate oxidation (- 87 ± 4 %; P < 0.001). Net carbohydrate balance was maintained (- 13 ± 14 %; NS), and fat oxidation was increased 6-fold. In 5 additional subjects, the experimental protocol was modified (identical rates of glucose infusion for both tests + pancreatic clamp) so that fasted subjects had the same glucose disposal rate as the nonfasted through the intervention of hyperglycemia. Under these conditions, prior fasting stimulated glycogen synthesis (+ 30 ± 4 %; P < 0.005) and inhibited glycolysis (- 27 ± 5 %; P < 0.01) in relation to a decrease in oxidation (- 40 ± 4 %; P < 0.001) with no change in nonoxidative glycolysis (- 1 ± 14 %; NS). Results of these two series of experiments suggest that in normoglycemia, the main effect of fasting is to inhibit insulin-mediated glucose transport-phosphorylation. When a normal glucose disposal is restored through hyperglycemia, fasting stimulates glycogen synthesis and inhibits glycolysis mainly in its oxidative component. These effects tend to optimize glucose carbon retention.

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NORMALIZATION OF SKELETAL MUSCLE BLOOD FLOW DOES NOT IMPROVE GLUCOSE UPTAKE IN OBESITY

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Defects in insulin stimulation of blood flow have been suggested to contribute to insulin resistance of glucose uptake. To directly examine whether amelioration of a blood flow defect in obesity reverses insulin resistance in obesity, we studied 7 obese (age 26±1 years, BMI 36±1 kg/m^2) and 7 nonobese males (age 25±2, BMI 22±1). Muscle blood flow was increased in one leg by an intrafemoral artery infusion of bradykinin (48 μg to obese, 27 μg to normal subjects over 100 min) during normoglycemic hyperinsulinemic (insulin infusion rate 1 mU·kg^{-1}·min^{-1}) conditions. Blood flow was measured in both legs before and during hyperinsulinemia using [^{15}O]-labelled water and positron emission tomography (PET). Glucose uptake was measured immediately thereafter using [^{18}F]-fluoro-deoxy-glucose and PET. Basal muscle blood flow was lower in the obese (1.5±0.1 ml·kg^{-1}muscle·min^{-1}) than in the nonobese (2.1±0.3, ml·kg^{-1}muscle·min^{-1}, p<0.05). During hyperinsulinemia, glucose uptake in the control leg was 63 % lower in the obese (21±5 μmol·kg^{-1}muscle·min^{-1}) than in the nonobese (56±10 μmol·kg^{-1}muscle·min^{-1}, p<0.01). Bradykinin increased blood flow in the obese to a level (3.0±0.4 ml·kg^{-1}muscle·min^{-1}) which exceeded flow in the control leg in the nonobese (2.4±0.5 ml·kg^{-1}muscle·min^{-1}). Despite this, glucose uptake was unaltered in the bradykinin infused leg (19.4±4.6 μmol·kg^{-1}muscle·min^{-1}) compared to the control leg (20.9±5.0 μmol·kg muscle^{-1}·min^{-1}) in the obese. In the nonobese subjects, bradykinin increased flow by 58 % (3.8±0.9 vs 2.4±0.5 ml·kg^{-1}muscle·min^{-1}, p<0.005) but had no effect on muscle glucose uptake (58±11 vs 56±10 μmol·kg^{-1}muscle·min^{-1}). These data demonstrate that normalization of blood flow in skeletal muscle of obese subjects has no effect on insulin stimulated glucose uptake.

3

MYOCARDIUM IS NOT RESISTANT TO THE EFFECT OF INSULIN TO INCREASE GLUCOSE UPTAKE IN NIDDM

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Insulin resistance of skeletal muscle glucose uptake characterizes patients with non-insulin-dependent diabetes mellitus (NIDDM). It is, however, unclear whether the myocardium also is affected by insulin resistance. We used [^{18}F]-2-deoxy-glucose combined with positron emission tomography to quantitate skeletal muscle and myocardial glucose uptake, and the euglycemic insulin clamp technique (5 mU/kg·min insulin infusion) to measure whole body glucose uptake in patients with NIDDM (n = 10, HbA_{1c} 8.1 ± 0.5%, age 43 ± 2 yrs, BMI 27 ± 1 kg/m^2) and in normal subjects (CTRL, n = 7, age 47 ± 2 yrs, BMI 26 ± 1 kg/m^2). All patients had a normal exercise test and resting ECG. In patients with NIDDM, rates of whole body (35 ± 3 *vs* 44 ± 3 μmol/kg body weight·min, NIDDM *vs* CTRL, p < 0.05) and skeletal muscle (71 ± 6 *vs* 96 ± 7 μmol/kg muscle·min, respectively, p < 0.02) glucose uptake were significantly decreased. However, no significant difference was found in myocardial glucose uptake (761 ± 82 *vs* 672 ± 63 μmol/kg muscle·min, respectively, p = 0.4). Myocardial glucose uptake was positively correlated with the left ventricular wall stress (r = 0.47, p < 0.05) and inversely with the ejection fraction (r = -0.61, p < 0.01) and stroke volume (r = -0.54, p < 0.05). There was no correlation between myocardial, and skeletal muscle (r = 0.05) or whole body (r = 0.00) glucose uptake. Serum FFA concentrations were comparable during the insulin infusion (205 ± 23 *vs* 168 ± 23, NS). We conclude that skeletal muscle and heart insulin resistance do not coexist in patients with NIDDM.

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ANALOG EFFECTS OF FDG IN HUMAN SKELETAL MUSCLE: PHYSIOLOGIC IMPLICATIONS OF INSULIN INDUCED REDUCTIONS OF "LC"

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Though positron emission tomography (PET) imaging of 18-fluoro-deoxy glucose (FDG) metabolism in skeletal muscle offers the potential to examine *in vivo* regulation of transport and phosphorylation, delineation of analog effects of FDG *vis a vis* glucose for human skeletal muscle has not been examined across a range of insulin doses. In general, FDGis transported more avidly but phosphorylated less efficiently than glucose; the lump constant (LC) expresses the net effect, each step weighted by its relative role in controlling glucose metabolism. To examine whether the LC in human skeletal muscle is altered by insulin, 16 lean healthy volunteers underwent simultaneous measurements of leg glucose uptake (A-V * blood flow) and 90 min dynamic PET imaging of FDG uptake at euglycemic insulin infusions rates of 0, 20, 40, and 120 mU/m2-min; (n=4 per dose). Insulin induced a highly significant (p<0.001) 4-fold step-wise decrement in LC. Net glucose influx (K) determined by PET was correlated with LGU (r=0.78, p<0.01) and K was identical whether calculated by Patlak analysis or derived from 3-compartmental modeling of dynamic tissue activity (r=0.99,p<0.01). Insulin stimulated an increase in the volume of distribution of FDG in muscle (K1/k2 ratio), and dose-dependent increases of the phosphorylation fraction (PF=k3/ (k2+k3);p<0.001). In step-wise multiple regression, K1/k2 and k3 accounted for 90% of individual variance in LGU. In summary, insulin stimulates a decrement in the LC for FDG; a physiologic implication being that insulin progressively induces re-alignment in the locus of control of glucose metabolism in skeletal muscle toward glucose phosphorylation, an interpretation further supported by the incremental insulin stimulation of PF.

5

EFFECTS OF PRIOR PROTEIN MALNUTRITION ON GLUCOSE INTOLERANCE AFTER HIGH-ENERGY/HIGH-FAT FEEDING

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Impaired pancreatic development during early life may predispose individuals to the development of glucose intolerance and non-insulin-dependent diabetes in later life, particularly if nutrition is inappropriate. The present study investigated whether impaired pancreatic development induced by exposure to suboptimal protein nutrition during early life exacerbated the development of glucose intolerance in response to the provision of a high-energy/high-fat (HEF) diet of normal protein content. Rats were exposed to either a standard (20% protein) diet (control, C) or a low (8%) protein diet (LP) during fetal life, early development and after weaning. At 20 weeks of age, both C and LP groups were transferred to HEF diet. After 8 weeks HEF feeding, glucose tolerance was assessed after an i.v. glucose load (0.5 mg/kg body wt.) in conscious, unstressed rats. HEF feeding elicited 24% (n.s.) and 53% ($P<0.05$) increases in incremental areas under the curve (IAUC values) for glucose in the C and LP groups respectively. The rate of glucose disappearance (K value, calculated from data obtained between 2 and 15 min after the intravenous glucose load) was unaffected by HEF feeding in the C group, but there was a 40% ($P<0.05$) decline in the K value for LP rats. The study therefore demonstrates that exposure to a high-fat/high-energy diet for a prolonged (8 week) period leads to greater loss of glucose tolerance in rats exposed to suboptimal protein nutrition in early life.

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ANALYSIS OF PPARγ, C/EBPα, GLUT4, LPL AND *OB* mRNA LEVELS IN ADIPOSE TISSUE OF RHESUS MONKEYS.

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Many, but not all, rhesus monkeys (*Macaca mulatta*) become obese after sexual maturation. Obesity is usually associated with insulin resistance and hyperinsulinemia, and NIDDM often develops at a later stage. Peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer-binding protein α (C/EBPα) coordinate the expression of genes involved in creating and maintaining the adipocyte phenotype such as GLUT4 glucose transporter. We measured the mRNA levels of PPARγ, C/EBPα, lipoprotein lipase (LPL), GLUT4 and *ob* in adipose tissue to examine whether these genes were differentially expressed in normal, obese hyperinsulinemic and NIDDM monkeys. The levels of mRNAs were quantitated by slot blot hybridization analysis. For probes, the cDNA fragments were amplified from monkey adipose tissue RNA and cloned. The mRNA levels of PPARγ, LPL and GLUT4 were approximately 2-fold higher in normal monkeys compared with obese hyperinsulinemic and NIDDM monkeys ($p's<0.05$). We observed no differences between hyperinsulinemic and NIDDM monkeys. In contrast, the level of *ob* mRNA was highest in hyperinsulinemic monkeys ($p<0.05$). In addition, LPL, GLUT4 and C/EBPα mRNA levels were significantly correlated to PPARγ mRNA levels (C/EBPα; $r=0.775$, LPL; $r=0.888$, GLUT4; $r=0.945$, $p's<0.001$). In contrast, *ob* mRNA levels were not related to PPARγ mRNA. Our data suggested that continued high-level expression of mRNAs of PPARγ, C/EBPα, LPL and GLUT4, levels may not be required for the maintenance of the obese or NIDDM states and that expression levels of PPARγ, C/EBPα, LPL and GLUT4, but not the *ob* gene, are highly coordinated.

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IMPAIRED EFFECT OF INSULIN ON BOTH GLUCOSE TURNOVER AND LIPOLYSIS IN NIDDM RELATIVES

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To elucidate early mechanisms in the pathogenesis of NIDDM we studied 10 young healthy individuals with two first-degree relatives with NIDDM (**R**) and 10 control subjects (**C**) pairwise matched for sex (M/F 4/6) age (35±1 vs 35±1) and BMI (23.6±0.6 vs 23.2±0.4). During a euglycemic clamp at two insulin levels (**L**ow~20 and **H**igh~90 mU/L) glucose turnover was assessed and abdominal subcutaneous adipose tissue (SAT) lipolysis and blood flow were studied with microdialysis and ^{133}Xe-clearance. Fasting glucose, insulin and C-peptide levels were similar in **R** and **C**. During the clamp, insulin sensitivity index for glucose disposal was lower ($P<0.03$) in **R** than **C** (**L** 12.0±1.6 vs 18.1±1.4, **H** 9.4±0.8 vs 12.9±0.6 (100*mg*L/mU/min)) and this was partly attributed to slightly higher insulin concentrations in **R** ($P<0.05$) indicating an impaired insulin clearance. SAT lipolysis measured as local glycerol release did not differ under basal conditions (2.0±0.2 vs 2.1±0.2 μmol/kg/min), but the inhibition during insulin infusion was attenuated in **R** compared to **C** (glycerol release: **L** 0.92±0.09 vs 0.68±0.16, **H** 0.71±0.10 vs 0.34±0.10 $P<0.03$). Plasma FFA also tended to be higher in **R** than **C** (basal 498±27 vs 483±41 μmol/L, **L** 145±37 vs 94±19, **H** 77±24 vs 55±18, NS). In contrast, isolated subcutaneous adipocytes obtained through needle biopsies displayed no differences between **R** and **C** with respect to in vitro effects of insulin on ^{14}C-glucose uptake or lipolysis. Moreover, muscle sympathetic nerve activity (MSA) was assessed with microneurography of the peroneal nerve. Under resting conditions no difference was found (**R** 27±4 and **C** 31±4 bursts/min) whereas the increase in activity during a standardized stress test (immersion of one hand in ice-cold water) was clearly higher in **R** than in **C** (364±152 vs 150±15 %, $P<0.05$). **Conclusion:** Resistance to insulin action in vivo on both glucose turnover and lipolysis is an early feature in the development of NIDDM. This may in part be caused by neural or humoral factors. An enhanced sympathoadrenal activity, which impairs insulin action, may play a role.

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METABOLIC IMPACT OF FAMILY HISTORY OF NIDDM

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Some previous studies found evidence of insulin resistance in non-diabetic relatives of NIDDM patients, while others did not. However, the numbers of subjects in previous studies were relatively small, and different techniques were used to measure insulin action. The euglycaemic hyperinsulinaemic clamp represent the "golden standard" technique for measuring insulin action. EGIR data base contain measurements of insulin action using the insulin clamp technique (1mU/kg/min) in normal glucose tolerant subjects from 20 European centers. The aim of this study was to determine the impact of family history of NIDDM on insulin action, and on glucose and lipid oxidation, based on the EGIR data base. Family history of NIDDM was positive in 235 (FH+) and negative in 564 individuals (FH-) respectively. BMI was slightly higher in FH+ compared with FH- (26.7±0.3 vs 25.8±0.2 kg/m^2, $p<0.02$). Age, waist-to-hip ratio, lean body mass, weight and height were similar in FH+ and FH-. After correction for covariates according to differences between investigators and subject characteristics including BMI (ANOVA), insulin stimulated glucose disposal was lower in FH+ vs FH- (30.3±0.6 vs 34.3±0.4 μmol/kg/min, $p<0.00001$). Despite this, insulin stimulated glucose oxidation (as determined using indirect calorimetry in some individuals) was elevated in FH+ vs FH- (16.3±0.5 vs 14.9±0.3 μmol/kg/min, $p<0.05$, N=76 vs 213). Consequently, insulin stimulated non-oxidative glucose metabolsim (= disposal - oxidation) was markedly reduced in FH+ vs FH- (15.2±0.9 vs 20.0±0.5 μmol/kg/min, $p<0.0005$, N=76 vs 213). After adjusting for BMI, basal lipid oxidation was lower in FH+ vs FH- (3.1±0.1 vs 3.5±0.1 μmol/kg/min, $p=0.05$, N=76 vs 181). Basal glucose oxidation and insulin stimulated lipid oxidation rates were similar in FH+ and FH-. In conclusion, insulin resistance is present in European non-diabetic relatives of NIDDM patients. The insulin resistance is restricted to the pathway of non-oxidative glucose metabolism, whereas insulin stimulated glucose oxidation is compensatorily increased in some NIDDM relatives. The tendency to obesity in NIDDM relatives may be increased by a lower oxidation of fat.

OP 2

Insulin Secretion in Vitro: Ion Channels and Oscillation

9

REGULATION OF FREE Ca^{2+} IN NON-MITOCHONDRIAL STORES OF INDIVIDUAL PANCREATIC ß-CELLS.

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To study the regulation of intracellular Ca^{2+} stores, single mouse pancreatic ß-cells were loaded with the low-affinity Ca^{2+}-indicator furaptra and permeabilized with digitonin to release cytoplasmic dye. After permeabilization, the 340/380 nm fluorescence excitation ratio was stable, corresponding to a free Ca^{2+} concentration ($[Ca^{2+}]$) of 249±10 µmol/l (n=84) in the presence of 3 mmol/l ATP and 200 nmol/l Ca^{2+}. The ratio was insensitive to ruthenium red and changes in the Mg^{2+} concentration in the 0.1-1 mmol/l range. Moreover, removal of ATP or Ca^{2+} resulted in a marked and sustained decrease, indicating that the signal reflects changes of $[Ca^{2+}]$ in non-mitochondrial ATP-dependent stores. More than 90% of the stored Ca^{2+} was released by the intracellular Ca^{2+}-ATPase inhibitor thapsigargin, the rest being mobilized by omission of ATP or addition of Br-A23187. Inositol-1,4,5-trisphosphate (IP_3) mobilized Ca^{2+} in a dose-dependent manner, releasing 52± 4% of the thapsigargin-sensitive Ca^{2+} at a maximally stimulating concentration (10 µmol/l). It was possible to differentiate between various Ca^{2+} stores on the basis of their resistance to digitonin treatment. After plasma membrane permeabilization, prolonged exposure to the detergent resulted in a further release of furaptra. In 5 of 9 cells this release resulted in a decrease of both IP_3-sensitivity and $[Ca^{2+}]$. In the remaining cells the IP_3 sensitivity persisted while $[Ca^{2+}]$ increased, probably reflecting loss of furaptra from a store with low $[Ca^{2+}]$. It is concluded that the pancreatic ß-cell exhibits at least two non-mitochondrial ATP-dependent Ca^{2+} stores differing with regard to their sensitivity to IP_3.

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HUMAN α ENDOSULFINE, ENDOGENOUS LIGAND FOR THE SULFONYLUREA RECEPTOR: CLONING AND EXPRESSION.

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Endosulfine, the endogenous ligand for the sulfonylurea receptor, exists under two molecular forms, α and ß. We have isolated from porcine brain the 13KDa α form which presents strong structural similarities with a bovine 12-13KDa cyclic AMP-regulated phosphoprotein, called ARPP-19. Based on the sequence of a partial cDNA clone coding for bovine α endosulfine, we have shown recently that α endosulfine and ARPP-19 are distinct molecular entities. In the present studies, we used primers derived from the known structures in heterologous species to amplify by PCR a 233 bp human cDNA fragment. This fragment was then used as a probe for cloning the full length cDNA from a human brain λGT11 library. We confirm that α endosulfine and ARPP-19 are distinct proteins, encoded by different genes, with a 71% degree of identity in their aminoacid sequences. We have expressed α endosulfine in a bacterial system and compared its properties to those of the protein isolated from porcine brain. The recombinant and the extracted proteins display the same qualitative and quantitative features in inhibiting the binding of [^{3}H]-glibenclamide to both rat cortex and ß cell membranes. Mutational studies and enzymatic fragmentation of the recombinant protein are now being carried out to determine the molecular moiety required for interaction with the sulfonylurea receptor. Considering the importance of the sulfonylurea receptor/K-ATP channel complex in regulating insulin secretion and action, our results open up a new avenue of research on the biological role of the now complete endosulfine-receptor-channel regulatory system. It will also be of interest to look for mutations in the human α endosulfine coding sequence in various pathological states, particularly in non-insulin dependent diabetes.

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INTERACTIONS BETWEEN TOLBUTAMIDE AND NUCLEOTIDES ON CLONED K-ATP CHANNELS.

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Sulphonylureas stimulate insulin secretion by blocking ATP-sensitive K-channels (K-ATP channels). It has previously been shown that intracellular MgADP enhances the sensitivity of K-ATP currents to tolbutamide (Schwanstecher et al., 1994 Br. J. Pharmacol. 111,302). The aim of this study was to investigate the molecular basis of this effect. The K-ATP channel comprises 2 types of subunit: Kir6.2 and the sulphonylurea receptor (SUR1). Mutations in the nucleotide binding domains of SUR1 (K719A/K1385M) abolish the activation of K-ATP currents by MgADP but do not alter the inhibitory effects of nucleotides (ATP or ADP). We therefore examined the effect of these mutations on the modulation of tolbutamide sensitivity by MgADP. We expressed Kir6.2 with wild-type SUR1 or mutant SUR1 (K719A/K1385M) in *Xenopus* oocytes, and measured K-ATP currents in giant inside-out patches. In the absence of nucleotides, tolbutamide dose-inhibition curves for wild-type and mutant currents suggested the presence of 2 binding sites, with K_i of 1.6 µM (0.6-4.5) and 1.8 mM (1.3-2.5) (n=8; geometric mean (SD range)). High-affinity binding reduced the current by ~50%. In the absence of tolbutamide, 100µM MgADP enhanced wild-type K-ATP currents by 55 ± 13% (n=5; mean ± SEM). Tolbutamide inhibited MgADP-activated currents and at >20 µM tolbutamide, K-ATP currents were smaller than in the absence of MgADP. MgADP did not significantly alter the K_i for tolbutamide at either binding site. 100µM MgADP reduced mutant currents (by 68 ± 3%, n=5), without otherwise affecting the tolbutamide dose-response curve. We conclude that, in oocytes, tolbutamide inhibits K-ATP currents by interacting with 2 separate binding sites. Further, the enhancement of tolbutamide block by MgADP is apparent rather than real: it results from tolbutamide, acting at the high-affinity site, producing a dose-dependent inhibition of MgADP-induced activation. At high tolbutamide concentrations this is sufficient to unmask the inhibitory action of MgADP.

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FREQUENCY OF SUR1 AND KIR6.2 MUTATIONS ASSOCIATED WITH FAMILIAL HYPERINSULINISM (HI)

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The islet β-cell ATP regulated K^+ channel is composed of 2 subunits, the sulfonylurea receptor (SUR1) and the small inward rectifier K^+ channel (Kir6.2). HI is an autosomal recessive disease associated with function-altering mutations in either of these two genes. To define the spectrum of genetic defects in a large heterogeneous group of HI patients, 78 probands were screened for SUR1 (39 exons) and Kir6.2 (1 exon in 6 fragments) mutations by single strand conformation polymorphism analysis (SSCP). Variants were confirmed by direct sequencing and by PCR-RFLP analysis of genomic DNA. Nineteen mutations were found in SUR1, including 4 frameshift, 1 nonsense, 9 missense and 5 intronic mutations. The missense mutations were at highly conserved regions, and some were found to produce functional disruptions. One nonsense mutation was found in Kir6.2. A total of 103 HI probands were then screened by PCR-RFLP assays developed for each mutation. Two of the SUR1 variants, accounting for ~90% of HI, were found in the 36 Ashkenazi Jewish probands, with 15 homozygotes, 8 compound heterozygotes, and 11 simple heterozygotes. Of 9 Arab probands 2 were homozygous and 1 was heterozygous for different SUR1 mutations, and 1 was homozygous for a Kir6.2 mutation. In 29 Northern European Caucasian HI probands, 2 compound heterozygotes and 6 simple heterozygotes were found. Combined results of 36 other probands from other ethnic backgrounds yielded 3 homozygotes, 2 complex heterozygotes, and 4 simple heterozygotes. Except for the 2 mutations found commonly in the Ashkenazi Jewish patients, all others were private mutations which were found in the homozygous state only in consanguineous families. In summary, mutations were found in SUR1 in 34/36 (95%) Ashkenazi HI patients, while they were found in SUR1/Bir6.2 in 21/74 (28%) patients from other populations. Functional analysis of the missense mutations will provide important new information on the structure/function relationships of the islet KATP channel.

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POTASSIUM CURRENTS IN NORMAL AND DEFECTIVE HUMAN B-CELLS.

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K_{ATP} channels have a key role to play in depolarization-response coupling of insulin release. In β-cells these channels are composed of the subunits, SUR1 and $K_{IR}6.2$. Mutations in the genes encoding these proteins cause altered β-cell function in humans leading to persistent hyperinsulinaemic hypoglycaemia of infancy (PHHI); a disorder associated with hypersecretion of insulin in infancy. In this study we have examined for the first time recordings of patch-clamp whole-cell K^+ currents in β-cells isolated from two patients with PHHI and compared these with the properties of normal human insulin-secreting cells *in vitro*. Control tissue was obtained from cadaveric organ donors, and PHHI β-cells isolated from the pancreas following surgery to alleviate hypoglycaemia. Three series of experiments were performed under standard whole-cell electrophysiological conditions. First, when cells were dialysed with an 'ATP-free' solution, this led to a marked increase in K_{ATP} channel currents in normal tissue due to wash-out of endogenous ATP(n=8), but the procedure was without significant effect in PHHI β-cell experiments (n=16). This alludes to an alteration in the function of K_{ATP} channels. Secondly, when 'ATP-containing' solutions were used in parallel experiments, no increase in K_{ATP} currents was obtained upon dialysis of normal β-cells (due to the presence of ATP) and K_{ATP} channels were by activated by diazoxide (0.5mM) and inhibited by the tolbutamide (0.5mM) (n=4). By contrast, diazoxide, tolbutamide and somatostatin (0.1mM) were all without effect in PHHI β-cell experiments (n=9). Finally, outward K^+ current recordings were used to assess the function of two K^+ channels in PHHI β-cells - the large conductance K_{Ca} channel and the delayed-rectifier K^+ channel. These channels were activated by a depolarisation, and their pharmacological properties assessed by TEA^+ (20mM) and quinine (0.01mM). There were no significant alterations in the properties of these channels in PHHI β-cells (n=11). We conclude that loss of function in β-cells of PHHI patients is specific to a defect in K_{ATP} channel activity.

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'KNOCK-OUT' OF K_{ATP} CHANNEL ACTIVITY IN HUMAN β-CELLS: IMPLICATIONS FOR STIMULUS-SECRETION COUPLING.

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Depolarization-response coupling mechanisms in β-cells are dependent upon the function of K_{ATP} channels, which are composed of two subunits $K_{IR}6.2$ and SUR1. Here, we have isolated intact islets from two neonates with persistent hyperinsulinaemic hypoglycaemia of infancy (PHHI) and studied the functional properties of β-cells *in vitro*. Both children were from families with a previous history of PHHI and both were unresponsive to medical therapy, necessitating a near-total pancreatectomy to alleviate hypoglycaemia. Post-operatively islets were isolated from the tissue. Analysis of genomic DNA from both patients revealed a mutation in exon 35 (G1398A) of the SUR1 gene, which is predicted to truncate the second nucleotide binding fold of SUR1. Patch-clamp studies of isolated β-cells revealed an absence of K_{ATP} channels in both children and the appearance of Ca^{2+}-dependent action potentials at rest (n=34). Diazoxide (200-500μM) had no effect on either K^+ currents (n=8), or changes in cytosolic Ca^{2+} (n=10) when measured by microfluorimetry with fura-2 loaded cells. The loss of K_{ATP} channel activity was confirmed in reconstitution studies using an engineered mutation in SUR1, which when expressed with $K_{IR}6.2$ in COS cells, failed to reconstitute functional K_{ATP} channels as assayed by $^{86}Rb^+$ efflux (n=4). By measurements of membrane capacitance from isolated PHHI β-cells, we found that exocytosis of secretory granules was augmented by a rise in intracellular Ca^{2+} (n=8) and that this was *inhibited,* and not stimulated as in normal β-cells by the sulphonylurea tolbutamide (5-100μM) (n=11). Finally, by directly measuring insulin secretion from intact islets using radioimmunoassay, we found that glucose (20mM) promoted insulin release: a response that occurs despite the absence of functional K_{ATP} channels, and the inability of glucose to elevate $[Ca^{2+}]_i$ (n=10). In summary, our data show how β-cells are severely compromised by a gene mutation in SUR1 and that glucose-induced secretion of insulin can also occur via non-Ca^{2+}-dependent mechanisms.

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GLUCOSE- AND SULPHONYLUREA-EVOKED PULSATILE INSULIN RELEASE FROM SINGLE ISLETS AS DETECTED BY 5-HT MICROAMPEROMETRY

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Pancreatic islets secrete insulin in a pulsatile fashion in response to a rise in glucose concentration. However, previous insulin measurements have had minimal temporal resolution and consequently little is currently known about the true dynamics and metabolic requirements of the oscillatory signalling pathway encoding for bursts of exocytotic secretion. Using simultaneous real time measurements of cytosolic free calcium ($[Ca^{2+}]_i$) and pre-loaded 5-hydroxytryptamine (5-HT) release as an electrochemical marker of insulin secretion from single mouse islets, we show that glucose (11-20 mM)-stimulated islets secrete 5-HT/insulin in a pulsatile fashion under physiologic conditions, and that this activity is, for the most part, encoded by regular high-frequency (1-6 min-1) $[Ca^{2+}]_i$ oscillations. The amount of 5-HT/insulin secreted per cycle bears a linear relationship with the duration of the underlying $[Ca^{2+}]_i$ oscillation regardless of the glucose concentration in the range 11-20 mM. In addition, we demonstrate the occurrence of low-frequency variations in global 5-HT/insulin release (0.2-0.5 min^{-1}), supported by intermittent high-frequency oscillations. Tolbutamide (100 μM) evokes pulsatile 5-HT/insulin release transiently in the absence of glucose, in spite of the fact that it triggers sustained (but brief) $[Ca^{2+}]_i$ oscillations. Pulsatile 5-HT/insulin release can, however, be reactivated following reintroduction of glucose, which enhances the duration of the $[Ca^{2+}]_i$ oscillations. We conclude that pulsatile insulin release is stringently dependent upon the dynamics of the $[Ca^{2+}]_i$ oscillations. In the case of sulphonylureas, the effectiveness of the latter requires minimum metabolic input (presumably through metabolic activation of voltage-sensitive Ca^{2+} channels).

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IN HUMANS AT LEAST SEVENTY-FIVE PERCENT OF OVERALL INSULIN SECRETION ARISES FROM PUNCTUATED HIGH-FREQUENCY INSULIN SECRETORY BURSTS

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Insulin is secreted in a pulsatile manner, resulting in small oscillations in its circulating concentrations. Detection of insulin secretory bursts in the peripheral blood is hampered by hepatic insulin extraction, dilution and a time-delayed damping of the amplitude. To examine human pulsatile insulin release we used a high-sensitivity, specificity and precision insulin ELISA, and optimized an established deconvolution methodology to quantify the frequency, mass and amplitude of insulin secretory bursts, as well as estimate the relative contribution of pulsatile insulin release. Four protocols were performed. 1) Insulin kinetics were determined following intravenous insulin bolus injection. 2) Criteria for detecting insulin secretory pulses were refined through analysis of insulin concentrations resulting from repetitive assay of pooled plasma, and from sampling during ablated insulin secretion and a constant insulin infusion. 3) Optimal sampling intensity was examined by sampling every 30 seconds and performing pulse analysis using the uncensored series, and every second, fourth or sixth sample. 4) The above defined optimal parameters were applied to 20 healthy humans to examine pulsatile insulin secretion during glucose stimulation (4.5 mg/kg/min). Using minutely sampled insulin concentrations measured by highly sensitive insulin ELISA, and insulin kinetics of 2.8 minutes (first half life), 5.0 minutes (second half life) and a fractional slow component of 0.28, the deconvolved insulin secretion rates could be resolved into a series of high frequency (4.7 ±0.1 minute/pulse) approximately symmetric insulin secretory bursts with a mean mass of 87 ±12 pmol/L/pulse and a mean amplitude of 35 ±4.7 pmol/L/minute. The relative contribution of pulsatile to overall insulin secretion was 75 ±1.6% (range 59 to 85%). We conclude that human insulin secretion during glucose stimulation consists of a series of insulin secretory bursts accounting for at least 75% of total insulin secretion.

OP 3
Genetics of NIDDM and Obesity

17

IDENTIFICATION OF MUTATIONS IN THE HNF-1α GENE (MODY3) IN GERMAN SUBJECTS WITH EARLY-ONSET NIDDM

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Recent studies have shown that mutations in the gene encoding the transcription factor HNF-1α are the cause of one form of maturity-onset diabetes of the Young (MODY3). We have screened the HNF-1α gene for mutations in a group of 12 subjects of German ancestry who were diagnosed with NIDDM before 35 years of age (mean age = 24.1 ± 10.4 years) and had another first degree relative with NIDDM. Although not meeting the strict diagnostic criteria for MODY, it is likely that several of these subjects had this form of diabetes. PCR amplification and direct sequencing of the promoter region and 10 exons revealed three mutations: Dresden-1, P291fsinsC; Dresden-2, R131Q; Dresden-3, R229Q. The age at diagnosis of NIDDM in these three subjects was 17, 18 and 21 years of age, respectively. Moreover, the subjects were not obese.
This result suggests that it may be worthwhile to screen subjects with early-onset NIDDM for mutations in the HNF-1α gene even in the absence of a strict diagnosis of MODY.

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A MUTATIONAL HOTSPOT IN THE POLY-C TRACT OF EXON 4 OF THE HEPATIC NUCLEAR FACTOR 1ALPHA GENE; SCREENING IN MATURITY ONSET DIABETES OF THE YOUNG.

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Mutations in the HNF-1α gene have recently been shown to cause Maturity Onset Diabetes of the Young (MODY). A common mutation has not been found. Patients are defined as having MODY if diagnosed as having non-insulin-dependent-diabetes before the age of 25 with an autosomal dominant mode of inheritance. We performed mutation detection on 15 probands from MODY families by PCR amplification and semi-automated sequencing of the 10 exons and adjacent splice sites of HNF-1α. A common mutation, P291fsinsC, occurring in the poly-C-tract of exon 4, was identified in 4 of 15 probands. Specific screening of exon 4 in a further 32 less strictly defined probands, aged <40 years, identified this mutation in 2 further pedigrees, giving an overall frequency of 13% (6/47). The mutation was found to cosegregate with 20 additional diabetic family members and was also found in 2 non-diabetic subjects, aged 22 and 26; these may develop diabetes later in life. Analysis of intragenic polymorphisms showed this mutation had occurred on at least three different haplotypes. This suggests the high frequency of this mutation is not due to a founder effect resulting from a single common ancestor, but that this region represents a mutational hotspot. Our results therefore suggest that this mutation is the most common single mutation causing MODY in UK families. Rapid screening for this mutation should now be carried out in MODY pedigrees before commencing mutation screening in other exons.

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DISRUPTION OF THE HNF-4α BINDING SITE IN THE HNF-1α PROMOTER IN AN ITALIAN FAMILY WITH MODY

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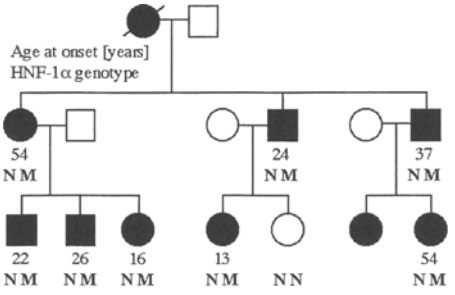
Mutation screening of the promoter and 10 exons of the HNF-1α gene in an Italian MODY family revealed an A→C substitution in the proximal promoter region. This mutation which is located in a putative HNF-4α binding site, cosegregates with MODY/NIDDM in this family.

normal allele: GGCTGAAGTCCAAAGTTCAGTCCCTTCGCT
mt. HNF-4α binding site: GGCTGAAGTCCCAAGTTCAGTCCCTTCGCT



We propose that this mutation affects transcriptional regulation. This mutation is associated with a progressive form of NIDDM characterized by increasing severity with age and the appearance of diabetes complications around 50 years of age.

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MITOCHONDRIAL DNA MUTATIONS ARE HIGHLY ASSOCIATED WITH TYPE 2 DIABETES MELLITUS IN JAPANESE PATIENTS

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Since inheritance of mitochondrial DNA (mtDNA) is exclusively maternal, mtDNA mutations have been implicated in this maternal inheritance of diabetes mellitus. The prevalence studies in a large population of diabetic patients revealed about 1 to 1.5% of mtDNA mutation at 3243 in different countries including Japan. However, it is possible that we are underestimating the precise prevalence of mtDNA mutation(s) associated with type 2 diabetic patients, because we have data on only 3243 mutation. Recently, we found at least 56 mtDNA mutations in Japanese by polymerase chain reaction-restriction fragment-single strand conformation polymorphism (PCR-RF-SSCP) analysis compared with the Cambridge Sequence. We investigated the prevalences of these mtDNA mutations in randomly selected 99 patients with type 2 diabetes mellitus and in 115 healthy control subjects by PCR-SSCP or PCR-RFLP analyses. We found that 8.1% (8/99) of Japanese patients with type 2 diabetes mellitus were associated with A to C mtDNA mutation at 1382, which was significantly higher than 1.7% (2/115) of control subjects. In addition, 14.1% (14/99) of diabetic patients were associated with mutations either at 1382 or A to G at 12026. Since none of the 14 patients with mutations either at 1382 or 12026 had mutation at 3243, we think that these two sites are new mtDNA mutations highly associated with type 2 diabetes mellitus in Japanese. The mutations were all homoplasmic. The mutation at 1382 is located in 12S rRNA and mutation at 12026 is expected to cause amino acid replacement from isoleucine to valine in NADH dehydrogenase 4. In conclusion, our investigation demonstrates that mtDNA mutations are much more frequently associated wih type 2 diabetic patients in Japanese than it has previously been reported. The prevalences of these mtDNA mutations needs to be examined in other countries or in other ethnic groups.

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GENETIC POLYMORPHISM OF LIPOPROTEIN LIPASE IS ASSOCIATED WITH NIDDM

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NIDDM is a polygenic disease commonly associated with lipid abnormalities, particularly elevated triglyceride levels. Lipoprotein lipase (LpL) is a key enzyme for triglyceride metabolism. This study explored the possible association between NIDDM and lipoprotein lipase (LpL) gene polymorphism in people with NIDDM. 597 Australian Caucasians were studied [mean (SE) age 58 (0.3)]. 214 had confirmed NIDDM. All were genotyped for LpLPvuII using PCR technique. Polymorphism of LpLPvuII is caused by the presence (+) or absence (-) of a PvuII recognition site in intron 6. The frequency distribution of PvuII +/+, PvuII +/- and PvuII -/- was 0.304, 0.502 and 0.194 respectively, and was in Hardy-Weinberg equilibrium. Using logistic regression analysis, there was a significant association between the PvuII polymorphism and the presence of NIDDM (r=0.137, p=0.0055) independent of age, gender and other circulating lipid variables. Whilst PvuII +/+ was associated with higher circulating triglyceride levels, the association between NIDDM and the Pvu II polymorphism was not affected by triglyceride levels. The odds ratio (OR) for patients homozygous for PvuII +/+ to have NIDDM was 2.51 (95% CI: 1.36-4.62) compared with PvuII -/- homozygotes. Among heterozygotes (PvuII +/-) there was also a trend towards increased NIDDM risk (OR:1.44, 95% CI: 0.82-2.54) compared with PvuII -/-. Furthermore in NIDDM patients with the Pvu II +/+ genotype there was an independent association with the presence of coronary artery disease in logistic regression analysis (p=0.0155). The frequency of PvuII +/+ in NIDDM patients with coronary heart disease was significantly higher than in those without (51.7% v 30.5%, $\chi^2 = 8.52$, p=0.014). LpLPvuII polymorphism and the occurrence of NIDDM are significantly associated and NIDDM with PvuII +/+ are more likely to have coronary artery disease.

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GENOMIC SCAN FOR GENETIC MARKERS LINKED TO DIABETES IN PIMA INDIANS.

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Genetic factors are likely to play a role in the etiology of non-insulin-dependent diabetes mellitus (NIDDM), but susceptibility loci for the most common forms of NIDDM have not been identified. A genome-wide scan was conducted to identify genetic markers linked to NIDDM in Pima Indians. Among 264 nuclear families containing 1862 sibling pairs, 517 anonymous genetic markers were genotyped from all 22 autosomal chromosomes. In 563 of these pairs, both siblings had NIDDM with age of onset < 45 yrs and analyses of the mean proportion of marker alleles identical by descent (IBD), which is expected to be 0.5 under the null hypothesis of no linkage, were conducted. The Haseman-Elston test of linkage, which regresses the proportion of marker alleles IBD against the squared difference in the sibs' trait values, was also conducted among all sib-pairs, using a cumulative incidence method to account for age of onset. Two chromosomal regions showed "suggestive" linkage to diabetes ($p < 0.0007$). Chromosome 7, in the vicinity of D7S1799 was linked in the affecteds-only analysis ($p = 0.0004$) and chromosome 11, in the vicinity of D11S4464, was linked by the cumulative incidence method ($p = 0.0003$). Seven other regions also had tentative evidence for linkage ($p < 0.01$). These results suggest that diabetes-susceptibility genes may be located at 7q21-22 and 11q23-25. Several other genetic loci may also contribute to NIDDM. These findings need to be extended in other studies of Pima Indians and, if possible, replicated in other populations. Additional fine mapping of these areas is needed to localize further the genetic elements involved.

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PPARγ AND HUMAN ADIPOCYTE DIFFERENTIATION: EVIDENCE FOR SITE-SPECIFIC EFFECTS OF THIAZOLIDINEDIONES AND PG$\Delta^{12,14}$J2

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The gamma isoform of peroxisome proliferator activated receptor (PPARγ) is highly expressed in adipocytes and promotes the differentiation of murine preadipocyte cell lines. Thiazolidinediones, a novel class of antidiabetic agent, enhance insulin action and bind to PPARγ with high affinity. The prostanoid 15-deoxy-D12,14 prostagandin J2 ($\Delta^{12,14}$ PGJ2) may represent the natural ligand for PPARγ. We have examined the effects of these agents on the differentiation of human Omental (Om) or Subcutaneous (Sc) preadipocytes from non diabetic subjects. Under control conditions, Om or Sc preadipocytes exhibited negligible differentiation, as assessed by lipid accumulation, glycerol-3-phosphate dehydrogenase (G3PDH) enzyme activity and G3PDH gene expression. Following BRL 49653 or troglitazone exposure, G3PDH activity and gene expression in Sc preadipocytes were markedly increased and differentiation was also evident morphologically. At 1mM, BRL 49653 was 100-fold more potent than troglitazone, stimulating G3PDH activity 16-fold ($p<0.001$) vs 6-fold for troglitazone ($p<0.05$). Furthermore, the rank order of potency of these agents to induce differentiation correlated with their ability to stimulate reporter gene activity via human PPARγ. In contrast, Om preadipocytes were refractory to differentiation by thiazolidinediones. Western blotting showed abundant PPARγ expression in both Om and Sc preadipocytes and adipocytes, and nuclear PPARγ expression, as assessed by immunofluorescence confocal microscopy, was equally enhanced by thiazolidinediones in Om and Sc preadipocytes. The mechanism of the divergent cellular responsiveness to these compounds remains to be elucidated. Given the close link between central fat distribution and insulin resistance, a site-specific effect of the thiazolidinediones may have clinical relevance.

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THIAZOLIDINEDIONES INCREASE EXPRESSION OF UNCOUPLING PROTEIN, TYPE II IODOTHYRONINE 5'-DEIODINASE AND MITOCHONDRIA IN C3H10T1/2 CELLS.

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The thiazolidinediones (TZDs) are a new class of oral antidiabetic agents whose mechanism of action is not fully understood. Recently, the TZDs (*e.g.,* troglitazone) were identified as high affinity ligands for the peroxisome proliferator-activated receptor γ (PPARγ), a ligand-activated transcription factor in adipocytes. This suggests that the antidiabetic action of these compounds may involve PPARγ-mediated transcription of adipose genes involved in lipid and carbohydrate metabolism. To further understand the action of TZDs, we explored the effects of these agents on differentiation of C3H10T1/2 cells, a pluripotent stem cell-line of mesodermal origin. Histochemical and biochemical analysis revealed that when these cells were treated with TZDs and insulin, they accumulated lipid and expressed the adipocyte marker aP2, indicating they differentiated into adipocytes. Treatment during the growth phase with TZDs resulted in maximal lipogenesis, indicating a need for clonal expansion for efficient adipogenic differentiation. Further analysis revealed that addition of TZDs to the cells increased i) the lipolytic response of the cells to $ß_3$-agonists, ii) mitochondrial-staining, iii) expression of uncoupling protein (UCP) and, iv) expression of mRNA for type II iodothyronine 5'- deiodinase (5'D-II). The presence of UCP and 5'D-II are hallmarks of brown adipose tissue (BAT). Thus, this represents the first direct evidence indicating that brown adipocytes may arise from pluripotent stem cells of mesenchymal origin. Furthermore, these results suggest the antidiabetic effects of TZDs may involve BAT differentiation. This, in turn, may lead to increased $ß_3$- and UCP-mediated thermogenesis resulting in increased fuel metabolism.

OP 4

Insulin Therapy in IDDM

25

INTENSIVE INSULIN THERAPY ALTERS DAILY SUBSTRATE OXIDATION IN INSULIN-DEPENDENT DIABETES MELLITUS.

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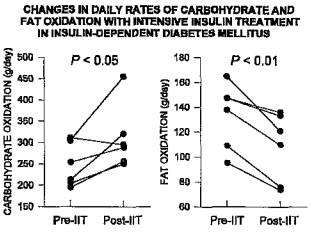
Weight gain and increased adiposity often accompany the improved glycemic control with intensive insulin therapy (IIT) in IDDM due to reductions in glycosuria, daily energy expenditure (EE), and basal fat oxidation. To investigate the role of changes in energy metabolism and metabolic efficiency during exercise in the propensity for weight gain with IIT, we studied 6 adult IDDM patients (gender 2M/4F, age 33±4 yrs, weight 75±7 kg, body mass index [BMI] 24±1 kg/m^2, diabetes duration 14±4 yrs) before and after 4 wks of insulin pump therapy. Daily EE, sleeping metabolic rate (SMR), basal metabolic rate (BMR), and EE and mechanical work (MW) during a standardized exercise protocol were measured during 24h stays in a respiratory chamber equipped with a novel force platform floor system, allowing simultaneous measurement of the amount and energy cost of physical activity. Metabolic efficiency during exercise was calculated as MW/EEex, where EEex is the increment in EE with exercise. After 4 wks of IIT, HbA1c improved (10.4±0.7 to 8.7±0.6%, $P < 0.05$) and glycosuria decreased (217±89 to 89±28 mmol/d, $P < 0.05$). Daily EE (9.58±0.78 vs 9.73±0.82 MJ/d), SMR (5.87±0.54 vs 6.00±0.45 MJ/d), BMR (6.32±0.61 vs 6.46±0.59 MJ/d), EEex (1.89±0.15 vs 1.89±0.19 MJ/d), and metabolic efficiency during exercise (23.1±3.2 vs 23.1±2.8%) were similar before and after IIT. However, daily rates of carbohydrate oxidation increased (246±21 vs 311±31 g/d, $P < 0.05$) and fat oxidation decreased (133±11 vs 108±11 g/d, $P < 0.01$). Thus, IIT alters substrate oxidation but does not appear to alter metabolic efficiency or EE during exercise in patients with IDDM. These results suggest that the reductions in fat oxidation and glycosuria with IIT are the major factors that contribute to the propensity for weight gain and increased adiposity in intensively treated patients with IDDM.



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SUBCUTANEOUS GLUCOSE MONITORING DURING 8 DAYS IN IDDM PATIENTS: WHAT HAVE WE LEARNT?

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Introduction: The hypothesis that hypoglycemia as well as hyperglycemia occur in IDDM patients much more frequently than what patients themselves may recognise whether by symptoms or by measuring their capillary glucose is one of many questions we may answer with the help of continuous tissue glucose (TG) monitoring. We designed a protocol to observe the glucose changes in IDDM patients during 48h under daily life conditions. Methods: We developed a glucosensor based on the enzymatic-amperometric method in combination with a microdialsyis probe (Ulmer Zuckeruhr-System) to monitor on-line the TG changes on a continuous basis. We performed 4 series of TG monitoring in 2 IDDM patients (sex: 1:1, age: 39.5±10.6 years; BMI: 23.7±3.0 kg/m^2, HBA1$_c$: 7.2±0.3) under intensified insulin therapy (injection therapy), each monitoring lasting 48 hours. Patients stayed during the first 24h within the facilities of our institute. During this time blood glucose was hourly measured with a reflectometric glucometer. In the following 24h the patients were allowed to go to their work or just to carry their normal life and were asked to control at least 5 times their capillary glucose. Results: Blood glucose values fluctuated between 35 mg/dl and 350 mg/dl. The sensor signal correlated well with the glucose reference values. Hypoglycemia, i.e. glucose values <54 mg/dl occurred 21 times, but it was recognised only 12 times by the patients. Most of the episodes occurred during day time (n=18). Additionally, hyperglycemic values >250 mg/dl occurred 12 times (9 episodes during day time) while patients detected only 7 episodes. Conclusions: Our patients detected just above half of their blood glucose extreme variations, which mostly occurred during the day. On the other hand, our glucosensor showed to be reliable to monitor the TG for 48h in all the cases.

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IMPACT OF RECENT CHANGES IN THE MANAGEMENT OF IMPLAN TABLE PUMPS. REDUCTION OF DYSFUNCTIONING

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Recent changes in insulin stability have led to an increase in catheter (KT) and/or pump related problems from 1993 to 1994. Simultaneously, physicians and manufacturers have introduced several improvements in the management of implantable pumps including changes in the refill frequency, in refill and rince procedures and development of a new KT (MiniMed 4027) with direct access (side-port : SP).

The aim of the study was to evaluate the impact of these changes on the functionnality of pump (from 01/93) and KT (from 11/94) together with the consequences on the patients including rate of surgical reintervention. Therefore, data from our group have been analysed within 3 periods : period I as a reference (before insulin stability decrease) : from 1991 to 1992, period II : from 1993 to 1994 (decrease in insulin stability), period III : from 1995 to 1996 (effects of technical improvements). All the patients were treated with MIP 2001 pumps, using U400 Hoechst 21 PH insulin. Results are summerized in the following table :

	Period I	Period II	Period III
Nb of pumps	43	51	55
Study duration (PxY)	50.04	57.96	56.02
Nb of KT flush/PxY	NA	NA	0.54
Nb of NaOH rince/PxY	NA	0.66	1.32
Nb of Kt exchanges/PxY	0.16	0.17	0.05
Discontinuation (%pumps)	0	37	0

PxY : patient x year

These data clearly demonstrate that the changes developed have fully restaured the safety and the feasability of intraperitoneal insulin infusion by means of implantable pumps.

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INADEQUATE USE OF INSULIN AS A CAUSE OF POOR GLYCAEMIC CONTROL AND KETOACIDOSIS

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A variety of manipulations of insulin treatment are perceived as a cause of poor glycaemic control and ketoacidosis. There are no direct data confirming poor compliance in IDDM. We present data (April 1993 to September 1994) comparing the known prescribed insulin dose (units) with insulin supplied using the Diabetes Audit and Research in Tayside Study (DARTS) database. DARTS contains complete data on all prescribed drugs encashed at all Tayside pharmacies. The cumulative volume of insulin prescriptions supplied was used to calculate the days of total insulin coverage, expressed as the compliance index. Relationships between compliance index, glycaemic control (HbA1c) and episodes of ketoacidosis were investigated in all patients attending our young adult and paediatric diabetes clinics. Eighty-nine patients aged 16±7 (mean ± S.D.) years, diabetes duration 8±4 years, HbA1c 8.4±1.9% were studied. Taken as a whole, mean insulin dosage prescribed was 48±19 units/day; mean insulin encashed was 58±25 units/day. Twenty-five patients (28%) encashed less insulin than their prescribed dose (mean deficit 115±68 insulin-free days per annum; range 9-246 days). A strong negative relationship existed between the compliance index and HbA1c (R^2 0.39; $p<0.001$) which remained when adjusted for all other covariates (e.g. age, weight, diabetes duration; $p<0.001$, multiple linear regression). In the top quartile (HbA1c>10%), 14 (64%) of subjects had insulin encashment records suggestive of poor compliance (mean deficit 55 insulin free days per annum) compared with only 2 (9%) in the lowest quartile (HbA1c<7%; mean excess 296 insulin days p.a.). Of all 10 ketoacidosis episodes, 9 occurred in subjects with a poor compliance index ($p<0.001$; mean deficit 108 insulin free days per annum). These data represent the first direct evidence of insulin under-utilisation in young patients with IDDM. They suggest poor compliance is a major factor contributing to long-term poor glycaemic control and ketoacidosis.

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VARIABILITY OF THE METABOLIC EFFECT OF S.C. INJECTED REGULAR INSULIN AND THE RAPID ACTING INSULIN-ANALOGUE B28ASP

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We studied the intra- and interindividual variability of the metabolic activity of regular insulin (R) and of the rapid acting insulin analogue B28Asp. Nine healthy male volunteers (age 25±1 years, BMI 22.6±2.1 kg/m²) recieved s.c. injections of R (Actrapid HM, NOVO NORDISK; U100; 0.2 U/kg, 14.4±1.6 U) during euglycaemic glucose clamps on 4 study days (blood glucose 5 mmol/l, basal i.v. insulin infusion 0.15 mU/kg/min). Ten other volunteers (age 26±2 years, BMI 22.9±1.9 kg/m²; N.S.) received on the 4 days injections of B28Asp (NOVO NORDISK). In the 600 min after the injections glucose infusion rates (GIR) and serum insulin concentrations were determined. In comparison to R s.c. injection of B28Asp resulted in a more rapid onset of action and a shorter duration of action. We investigated the variation in action of these insulin by calculating coefficents of variation (CV). Even under the strictly controlled experimental conditions of this study, the s.c. injection of R resulted in a intra-individual variability of the metabolic activity of 10-25 % in healthy volunteers (see table). Injection of B28Asp resulted in a lower variability in the time needed until maximal serum insulin concentrations were reached than with R. The inter-individual variability was approximately 10 % higher than the intra-individual with both insulin preparations.

	intra-indivi. CV (%)			inter-indivi. CV (%)	
parameter	R	B28Asp	p-value	R	B28Asp
GIR_{max} (mg/kg/min)	14.9	15.6	0.866	26.2	28.1
C_{max} (pmol/l	19.4	14.2	0.187	27.8	17.7
t_{max} - GIR (min)	13.9	11.1	0.473	23.2	18.5
t_{max} - Insulin (min)	24.4	15.2	0.026	37.2	19.6
AUC 0-90 min - GIR	32.3	25.7	0.480	51.7	41.7
AUC 0-90 min - Insulin	19.8	18.1	0.840	33.4	27.1
AUC 0-600 min - GIR	12.7	17.7	0.258	20.9	25.2
AUC 0-600 min - Insulin	14.3	15.2	0.758	17.4	18.9

(paired t-test for intra-individual comparisons)

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REDUCED NOCTURNAL HYPOGLYCAEMIA WITH INSULIN ANALOGUE LISPRO IN TYPE 1 DIABETIC PATIENTS

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Unmodified insulin has a long absorption tail, unlike short-acting insulin analogues, and may contribute to hypoglycaemia in the early part of the night. A randomized, crossover, double-blind study was therefore performed to compare blood glucose concentrations in the early part of the night in Type 1 diabetic patients, receiving lispro or unmodified insulin, in random order, on two separate study days. Twenty three C-peptide negative patients, 12 using a basal-bolus insulin regimen, and 11 using twice-daily insulin, were studied. The patients were admitted to the investigation unit at 1700 h, and received a single dose of lispro or unmodified human insulin before the evening meal. In both groups the NPH insulin doses remained unchanged. Identical meals and snacks were eaten at the same time during both study days. Average postprandial (1800 - 2400 h) blood glucose concentrations were significantly lower after lispro therapy compared to unmodified insulin therapy (8.0 ± 0.3 (SE) vs 9.0 ± 0.3 mmol l^{-1}, p = 0.003). Night-time (2400 - 0400 h) blood glucose concentrations were significantly higher after lispro compared to unmodified insulin therapy (10.3 ± 0.4 vs 9.1 ± 0.4 mmol l^{-1}, p = 0.02). This difference was greater in patients on the basal-bolus insulin regimen (11.6 ± 0.5 vs 8.7 ± 0.4 mmol l^{-1}, p = 0.000). The incidence of nocturnal hypoglycaemia (2400 - 0400 h, blood glucose < 3.5 mmol l^{-1}) was less with lispro compared to unmodified insulin (1 vs 6 patients, p = 0.04). Thus on a basal bolus insulin regimen, evening lispro gives less hypoglycaemia in the early part of the night at the expense of average blood glucose control.

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IMPROVEMENT OF HBA1C WITHOUT INCREASING HYPOGLYCEMIA RISK IN DIABETIC PATIENTS TREATED WITH INSULIN LISPRO IN EXTERNAL PUMPS.

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The aim of this 6-month randomized, open, crossover, multicenter trial was to compare the effects of insulin lispro (LP) to Actrapid HM 100 pump formulation (Actr.) on glycemic control in Type I diabetics. These diabetic patients have been using a continuous subcutaneous insulin infusion (CSII) for at least 1 year. Thirty-nine patients (mean age ± SD: 38.9 ± 9.9 yr; BMI: 24.5 ± 2.4 kg/m²; mean disease duration: 22.2 ± 9.6 yr; mean duration of pump treatment: 5.1 ± 3.0 yr; mean HbA1c= 7.84% ± 0.76) were treated on CSII using an external pump (Minimed 506). The patients were randomized to LP or Actr. for 3 months then switched to the other insulin for 3 months. Boluses were given 0 to 5 min. before 3 meals for LP and 15-30 min. before for Actr. Six blood glucose (BG) measurements were taken daily (3 pre- and postprandial) and stored in a memory meter. At the end of the 3-month period, HbA1c had a larger decrease with LP (0.62% ± 0.58 [7.74% ± 0.90 to 7.11% ± 0.69]) compared to Actr. (0.09% ± 0.63 [7.97% ± 0.53 to 7.88 % ± 0.65]) (p=0.01). The standard deviation (SD) of BG values, an index of glycemic fluctuation and the main criteria of the study, is 3.46 ± 0.55 mmol/l with LP compared to 3.79 ± 0.66 mmol/l with Actr. (p=0.0001). Mean postprandial BG decreased significantly with LP (8.19 ± 1.04 mmol/l compared to 9.79 ± 1.26 mmol/l) (p=0.0001), as well as the SD of the postprandial BG : 3.55 ± 0.55 mmol/l (LP) compared to 3.88 ± 0.66 mmol/l (Actr.) (p=0.0002). The mean and SD of preprandial BG were similar in the 2 groups. The incidence of hypoglycemia per 30 days (BG<3.3 mmol/l) was lower but not significantly decreased in the LP group (10.89 ± 7.16 compared with 12.90 ± 6.07) (p= 0.36). At the end of the crossover study, all but one patient chose insulin lispro for the extension phase. In conclusion, insulin lispro optimized the metabolic control of Type I diabetics treated by CSII, reducing significantly the HbA1C levels, without increasing the risk of hypoglycemia. These results could be related to the pharmacokinetic profile of LP used in CSII as basal and bolus insulin therapy.

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DOSE RESPONSE WITH LONG-ACTING INSULIN ANALOG NN304 FOLLOWS ANALOG DYNAMICS IN HINDLIMB LYMPH IN DOGS.

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The new long-acting insulin analog, Lys^{B29}-tetradecanoyl, des(B30) human insulin (NN304) has shown a protracted action profile with I.V. injection and infusion. This protraction is assumed to be due to albumin binding in plasma and interstitial fluid. However, the temporal profile for dose-dependent activation and deactivation of glucose turnover by NN304 has not been examined. This study examined glucose utilization (Rd) and glucose production (HGO) in response to NN304 infusion at three doses. Euglycemic clamps (Hot GINF), with arterial and hindlimb lymph (HL) sampling, were performed in normal dogs during somatostatin infusion (0.8 µg/min/kg) plus basal insulin replacement (1.2 pmol/min/kg) for 660 min. NN304 was infused at 3.6 (N=4), 10 (N=4), or 18 (N=1) pmol/min/kg for 0-320 min (activation), then suspended from 320-660 min (deactivation). Tracer (3-^{3}H-glucose) was infused throughout starting at -120 min.

	3.6 pmol/min/kg	**10** pmol/min/kg	**18** pmol/min/kg
NN304, plasma (pM)	2412 ± 522	4958 ± 456	14397
HL (pM)	176 ± 27	430 ± 26	1687
Rd (mg/min/kg)	7.2 ± 1.2	14.2 ± 0.6	15.6
HGO (mg/min/kg)	1.2 ± 0.3	1.5 ± 0.6	0.2

During activation, steady state (SS) plasma and HL NN304, Rd and HGO differed by dose (P<0.02). Activation/deactivation times to 50% of SS ($t_{1/2}$) were similar within each dose with a tendency toward delayed deactivation (P≥0.10, limited power of detection). Activation of Rd and suppression of HGO were similar to NN304 $t_{1/2}$ in hindlimb lymph, but not plasma (P<0.01). These data 1) indicate that transendothelial transport is rate-limiting for NN304 as with native insulin, and 2) are consistent with protracted action of the analog due to albumin binding.

OP 5
Current Management of NIDDM

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METFORMIN ASSOCIATED TO INSULIN THERAPY FOR TREATMENT OF OBESE NIDDM.

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Albeit its widespread use, the advantages of combined sulfonylurea-insulin (SU) treatment in NIDDM are questionable. On the other hand, metformin-insulin (MI) combination would be potentially beneficial for NIDDM patients, because of peripheral action of metformin. To assess the effects of MI as compared to SI on fasting plasma glucose (FPG), Hba_{1c}, body weight and insulin dose, we studied 100 patients with NIDDM (35M, 65F), treated with MI (N=27 obese, BMI=34.5±0.7, and N=23 nonobese, BMI=26.9±0.6) or SI (N=22 obese, BMI=34.8±0.9, and N=28 nonobese, BMI=26.8±0.4) for 6 months. Both treatments decreased to a similar extent FPG (MI, 11.4±0.6 and SI, 10.9 ±0.6 mmol/L, p=NS) and Hba_{1c} (MI, 8.1±0.2 and SI, 7.7±0.3 %, p=NS) in nonobese NIDDM patients, but weight gain (MI, 0.4±0.1 vs SI, 3±0.1 kg, $p<0.05$) resulted lower after MI. In obese NIDDM patients, FPG (MI, 9.1±0.4 vs SI, 9.9±0.8 mmol/L) and Hba_{1c} (MI, 7.4±0.2 vs SI, 8.2±0.3 %, $p<0.05$) and body weight (MI, -2.3±1.5 vs SI, -1±0.8 kg, $p<0.05$) decreased to a greater extent after MI (all, $p<0.05$). Interestingly, daily insulin dose decreased after MI but not after SI, in nonobese (MI, -8±2.9 vs SI, 6±2.2 Units) and obese (MI, -12±1.7 vs SI, 3±2.1 Units) NIDDM patients (both, $p<0.05$). In conclusion, in nonobese NIDDM, MI prevents weight gain and increase in insulin dose; in obese NIDDM, MI determines a better glycemic control and weight loss. Therefore, MI appears to be more effective than SI in the treatment of obese NIDDM.

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METFORMIN-ASSOCIATED LACTIC ACIDOSIS

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Metformin was introduced in the USA in May 1995. Through June 30, 1996, the FDA received reports of 66 cases of lactic acidosis attributed to metformin. In 47 patients, the diagnosis was confirmed by a lactate level of 5.0 mM or greater. Characteristics of these patients are shown in the table. In the remaining 21 patients, 2 were found to have not been taking metformin, 3 had no lactate reported, 7 had normal lactate levels (under 2.7 mM), and 7 had mild hyperlactatemia with levels between 2.7 to 4.9 mM. All patients with mild hyperlactatemia recovered.

	DIED	RECOVERED
number (male/female)	20 (9/11)	27 (9/18)
age, mean ± SEM	70.5 ± 2.7	67.3 ± 2.1
lactate, mM	15.9 ± 1.9 *	11.2 ± 1.3 *
pH	7.085 ± 0.01 *	7.29 ± 0.01 *
dose, mg/day	1259 ± 145	1349 ± 115
time to onset, days	64.6 ± 26.1	56.3 ± 18.1
creatinine> 1.4	16 **	7 **
creatinine< 1.5	1 **	8 **

* $p<0.05$ by t test ** $P<0.05$ by exact permutation test

Based on an estimate of about 1 million patients who have been treated through June 30, 1996, the reporting rate of confirmed lactic acidosis was about 5 per 100,000. Mortality was 43%.

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DO SULFONYLUREAS INCREASE GROWTH HORMONE SECRETION IN MAN?

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It has been recently demonstrated that glipizide stimulates growth hormone (GH) release and antagonizes somatostatin (SRIF) action in cultured somatotrophic cells. To determine the relevance of this observation to humans, we measured GH secretion in 12 healthy subjects following ingestion of glipizide (5mg) or placebo at time 0 min. On each occasion, GHRH (i.v.bolus 0.1μg/kg) was given at 60 and 210 min, with SRIF being infused from 180-330 min. Glucose was clamped (glipizide day) at 5mM. The order of the studies was random. As expected, glipizide induced a profound increase in insulin that was completely suppressed by SRIF. In the presence of normal plasma glucose concentrations, glipizide (vs. placebo) produced 20% increase in GH secretion over entire study duration (AUC 2961±456 vs. 2469±334ng/ml/6hrs, $p<0.05$) and nearly doubled GH secretion during SRIF suppression period (AUC 1105±260 vs. 656±90 ng/ml/2.5hr, $p<0.05$). Our data therefore indicated a stimulatory effect of sulfonylureas on GH secretion *in vivo*. Since excess GH secretion may accelerate diabetic complications, the present findings if confirmed in diabetic patients, may have significant clinical implications to patients treated with sulfonylureas.

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EFFECT OF SULPHONYLUREA DRUGS ON ARRHYTHMIAS AND DEATH RATE INDUCED BY ISCHAEMIA AND REPERFUSION.

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Influence of glimepiride (GM), glibenclamide (GB), gliclazide (GC) and tolbutamide (TB) was analyzed on incidence of ventricular ectopic beats (VEB), on duration of ventricular fibrillation (VF) and on death rate (DR; vs. solvent:23±1%) induced by coronary clamping (CC;30 min) or reperfusion (CR ; 50 min following 10 min CC) in healthy (H) and streptozotocin-diabetic (D) rats. Drugs (0.1-1000 μmol/kg) were administered i.p. 30 min before CC. Data were compared using Student's t-tests and regression analysis, $p<0.05$ was considered significant. During CC only GB decreased these parameters (VEB: y= -400logx+635; VF:y=-45logx+97; DR:28±6%) in H rats, whereas GC (VEB:y=732logx +1350; VF:y=174logx+179; DR:65±5%), GM (VEB:y=573logx+739; VF:y=24logx+82; DR:74±7%) and TB (VEB:y=626logx+1635; VF:y=285logx+260; DR:66±5%) increased them. During CR only GM decreased these variables (VEB:y=-61ogx+13; VF:y= -5logx+13; DR:21±6%), while GB (VEB:y=20logx+32; VF:y=3logx+22; DR:60±6%), GC (VEB:y=9logx+46; VF:y=3logx+22; DR:53±5%) and TB (VEB:y=19logx+46; VF:y=8logx +42; DR:60±8%) enhanced them. In D condition the effects of GM and GB changed. Namely, GM decreased these parameters (VEB:y=-269logx+425; VF:y=-146logx+251; DR:13±6%) during CC and GB (VEB:y=-141ogx+31; VF:y=-17logx+29; DR:10±6%) during CR. Suggesting that only GB and GM should be preferred in oral antidiabetic treatment of type-2 diabetic patients suffering from ischaemic and reperfusion events.

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WHITE COAT EFFECT IN DIABETES: AMBULATORY BLOOD PRESSURE MEASUREMENT IN RELATION TO CLINIC BASED MEASUREMENT.

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Aim: To quantify differences between clinic and ambulatory blood pressure (AMBP) recordings in diabetic patients with suspected hypertension, and to relate the white coat effect to renal function, gender, age, and antihypertensive treatment. **Methods and Patients:** Within 3½ years (1.1.93 - 5.31.96) 119 diabetic patients were referred to AMBP monitoring from our out-patients' clinic. 103 fulfilled WHO's criterion of hypertension (Systolic BP > 140 mmHg and/or diastolic BP > 90 mmHg) at two blood pressure measurements obtained in the usual out-patient clinical setting. AMBP was measured using Spacelab apparatus (model 90202). Sixty-one had IDDM, 42 NIDDM, 64 males, 39 females. The mean age for the whole population was 48.7 years (16.6 - 80.2 years), 44 patients received antihypertensive treatment. Forty-eight patients were normoalbuminuric (albumin/creatinine ratio <2.5 mg/mmol for males and <3.5 for females), 55 patients had microalbuminuria or nephropathy. Among the 48 normoalbuminuric patients 17 received antihypertensiva prior to the AMBP recording. **Results:** BP measurements recorded in the clinic was significantly higher compared with day-time AMBP: for the systolic BP in average 15.6 mmHg (5 and 95% percentiles: -13.1 and 51.3 mmHg, $p < 0.0001$) and for the diastolic BP in average 10.1 mmHg (5 and 95% percentiles: -7.5 and 29.7 mmHg, $p < 0.0001$). The differences between the BP measurements were not related to gender, age, diabetes duration, type of diabetes, UAE, or to antihypertensive treatment. In consequence of the AMBP measurement only 5 patients among 31 normoalbuminuric patients started antihypertensive treatment, in another 7 normoalbuminuric patients ongoing treatment was intensified. **In conclusion:** 1) The white coat effect in diabetic patients are in accordance with other studies of non-diabetics. 2) The white coat effect varies considerably among patients and is not predictable. 3) AMBP recording may be relevant especially in normoalbuminuric diabetic patients with high clinic BP recordings, since unnecessary treatment may be avoided in a large fraction of patients - in this material 83%.

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TREATMENT OF NIDDM: FAIR GLYCAEMIC CONTROL DURING 5 YEARS PREVENTS PROGRESSION OF ALBUMINURIA

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Aim: To compare the effect of insulin and sulphonylurea therapy in patients with NIDDM and study the effect of good glycaemic control on progression of albuminuria. **Material and methods:** Thirty-one men and 24 women aged 59.3 ± 6.2 (mean, SD) years, with a duration of NIDDM for 7.3 ± 3.1 years and BMI 26.7 ± 3.7 kg/m^2 were randomised to continue sulphonylurea therapy (SU, n=26) or start insulin (I, n=29) and followed for a median of 5 (3-6) years. Levels of HbA_{1c} and albuminuria was measured 3-6 monthly. Progression of albuminuria was defined as an increase in the overnight albumin excretion rate from normoalbuminuria (<20µg/min) to microalbuminuria (MA 20-200 µg/min), or from MA to proteinuria (>200 µg/min). **Results:** 1) In the I group HbA_{1c} was reduced from 8.7 ± 1.6 to 7.8 ± 1.1 % ($p=0.001$) after one year and remained unchanged thereafter. In the SU group, HbA_{1c} increased continuously, and 21 of 26 patients had to be given insulin after a mean of 2.1 years due to hyperglycaemic symptoms or HbA_{1c} >10 %. When changed to insulin therapy, these patients achieved similar glycaemic control as those originally randomised to insulin 2) Due to the change of therapy in the SU group, we analysed the data according to the glycaemic control achieved, and found that 23 subjects had fair glycaemic control (average HbA_{1c} from year 1-5 < 8.0%). None of these patients had progression of albuminuria during the study, while 6 of the patients with average $HbA_{1c} \geq 8.0\%$ had such a progression. 3) The insulin dose at the end of the study was 0.82 ±0.4 U/kg. There was a significant negative correlation between the insulin dose and the insulin sensitivity index (GDRI) measured with the euglycaemic clamp technique at baseline ($p<0.01$). However, variations in GDRI predicted only 22% of the variations in insulin dose among patients. **Conclusion:** Insulin, but not SU therapy is able to achieve and maintain good glycaemic control during long term and good glycaemic control may prevent progression of albuminuria in patients with NIDDM.

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A RANDOMIZED TRIAL OF COUNSELING FOR FAT RESTRICTION VERSUS USUAL DIETARY ADVICES IN THE TREATMENT OF NIDDM

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Dietary fats play a major role in the development of obesity and insulin resistance leading to NIDDM. In this study we tested the assumption that promoting a high carbohydrate- low fat diet consumed ad libitum may be a more effective dietary treatment of newly diagnosed NIDDM patients, than is a caloric restriction with a defined amount of carbohydrates. Fourty NIDDM patients were randomized to one of the two treatment groups and so far received intensive dietary counseling over a period of 6 months (a 5 year follow up is planned). One group (FAT-group) was instructed in reducing dietary fat with the possibility to eat carbohydrates and protein ad libitum. The other group (CAL-group) underwent the usual dietary advices, as caloric restriction with a defined daily amount of carbohydrates. Dietary intake, body weight, body composition, diabetes control (HbA1c) and dietary compliance (difference between resting energy expenditure and energy intake in %) were measured.

In contrast to the CAL-group energy derived from fat decreased significantly in the FAT-group (43% to 28% vs. 43% to 36%). The carbohydrate component increased in the FAT-group but remained constant in the CAL-group (43% to 51% vs. 42% to 44%). The resulting weight reduction after 6 months was significantly higher in the FAT-group (-3,5kg vs. -1,7kg in the CAL-group; $p<0,05$). The FAT-group reduced mainly body fat mass (80%), the CAL-group mainly lean body mass (75%). Diabetes control improved in both groups (HbA1c: FAT-group 10,6% to 7,1%; $p<0,01$ vs. CAL-group 10,0% to 7,6%, $p<0,01$). Dietary compliance ranged from 20 to 98% and was positively correlated to the loss of body fat mass and negatively to the loss of lean body mass. It was significantly better in the FAT-group (mean 72% vs. 45% in the CAL-group; $p<0,01$).

In conclusion, a 6 month counseling for fat restriction with ad libitum carbohydrate and protein intake in the dietary therapy of NIDDM decreases HbA1c in the same amount as an usual dietary advice. Nevertheless the compliance to the dietary advices was significantly higher in the FAT-group, resulting in a significantly higher weight reduction, mainly of fat mass.

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CARBOHYDRATE MALABSOPTION FOLLOWING ACARBOSE ADMINISTRATION.

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Carbohydrate absorption during Acarbose administration was determined to investigate the action and side effects of this drug. In seven healthy volunteers, the breath hydrogen concentration was obtained at 15-min. intervals after administration of 6 g of lactulose, and the area under the concentration-time curve for four hours from the time that breath hydrogen exceeded the pretreatment level by ≥10 ppm was determined as the standard. Then the amount of undigested carbohydrate following administration of various doses of Acarbose and Ensure Liquid was calculated. In addition, breath hydrogen data were obtained before and after two and four months of Acarbose therapy in eight patients with non-insulin-dependent diabetes.There was a mean of 5.3 g, 7.7 g, and 10.8 g of carbohydrate unabsorbed after administration of 50 mg of Acarbose with 250 ml or 500 ml of Ensure and 100 mg of Acarbose with 500 ml of Ensure, respectively. Gastrointestinal symptoms (flatulence and loose stools) occurred when the amount of unabsorbed carbohydrate was 10 g or more.In the eight diabetic patients, breath hydrogen excretion decreased to 31.6% of the initial value following two months of Acarbose administration. Improvement of the hemoglobin A1C level was maintained after five months of administration.These results show that the increase of unabsorbed carbohydrate depends on the Acarbose dose and the carbohydrate load, as do the gastrointestinal symptoms experienced. Although the amount of unabsorbed carbohydrate decreases with continued Acarbose administration and gastrointestinal symptoms subside accordingly, improved glycemic control is maintained.

OP 6

Therapeutic Diabetes Education and Psychology

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LONG TERM BENEFITS OF IMMEDIATE, INTENSIVE EDUCATION AT DIAGNOSIS OF NIDDM

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In Bournemouth, all patients with new onset NIDDM are seen **within one week** of diagnosis in a group education programme. For the first 3 months, their sole care is given by the diabetes nurse specialist and dietitian according to pre-determined protocols. At 3 months, patients are screened for diabetic complications and the majority transferred to primary care for follow up as part of an integrated service. We have prospectively followed up a cohort of 156 patients referred between January and July 1994. 97% patients completed the programme and 92% were transferred to primary care within 12 months from diagnosis (of whom 8% have been referred back to secondary care).

Results	n	156	151	151	151	147
Outcomes(median)		Time from diagnosis(months)				
		0	3	6	12	24
HbAIc(%)		10.1	8.2	7.8	7.4	7.3
Weight(Kg)		80	79	76.9	77	77
Treatment(%)						
Diet only		100	49	51	46	46
Tablets		0	49	46	51	50
Insulin		0	2	3	3	4
Home monitoring(%)		Blood			42	38
		Urine			56	60
		None			2	2
Complications(%)	Retinopathy		8			8
	Nephropathy		10			12
	Neuropathy		11			13
	Macrovascular		17			17

Nurse led immediate patient management at diagnosis of NIDDM produces sustained, acceptable clinical outcomes.

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EFFECTIVENESS OF EDUCATIONAL COURSES FOR INSULIN-TREATED WORKING-AGED PERSONS WITH DIABETES

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The aim of the study was to evaluate the effectiveness of six day educational courses for insulin-treated working-aged persons with diabetes. The main focus in the study was on subjective evaluations of the outcomes of treatment, selfcare practices, attitudes towards diabetes and its selfcare, and quality of life. The measures used were based on the Health Belief Model and perceived control of selfcare. HbA_{1C}-values were used as measures of blood glucose levels. Four measurements were done at different time points: the first one month prior to the course, the second (HbA_{1C} not included) in the beginning of the course, the third three months after and the fourth 12 months after the course. The same measurements were performed on the intervention group (N = 110) attending the courses and the reference group (N = 80) not attending the courses. The HbA_{1C}-values of the intervention group were significantly ($p < .01$) lower three months after the course (M = 8.54, SD = 1.39) compared to the values before the course (M = 9.17, SD = 1.55). The course attendees also considered some aspects of selfcare, especially nutrition, more effective and less demanding and had more trust in their ability to practice selfcare in these areas. The reference group did not show similar changes. One year after the course there was not much left of the positive effects observed in the three month follow-up. The courses did have some significant positive effects, but they were not very lasting. The potential key to more lasting results could be closer and more frequent cooperation between the course attendee and his or her local health care unit even after the course. The aim would be to reinforce the altered attitudes and selfcare practices.

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LONGITUDINAL STUDY ON GLYCEMIC CONTROL AND QUALITY OF LIFE IN REFERRED NIDDM PATIENTS

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Aim of the study was to describe the impact of improved glycemic control on Quality of Life (QoL) in NIDDM patients referred for insulin therapy to an outpatient department (OPD). The study population consisted of 94 NIDDM patients referred to and treated by an OPD diabetes team aiming at achieving acceptable glycemic control by means of maximizing oral therapy, if necessary switching over to insulin therapy, information and education provided by a diabetes nurse and dietician. QoL was measured by using a disease-specific (Diabetes Health Profile - DHP) and a generic questionnaire (RAND-36). After one year the medical examination and QoL measurement were repeated. Changes in QoL during one year were compared between subgroups according to hyperglycemic complaints at baseline, switching over to insulin therapy, and achieving good metabolic control. Regression analysis included presence of hyperglycemic complaints at baseline, switching over to insulin therapy and change in glycosylated hemoglobin (GHb) as independent variables and changes in QoL-outcome as dependent variables. After one year, both GHb and QoL improved in the total group. Patients with hyperglycemic complaints (n=46) improved more in QoL than those without, especially on physical functioning, social functioning, vitality, and health change of the RAND-36, but at the final examination still scored lower on most of the DHP and RAND-36 dimensions. Patients switched over to insulin (n=61) improved in a similar manner as the others, but at the final examination they had more problems with social functioning and pain. Patients who achieved good glycemic control (GHb $\leq$ 8%, n=57) after one year improved in a similar manner as the others. At an OPD, the efforts of a diabetes team to achieve acceptable glycemic control in referred NIDDM patients resulted in improved glycemic control and increased QoL outcome. Reduction of hyperglycemic complaints seemed to be a determinant of improvement in QoL-outcome, whereas achieving good metabolic control was not. Changing to insulin therapy did seem to have a negative impact on the dimensions social functioning and pain, but not in other dimensions.

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DOCTOR-PATIENT COMMUNICATION DURING OUTPATIENT CONSULTATIONS IN NIDDM

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The treatment of NIDDM largely depends on self-management. Therefore, patients' needs need special consideration during medical consultations by means of effective communication. This will lead to higher levels of patient compliance and satisfaction, necessary in preventing complications and improving metabolic control. Patients' needs are likely to change through time. Ideally, doctor's communication patterns change accordingly. Our aim was to examine how communication behaviors actually change during a series of consecutive outpatient consultations in NIDDM. Therefore, the first 3 consultations between 18 newly referred patients with poorly controlled NIDDM and their internist were videotaped. Communication was measured by means of the Roter Interaction Analysis System. Changes in communication behaviors during these 54 consultations were analyzed using one-way analysis of variance and t-test. Results indicate that the doctor spoke longer than the patients during the repeat visits ($p<.05$). Accordingly, the amount of time the doctor looked at the patient declined gradually from initial to third consultation (58, 48 and 39% of the consultation time, resp., $p<.05$). In addition, during the first outpatient consultation the doctor was more attentive (e.g. he paraphrased more and requested more clarifications). Apparently, communication patterns do indeed change; during the initial consultation the doctor-patient interaction is more equal and reciprocal. To examine the impact of these communication patterns we are now investigating its relation with patients' attitude towards and intention to use insulin as well as on metabolic control by observing 120 consultations with 8 internists.

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INTERACTION STRATEGIES IN DIETARY COUNSELLING OF DIABETIC CHILDREN

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The purpose of this study was to analyse interaction strategies in naturally occurring dietary counselling encounters between a dietician, a diabetic child and his or her parent. The diabetics were either school starters (7 to 9 year-old) or teen-agers (13 to 15 year-old) who had had diabetes for at least half a year, and had had previous dietary counselling encounters. The research data consisted of 57 tape-recorded dietary counselling encounters at pediatric out-patient clinics of five hospitals in different regions of Finland. The recordings were transcribed according to the rules used in conversation analysis. The transcripts were then analysed in detail to trace the stable organized interaction formats in dietary encounters. The following formats were identified: opening sequence; definition of problems; dietary recall; picturing everyday-life of the diabetic and his or her family; information delivery; negotiating dietary change; and closing sequence. In most encounters several problems were raised and discussed, not only in the beginning but also in the course of the encounter. The following aspects were found to be important in order to make a dietary counselling encounter effective and interactive: 1) The dietician was flexible in switching from one format to another and in using formats within other formats according to the turns of speech of the other participants of the encounter. 2) The problems raised by a parent and especially those raised by the diabetic himself or herself were inmediately discussed. 3) The dietary changes were thoroughly negotiated and the opinion of the diabetic himself or herself was taken into account in a new dietary plan.

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DIABETIC FOOT: PATIENTS' PERCEPTIONS OF RISKS, AND BARRIERS TO FOOTCARE MAY BE FINAL DETERMINANTS OF ULCERATION

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Neuropathic foot ulceration remains common despite the use of screening and educational programs. Patients' attitudes and beliefs may be important in determining footcare behaviour. We therefore investigated patients' beliefs about risks (severity and personal vulnerability) of foot complications, and barriers to and benefits of footcare, using Likert response scales, based on the Health Belief Model. 129 patients without evidence of peripheral vascular disease (PVD) were included - 39 diabetic controls (C), 47 patients with diabetic neuropathy (DN), 29 patients with previous neuropathic foot ulceration (DNU) and 14 patients with Charcot neuro-arthropathy (CH). Scores for severity showed foot complications (ulceration and gangrene) rated as highly as cardiac, renal and retinal disease. Vulnerability scores (range 0-5) were also similar for the major complications (cardiac 3.2, blindness 3.0, renal 3.0, foot ulcer 2.6, PVD 3.1), but in the DN group, vulnerability to foot ulceration scored lower than to PVD (2.3 ± 1.5 vs 3.03 ± 1.33, $p<0.05$). Subjects from DN, DNU and CH scored personal vulnerability to foot ulceration no higher than that for the "average diabetic patient" (2.7 ± 1.7 vs 2.8 ± 1.6). Benefits of footcare were rated universally highly in all 4 groups. Scores for barriers (range 0-6) to wearing appropriate shoes were reported as significantly higher than barriers to performing other aspects of footcare (3.6 ± 2.1 vs 2.5 ± 2.2, $p<0.001$). In conclusion, patients recognise the severity of foot complications, but even those at "high risk" do not accept their personal vulnerability. Also, barriers to appropriate footwear may explain the high rate of shoe induced ulcers.

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GLUCAGEN® ADMINISTRATION - UNDEREVALUATED AND UNDERTAUGHT

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Glucagen® treatment requires a manually dextrous 'operator' who is composed, confident and competent in the whole procedure. Anecdotal reports of difficulty from parents in this emergency led us to investigate the 'techniques' of 136 parents (106 parents of teenagers (T) mean age 14.7yrs, duration of DM 4.7yrs, and 30 parents of young children (C) mean age 5.8 yrs, duration of DM 2.4 yrs). A simulated administration by parents using Glucagen® Kits was timed, rated and compared with a group of diabetes health professionals. Parent's 'real life' experiences of education and administration were obtained with a standard questionnaire. Glucagon residues in syringes and vials were assayed in a laboratory.

GLUCAGEN® ADMINISTRATION	% UNUSED
Parents of teenagers (T)	21.3
Parents of children (C) > 25Kg	30.3
Parents of children (C) < 25Kg.	20.7

ADMINISTRATION TIME IN MINUTES -(MEANS)

Duration of Diabetes >2yrs(T)	<2yrs(T)	>2yrs(C) *	<2yrs(C)
Fathers (n=28) 3.32	(n=16) 2.90	(n=4) 1.62	(n=7) 2.22
Mothers (n=41) 3.03	(n=15) 2.68	(n=10) 1.83	(n=9) 2.35
Professionals (n=10) Mean = 1.28 ± 0.20 (sig. faster for all groups except *)			

We found the following problems: no home Glucagen® - 8.7%, handling difficulties - opening pack , sheath removal, mixing, needle bending - 70%, total syringe separation 4.4%, injecting water only - freeze dried powder left in vial- 3.7%.

Conclusion The simulated administration and survey have identified some difficulties which could seriously alter the effectiveness of this treatment. These findings indicate families need hands on demonstration at diagnosis plus regular re-education to minimise the risks of severe hypoglycaemia.

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DRUG MISUSE IN TYPE 1 DIABETES

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Exposure to illicit drugs in the young has increased dramatically over the past five years. Case reports illustrate the risks to patients with IDDM who indulge in drug misuse. We tried to determine the prevalence of drug misuse amongst our population with Type 1 diabetes. A survey was sent to 245 patients with IDDM in our district as identified by computer records with anonymity preserved for reply. A total of 111 (45.3%) replied, age range 16-45, mean 29 years, sex ratio 1.04 male/female. The number of patients with a history of being offered drugs was 49 (44%). The number who have used illegal drugs was 37 (33.3%) with 20 (18%) admitting to current use. The number with experience of cannabis was 36 (32.4%), amphetamine 10 (9%), cocaine 6 (5.4%), LSD 6 (5.4 %) and MDMA (ecstasy) 4 (3.6%). Two admitted to abuse of temazepam with one each for heroin and solvents. In conclusion drug misuse is not uncommon in our group. Given the mean age of reply we strongly suspect under-reporting of misuse in those under 20 years. These drugs may impair control and complicate ketoacidosis, as recently reported with MDMA.We would recommend that all young patients with IDDM should be questioned about drug misuse and given advice accordingly. This is particularly appropriate where illicit drugs are easily available.

OP 7

Apoptosis

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CYTOKINES INDUCE DNA STRAND BREAKS AND APOPTOSIS IN HUMAN PANCREATIC ISLET CELLS C.A. Delaney[1], D. Pavlovic[2], A. Hoorens[2], D.G. Pipeleers[2] and D.L. Eizirik[1,2]. [1]Dept of Medical Cell Biology, Uppsala University, Uppsala, Sweden, [2]Dept of Metabolism and Endocrinology, Vrije Universiteit Brussel, Belgium.

We have previously shown that 6 days exposure of human pancreatic islets to a combination of cytokines (interleukin-1β 50 U/ml + tumour necrosis factor-α 1000 U/ml + interferon-γ 1000 U/ml), severely impairs β-cell function. In the present study we examined whether this condition affects the DNA integrity and viability of human islet cells. Cells were studied after 3, 6 and 9 days of cytokine treatment by both single cell gel electrophoresis (the "comet assay", a sensitive method for detection of DNA strand breaks) and by a cytotoxicity assay using the DNA binding dyes Hoechst 33342 and propidium iodide as indices for counting the number of viable, necrotic or apoptotic cells. Cytokine treatment for 6 and 9 days resulted in significant DNA strand breaks (n = 5; $P<0.05$ vs controls), and in the appearance of apoptotic cells (day 6, 46 ± 6% vs 13 ± 3% in controls, $P<0.001$; day 9, 56 ± 3% versus 20 ± 5% in controls, $P<0.001$). Treatment with cytokines did not increase the percentage of necrotic cells. Inhibitors of nitric oxide formation failed to prevent cytokine-induced DNA strand breaks and apoptosis.The present data suggest that prolonged (6-9 days) exposure of human pancreatic islets to a mixture of cytokines induces DNA strand breaks and cell death by apoptosis. These deleterious effects of cytokines appear to be independent of nitric oxide generation.

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ROLE OF FAS LIGAND IN THE DEVELOPMENT OF INSULITIS IN THE NON-OBESE DIABETIC MICE

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The aim of the present study was to study the role of Fas ligand-induced lymphocyte apoptosis in the development of insulitis in the non-obese diabetic (NOD) mice. Fas Ligand with a M_r of 45 kD appeared in the pancreas of NOD mice during onset of insulitis as demonstrated by western blot analysis. In situ DNA end-labelling (ISEL) of the pancreases of NOD mice demonstrated positive cells in the islets of Langerhans with an age-dependent increase in density and the frequency of animals with islets containing ISEL-positive cells. In the pancreatic islets of BALB/c mice, no ISEL-positive cells were observed in any of the studied age groups. In ultrastructural analysis degenerating cells with condensed or fragmented nuclei and plasma membrane detached from neighbouring cells were observed both in and around the islets. In some cases, these cells were being phagocytosed by the neighbouring islet cells. Degenerating cells with characteristics of lymphocytes were seen in contact with healthy lymphocytes around the islets. Neutralizing Fas-ligand antibodies affected ^{3}H-TdR incorporation of CD3+CD28+ pancreatic lymphocytes from 12-30-week-old NOD mice in one of three cultures. There was no difference in the effect of neutralizing Fas Ligand antibodies between the pancreatic and blood lymphocytes. The present results on the increase in density of apoptotic cells in the pancreatic islets of NOD mice simultaneously with the onset of insulitis and appearance of Fas Ligand suggest that the pancreas infiltrating lymphocytes may be destroyed by Fas-ligand-induced apoptosis.

OP 8

Epidemiology and Prevention of NIDDM

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COLORECTAL CANCER (CRC): ANOTHER COMPLICATION OF DIABETES MELLITUS (DM)?

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Delayed stool transit time and other gastrointestinal abnormalities are commonly observed in DM and are also known to be associated with CRC. Previous studies have generally included too few subjects with both DM and CRC to adequately assess the contribution of DM to CRC incidence after adjustment for important covariates. The 1959-1972 Cancer Prevention Study I, with more than one million respondents, provided a unique opportunity to explore whether the 15,500 eligible persons with DM were more likely to develop CRC during a 13 year follow-up period than were the 850,946 eligible persons without DM (CRC cases detected, DM=158, without DM=7069). Using proportional hazards analysis, we found that the age-adjusted incidence density ratio (IDR) for the association of DM with CRC among men was 1.27 (95% confidence interval [CI]=1.02,1.58) and 1.06 (CI=.84-1.34) for women. After adjustment for CRC risk factors such as race, educational level, body mass index, smoking, alcohol use, dietary intake, aspirin use, physical activity, and family history of CRC, the IDR was 1.30 (CI=1.03-1.65) for men and 1.16 (CI=0.87-1.53) for women. Restricting the analysis to persons aged 60 years and older (to eliminate persons who died early from heart disease, thus allowing a more equal comparison between persons with and without DM) resulted in a multivariable-adjusted IDR stronger for men (1.45,CI=1.07,1.97), but not for women (.99, CI=.66,1.49). In summary, a moderately increased risk for CRC among men with DM was observed. Future studies should clarify the intriguing possibility of a cancer-promoting gastrointestinal mileau, including delayed stool transit and elevated fecal bile acid concentrations, associated with hyperglycemia and neuropathy.

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SERUM ANTIOXIDANTS AND RISK OF NON-INSULIN DEPENDENT DIABETES

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Antioxidants are shown to be protective factors for many chronic diseases but the knowledge of their role in the etiology of non-insulin dependent diabetes mellitus (NIDDM) is very scarce. We studied the association of serum levels of alpha-tocopherol, beta-carotene and retinol with the incidence of NIDDM in a nested case-control study within a prospective population study. Serum levels of the antioxidants were determined in 106 patients with NIDDM and 201 controls matched for sex and age. High levels of serum beta-carotene and alpha-tocopherol were associated with a decreased risk of NIDDM. The relative risk of diabetes between the highest and lowest tertiles of the beta-carotene and alpha-tocopherol distributions were 0.45 (95 % confidence interval 0.22 - 0.92) and 0.61 (95 % confidence interval 0.32 - 1.15), respectively. The trend in the decrease of the risk was statistically significant ($p < 0.05$) with the serum level of alpha-tocopherol. The trend remained significant after adjustment for the strongest risk factors for NIDDM, body mass index and fasting blood glucose at baseline. No association was observed with the serum levels of retinol and the risk of NIDDM. Our results support the hypothesis that high levels of antioxidants may decrease the risk of NIDDM.

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NON INSULIN DEPENDENT DIABETES MELLITUS AND OBESITY IN MEXICAN PIMA INDIANS.

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The development of NIDDM and obesity depends on both genetic and environmental factors. To investigate the impact of the environment we compared the prevalence of NIDDM and the mean body mass index (BMI) in Mexican Pimas living in Maycoba in the Sierra Madre mountains of Mexico to those in the Pima Indians of Arizona. These two groups of Pima Indians are thought to have separated 700-1000 years ago. The Mexican Pimas of Maycoba still live a traditional lifestyle with non-mechanized agriculture and lumber milling activities. We also compared the Mexican Pimas with non-Pimas living in the same environment. The entire population of Maycoba and surrounding community, aged 20 and over, were invited to undergo a 75g oral glucose tolerance test and anthropometric measures (weight, height). NIDDM and IGT were classified using WHO criteria. 141 Pimas (70F/71M) and 194 non-Pima Mexicans (101F/93M) from Maycoba were compared to 1004 Arizona Pima Indians (593F/411M) from the Gila River Indian Community. The prevalence rates are age-standardized to the Mexican Pima population and presented with 95% confidence intervals.

	Mex Pimas	NonPima Mex	AZ Pimas
NIDDM %	6.4 (2.2,10.6)	3.4 (3.3,6.4)	38.2 (33.8,42.6)
IGT %	6.4 (2.2,10.6)	8.2 (3.8,12.5)	10.5 (8.4,12.6)

The mean BMI in the Mexican Pimas was 24.9±3.9 (SD). This was similar to the non-Pima Mexicans (25.7±4.6), and lower than in the Arizona Pimas (34.2±8.2). The prevalence of NIDDM in the Mexican Pimas was only one-sixth of that in the Arizona Pimas, whereas the rate in the Mexican Pimas was higher, but not significantly so, than in non-Pimas. These results indicate the importance of environmental factors in preventing the development of both NIDDM and obesity in populations who presumably carry a genetic susceptibility to these conditions.

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DECREASED TESTOSTERONE IS A PREDICTOR OF INCREASED INTRA-ABDOMINAL FAT IN SECOND-GENERATION JAPANESE-AMERICAN MEN

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Visceral adiposity is a risk factor for development of non-insulin dependent diabetes (NIDD) and coronary heart diseases (CHD). Because sex hormones also have been implicated in NIDD and CHD, we examined the association between baseline serum testosterone (T) levels and changes in visceral adiposity in a cohort of second-generation Japanese American (Nisei) men over 7.5 years (yr) of follow-up. At baseline, these men were assessed for glucose tolerance with a 75 g oral glucose tolerance test (WHO criteria); body mass index (BMI; weight in kg/height in cm^2); visceral adiposity measured as intra-abdominal fat area (IAF) at the level of umbilicus using computed tomography; and serum total T levels. IAF was re-measured at 7.5 yr follow-up. The outcome was defined as Δ IAF (7.5 yr IAF - baseline IAF). The final analysis included a total of 112 Nisei men with the following baseline characteristics (in mean±SD): age, 64±6 yr; BMI, 25.3±2.8; IAF, 120.9±49.2 cm^2; T, 16.8± 4.5 nmol/L. Also at baseline, impaired glucose tolerance (IGT) was diagnosed in 37 (33.0%) men and NIDD, 13 (11.6%). At the 7.5 yr follow-up, overall visceral obesity was increased by a mean ΔIAF of 6.0 (range 163 to -112). ΔIAF was significantly correlated only with: baseline T (r= -0.249, p=.004) and NIDD status (-0.247, .004), but not with age, baseline IAF, or BMI. In a multiple linear regression model with ΔIAF as the dependent variable, adjusting for baseline NIDD status, IAF, BMI, and age, baseline T was significantly related to ΔIAF (coefficient [β] = -8.60; 95% confidence limits [CL] -14.37 to -2.97; p=0.003). Therefore, in this Japanese-American male cohort, lower total testosterone is strongly predictive of an increase in visceral adiposity, independent of age, IAF, BMI, or NIDD status at baseline. This would suggest that visceral adiposity is one of the physiological manifestations through which testosterone could exert its influence on the development of NIDD and CHD, consistent with the role of this sex hormone in modulating body fat distributions.

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FASTING PROINSULIN LEVELS PREDICT THE DEVELOPMENT OF NIDDM IN KOREAN SUBJECTS

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To investigate whether baseline insulin and proinsulin levels can predict the development of NIDDM in Korean people, we conducted a prospective study on the non-diabetic cohort for 2 years in Yonchon County of Korea. Among the initial 1197 non-diabetic subjects, a total of 67 subjects became diabetic in two years. We compared the baseline fasting insulin and proinsulin concentrations of these 67 subjects(New-DM) with those of age, sex, BMI and WHR-matched subjects(Non-DM, n=66) who remain non-diabetic. The baseline insulin concentrations of the New-DM group were not different from those of the Non-DM group(8.0 ± 2.9 vs. 7.3 ± 1.8 uIU/ml, $p>0.05$). However, the baseline fasting proinsulin levels of the New-DM group were significantly higher compared to Non-DM group(15.3 ± 14.5 vs. 8.7 ± 5.7 pmol/L, $p<0.05$). When we observed the natural history of the initial IGT subjects(n=153), 13% became diabetic and 53% remain IGT and 34% reverted to normal glucose tolerance status. The initial fasting proinsulin levels in these 3 groups were 20.6 ± 19.0, 13.0 ± 9.5, and 11.9 ± 7.7 pmol/L respectively ($p<0.05$, ANOVA). These results suggest that beta-cell dysfunction (as reflected by higher proinsulin levels) rather than insulin resistance (reflected by fasting hyperinsulinemia) plays the most important role in the future development of diabetes in Korean population.

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HYPERINSULINAEMIA AND THE RISK OF STROKE: 22-YEAR FOLLOW-UP RESULTS OF THE HELSINKI POLICEMEN STUDY

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Several studies have shown that hyperinsulinaemia is associated with the risk of coronary heart disease, but little information is available on the association of hyperinsulinaemia with the risk of cerebrovascular disease. We have studied this association in a cohort of 1124 Helsinki policemen aged 34-64 years without previous stroke and diabetes at baseline examination in 1971-72 which included an oral glucose tolerance test (OGTT) with blood glucose and plasma insulin measurements at 0, 1 and 2 hours as well as measurements of other cardiovascular risk factors. The area under the plasma insulin response curve (AUCins) during OGTT was used as a composite variable reflecting plasma insulin levels. During 22-year follow-up 90 men had a fatal or non-fatal stroke. Age-adjusted and multiple-adjusted hazard ratios for stroke were calculated using the Cox proportional hazards model for men in the highest AUCins quintile vs those in the combined four lower quintiles. The age-adjusted hazard ratio for stroke risk was 1.68 (1.06-2.65;p=0.026). Adjustment, in addition to age, for the area under the blood glucose curve reduced the hazard ratio to 1.47 (0.90-2.42;NS), for body mass index to 1.23 (0.74-2.03;NS), for subscapular skinfold thickness to 1.27 (0.78-2.05;NS), and for systolic blood pressure to 1.55 (0.98-2.46;p=0.059). Similar adjustment for other factors potentially related to the risk of stroke (plasma cholesterol and triglycerides, smoking, degree of physical activity, and history of previous myocardial infarction) did not substantially reduce the hazard ratio. In a multiple adjustment including all the risk factors mentioned above the hazard ratio was reduced to 1.24 (0.74-2.09;NS). In conclusion, the association of hyperinsulinaemia with the risk of stroke was not independent of other risk factors. In particular, this association was closely linked with indices of obesity.

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METABOLIC CHARACTERISTICS OF INSULIN RESISTANT SUBJECTS WITH S_I=0: THE INSULIN RESISTANCE ATHEROSCLEROSIS STUDY

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We have previously shown in subjects from the Insulin Resistance Atherosclerosis Study (IRAS) that relatively few subjects with non-insulin dependent diabetes mellitus (NIDDM) are insulin sensitive (8% or 37/479) as judged by the median level of insulin sensitivity (S_I>1.67 10^{-4}•min•μU^{-1}•ml^{-1}) in non-diabetic subjects. One potential problem with the use of insulin modified frequently sampled intravenous glucose tolerance test (FSIGT) in diabetic subjects is that a relatively large number of NIDDM subjects have a S_I= 0. Characteristics of subjects with S_I=0 have not well characterized and could reflect either extreme insulin resistance or modeling problems. The distribution of insulin sensitivity in subjects with NIDDM was: insulin sensitive (S_I>1.67) 8%, n=37; insulin resistant (0<S_I≤1.67) 57%, n=270; and insulin resistant (S_I=0) 35%, n=172. Little is known about what characterizes these insulin resistant subjects with S_I>0 (n=270) vs. other insulin resistant subjects with S_I=0 (n=172). Results (by analyses of variance) are shown below:

	BMI	WHR	FI	TG	PAI-1
S_I=0	32.7	0.92	29.7	210	38.5
0<S_I≤1.67	31.0	0.91	21.0	178	29.8
p-value	<0.05	NS	<0.001	<0.001	<0.001

Abbreviations: *BMI=body mass index (kg/m²); WHR=waist/hip ratio; FI=fasting insulin (μU/ml); TG=triglycerides (mg/dl); PAI-1=plasminogen activator inhibitor-1 (ng/ml)*

Therefore, subjects with S_I=0 tended to be more obese, having higher FI and increased cardiovascular risk factors as suggested by high TG and PAI. These results suggest that NIDDM subjects with S_I=0 have metabolic characteristics associated with severe insulin resistance.

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EARLY DIABETES INTERVENTION TRIAL

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The Early Diabetes Intervention Trial (EDIT) is a nine-centre three-year trial which aims to determine whether progression to diabetes can be prevented or delayed in subjects, thought to be at risk of diabetes, with two successive increased fasting plasma glucose (fpg) levels (5.5 - 7.7 mmol l^{-1}). 668 self-referred subjects (50% male) have been randomised, in a factorial design, to double-blind treatment with acarbose or placebo and metformin or placebo. Their mean (SD) age was 52 (10) y, body mass index 28.6 (4.6) kg m^{-2}, fpg 5.9 (0.7) mmol l^{-1} and glycosylated haemoglobin (HbA_{1c}) 5.9 (0.6) % (normal ≤6.2%). WHO categorisation of mean glucose levels from two post-randomisation 75g oral glucose tolerance tests (OGTT), two weeks apart, showed 46% to have normal glucose tolerance (NGT), 37% impaired glucose tolerance (IGT) and 17% diabetes (NIDDM). Mean HbA_{1c} levels increased by OGTT category (NGT 5.7%, IGT 5.9%, NIDDM 6.4%, p=0.0001) with 11%, 19% and 59% respectively having a raised HbA_{1c} (>6.2%). Regression analysis showed a two-hour glucose of 11.1 mmol l^{-1} was equivalent to an fpg of 6.2 mmol l^{-1}. 53% of subjects with an fpg above this value had a raised HbA_{1c}. Conclusion: 54% of the subjects studied, with increased but not diabetic fpg values, have IGT or NIDDM on OGTT. Follow up will determine whether classification of dysglycaemia by fpg, HbA_{1c} or OGTT best predicts those will progress to diabetes and whether early acarbose or metformin therapy can reverse, prevent or delay this progression.

OP 9

Retinopathy and Nephropathy

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RETINAL NEOVASCULARIZATION FROM EXPERIMENTAL ISCHEMIA IS INHIBITED BY A PKCβ INHIBITOR, LY333531.

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We tested the inhibition of preretinal and optic nerve head neovascularization from an ischemic stimulus by PKCβ inhibition to determine the therapeutic potential of this strategy. Orally-administered LY333531 was employed in a porcine model of neovascularization from branch retinal vein occlusion. Branch retinal vein occlusion was produced in 20 eyes of 10 pigs using Rose Bengal photodynamic therapy and argon laser treatment. Five animals with branch retinal vein occlusion were untreated as controls and five were fed LY333531 1 mg/kg twice daily. The eyes were followed clinically for 12 weeks by fundus photography and fluorescein angiography. The neovascularization was graded from fundus photographs and histopathology in a masked manner. Untreated eyes had a mean neovascularization grade of 1.9, and LY333531-treated eyes had a mean grade of 3.1. Treatment resulted in significant inhibition of neovascularization (p=0.04 by t-test). The drug was well tolerated. PKCβ inhibition with LY333531 effectively inhibited preretinal and optic nerve head neovascularization in the pig model of branch retinal vein occlusion, consistent with known signal transduction pathways by angiogenic growth factors, including VEGF. PKCβ inhibition may be effective in the therapy of diabetic neovascular eye disease, which is also ischemia-mediated.

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EVALUATING RISK OF PROGRESSION TO PHOTOCOAGULATION BY RETINAL PHOTOGRAPHY OR DIRECT OPHTHALMOSCOPY.

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The UK Prospective Diabetes Study is a randomised controlled trial of therapies of Non Insulin Dependent Diabetes. Four field 30° stereo photographs of each eye are graded centrally using the modified Wisconsin '191' scheme. This analysis is restricted to 788 subjects who had both eyes photographed and graded at each of the diagnosis, three, and nine year visits and direct ophthalmoscopy at diagnosis.

		Progression to photocoagulation	
Retinopathy level	n	3 years	9 years
No retinopathy	478	0.4%	2.5%
Microaneurysms only	190	1.0%	6.3%
Haemorrhages or hard exudates	58	12.1%	32.8%
Cotton wool spots or IRMA	62	41.9%	71.0%

		Progression to photocoagulation	
Ophthalmoscopy report	n	3 years	9 years
No retinopathy	701	1.0%	5.1%
Microaneurysms	32	15.6%	33.0%
Haemorrhages	10	30.0%	40.0%
Hard exudates	21	42.9%	61.9%
Cotton wool spots or new vessels	24	66.7%	83.0%

The sensitivity of increasing retinopathy levels from retinal photographs for progression to photocoagulation at 9 years is 0.86, 0.72 and 0.51 respectively and for ophthalmoscopy severity 0.59, 0.43, 0.38 and 0.23 respectively. We conclude that: progression to photocoagulation can occur within 3 years in newly diagnosed NIDDM subjects even when there is none at baseline; that baseline retinopathy level is a strong predictor of progression; that graded photography is a more sensitive method of identifying those who will progress than routine ophthalmoscopy.

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EFFECT OF MODIFIED LOW DENSITY LIPOPROTEIN CHOLESTEROL AND α-TOCOPHEROL ON MESANGIAL CELL VIABILITY.

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Background: Low Density Lipoprotein (LDL) cholesterol has been implicated in the development of glomerulosclerosis. LDL modified by glycation and/or oxidation, may contribute to diabetic nephropathy. α-tocopherol may ameliorate vascular damage. Previously we have shown that modified LDL toxicity to cultured retinal microvascular cells is abolished by α-tocopherol enrichment of LDL.

Aims: To determine the effects of modified LDL on mesangial cell viability, and if cell death is observed to determine if it is by apoptosis or necrosis.

Methods: Mesangial cells were cultured from non-diabetic Sprague-Dawley rats. Pooled LDL from non-diabetic humans was enriched *in vitro* with α-tocopherol, and subsequently modified by glycation, and/or minimal oxidation, and/or marked (Cu^{++} catalyzed) oxidation. Non-enriched modified LDL was also studied. Passage 3-6 cells were studied in growth phase and in quiescence, in triplicate. After 3 days exposure to LDL (protein 200 μg/ml) cell counts were determined by hemocytometer. Differences between cell counts (day 3/day 0) were assessed by 1 way ANOVA, with post-hoc analysis by Tukey's test. Video microscopy, flow cytometry and DNA fragmentation were used to evaluate cell death mechanism.

Results: Relative to unmodified LDL, that modified by glycation, minimal oxidation, and by the combination (glycoxidation) did not affect cell viability of quiescent cells (n = 3) or of cells in growth phase (n = 2) $p>0.05$. Relative to these LDL conditions markedly oxidized LDL and oxidized-glycated LDL was highly toxic $p<0.001$. Supplementation with α-tocopherol had no effect. Preliminary results suggest that growing cells die by apoptosis, and quiescent cells by necrosis.

Conclusions: Mesangial cell viability is not reduced by glycated, minimally oxidized and/or glycoxidized LDL. Markedly oxidized and oxidized glycated LDL is highly toxic. α-tocopherol supplementation of LDL has no effect. Death of growth phase cells is by apoptosis, while that of quiescent cells is necrosis.

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THE EFFECT OF BLOOD PRESSURE RHYTHM ON THE PROGRESSION OF DIABETIC NEPHROPATHY.

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The aim of this study was to assess the contribution of an abnormal blood pressure diurnal rhythm on the progression of diabetic nephropathy. We retrospectively studied 26 patients with diabetic nephropathy between 1990 and 1996. Patients underwent ambulatory blood pressure monitoring and were classified as either 'dippers' or 'non-dippers' according to their blood pressure diurnal rhythm. Dippers were those patients whose mean sleeping blood pressure (systolic and diastolic) was 10% less than blood pressure whilst awake. Weight, glycated haemoglobin, serum creatinine,blood pressure and antihypertensive regime was recorded on a three monthly basis, with annual creatinine clearance and 24 hour urine protein excretion. The rate of decline of creatinine clearance was derived from serum creatinine estimation. Creatinine clearance was calculated using the Cockcroft-Gault formula. The two groups were compared using the Mann-Whitney U test,$p<0.05$. There were 13 patients in each group. There was no statistical difference between the the ages in each group (dippers:55, non-dippers:52), mean glycated haemoglobin (dippers: 7.4%, non-dippers: 8.5%), body mass index (dippers: 27kg/m^2, non-dippers: 29kg/m^2), 24 hour protein excretion (dippers: 2.7g/24hrs, non-dippers 3.5g/24hrs), starting serum creatinine (dippers: 105μmol/l, non-dippers: 95μmol/l) and numbers with Type 1 diabetes (Dippers:3, non-dippers:4). Mean clinic blood pressure and mean daytime ambulatory blood pressure were not statistically dirrerent in each group. Night time blood pressure was significantly higher in the non-dipper group as expected. The rate of decline of creatinine clearance (dipper: 2.9ml/min/yr, non-dipper 7.9 ml/min/yr, $p<0.05$) and the serum creatinine at the end of the study were significantly higher in the non-dipper group. In conclusion we found that there was a profound effect of non-dipping upon the rate of decline of creatinine clearance in patients with diabetic nephropathy.

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A NEW HUMAN ATRIAL NATRIURETIC PEPTIDE GENE C/T POLYMORPHISM CORRELATES WITH MICROALBUMINURIA IN IDDM PATIENTS.

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Diabetic nephropathy develops in 30% of type 1 (IDDM) diabetic patients and carries an increased risk of early mortality from renal failure and cardiovascular disease. Microalbuminuria predicts clinical nephropathy and increases the long-term risk of cardiovascular disease. Based on the physiological actions on the kidney and in searching for a candidate gene for susceptibility to diabetic nephropathy, we studied the gene encoding for the human Atrial Natriuretic Peptide (hANP) in IDDM patients and in healthy controls. A fragment of 640 bp (from exon I to exon II) of the hANP gene was amplified by Polymerase Chain Reaction (primers: sense 5'-AGAC-AGAGCAGCAA-GCAGTG-3', antisense 5'-CATTTCCATCCCCAGTTCC-3'). To detect for point mutations, non-isotopic Single-Strand Conformation Polymorphism and direct sequencing have been used. We detected a new point mutation (708 C/T) in the hANP gene, introducing a new restriction site for *Bst*XI. Alleles' and genotypes' frequencies of this polymorphism were studied by RFLP analysis in 442 IDDM unrelated patients (216 M, 226 F) and 58 healthy controls (C) (28 M, 30 F). 313 IDDM subjects were normoalbuminuric (NA, AER <20 μg/min), 79 persistently microalbuminuric (mA, AER 20-200 μg/min), 50 macroalbuminuric (MA, AER >200 μg/min). The four groups were well matched for sex, age and BMI. Diabetes duration (years) was: 16±9, 18±7, 24±6; SBP (mmHg): 120±14, 121±16, 155±15, 120±12; DBP: 77±9, 78±12, 95±9, 75±8; serum creatinine (mg/dl): 0.86±0.2, 0.86±0.1, 1.82±1.5, 0.88±0.1; HbA1c (%): 7.6±1.9, 7.8±2.3, 8.9±1.4, 5.2±0.8, in NA, mA, MA and C, respectively. Alleles' frequencies were: NA: allele wild (W) 95.3%; allele mutant (M) 4.7%, mA: W 89.3%, M 10.7%; MA: W 96%, M 4%; C: W 96.5%, M 3.5%, p=0.001. Genotypes' distribution was: NA: W/W 90.4%, W/M 9.6%, M/M 0; mA: W/W 77.2%, W/M 21.5%, M/M 1.3%; MA: W/W 92%, W/M 8%, M/M 0; C W/W 93.1%, W/M 6.9%, M/M 0; p=0.001. The significant difference of the polymorphism distribution between microalbuminuric subjects and the other groups (NA, mA and C) persisted also when within NA patients were included only patients with a diabetes duration above 20 years (low risk of diabetic nephropathy). No difference was observed in the C/T polymorphism distribution in IDDM with or without hypertension and in IDDM with different degree of diabetic retinopathy. In conclusion, the new polymorphism in the hANP gene is not associated with overt nephropathy, but results associated with microalbuminuria.

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METABOLIC AND GENETIC PREDICTORS OF PROGRESSION OF DIABETIC KIDNEY DISEASE.

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Hyperglycaemia, raised blood pressure and an insertion/deletion (I/D) polymorphism in the ACE gene, have all been implicated as risk factors for the development of diabetic kidney disease(DKD) but their relative role as promoters of progression remains unresolved. **Aim:** To assess in a cohort of IDDM patients with DKD the relative importance of blood glucose, blood pressure (BP) and the ACE genotype on disease progression. **Methods:** All IDDM patients with persistent albuminuria (> 300mg/24 hours) attending the diabetic clinic at Guy's Hospital between 1977 and 1993 were recruited. Measurements of BP, HbA_1 and GFR were performed six monthly until end - stage renal disease or death. The mean follow up period was 8 years (range 1.5 - 15.5 years). 58 patients were followed and ACE genotype was determined by PCR in the 34 surviving subjects still resident in the UK. Baseline and time dependent variables were related to the rate of change of GFR using weighted linear regression and stepwise multiple regression analysis. Analysis of co-variance was used to adjust for the effect of confounders. **Results:** Patients had well controlled BP throughout the observation period (mean arterial pressure 97 ± 8 mmHg) and the mean rate of fall of GFR was 4.32 ± 4.08 mls/min/year. Patients with a higher HbA_1 lost GFR at a faster rate. In stepwise multiple regression analysis both mean HbA_1 and mean diastolic BP were significantly and independently related with a faster rate of progression ($p< 0.0001$ and $p=0.019$ respectively). The I I genotype was independently associated with a slower rate of GFR fall ($p< 0.05$) **Conclusions:** In IDDM subjects who develop DKD, those with the I I genotype have a slower rate of progression, a process modifiable by blood glucose and BP control.

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INCREASED ALBUMIN EXCRETION RATE IN NON-DIABETIC OFFSPRING OF NIDDM PATIENTS WITH DIABETIC NEPHROPATHY.

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Diabetic nephropathy (DNP) clusters in families with both IDDM and NIDDM. Offspring of NIDDM patients have an increased risk of developing diabetes and have also been shown to have an increased albumin excretion rate (AER). It is not known whether this is a general feature of NIDDM or a consequence of increased AER in the diabetic parent. To address this question we studied non-diabetic offspring of 16 (10M/6F) NIDDM patients with (DNP+), and 49 (22/27) NIDDM patients without any sign of diabetic nephropathy (DNP-). Both groups of NIDDM patients had similar age (73±1 vs 70±1 yrs), BMI (28.2±1.1 vs 27.5±0.5 kg/m^2), and diabetes duration (9.1±1.3 vs 10.7±0.6 yrs). Data presented are mean±SEM. Offspring of DNP+ were more obese (p=0.0002) and had increased AER vs offspring of DNP- even after adjustment for BMI (p=0.01).

	OffspringDNP+	OffspringDNP-	p-value (adj.BMI)
n (M/F)	26 (8/18)	90 (30/60)	
Age (yrs)	42.0±1.2	41.4±0.8	
BMI (kg/m^2)	28.4±0.9	24.4±0.3	0.0002
Waist/hip	0.924±0.016	0.882±0.009	0.025 (0.3)
SBP / DBP (mmHg)	124±3 / 78±2	124±1 / 79±1	
FBG (mM)	5.1±0.1	5.0±0.1	
AUCgluc	165±30	144±13	
FSI (pM)	52±7	43±2	
AUCins	29040±2760	28020±1800	
lnAER (µg/min)	1.94±0.19	1.23±0.08	0.002 (0.01)

In conclusion, a genetic predisposition to both DNP and NIDDM, is associated with increased AER and obesity, particularly abdominal obesity, in the offspring.

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PROGRESSION OF DIABETIC NEPHROPATHY DURING LONGSTANDING ANGIOTENSIN CONVERTING ENZYME INHIBITION - THE IMPACT OF POLYMORPHISMS IN THE RENIN ANGIOTENSIN SYSTEM

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We tested the concept, that polymorphisms in the genes coding for angiotensin converting enzyme (ACE) (ACE/ID), angiotensinogen (Ang235 and Ang174) and the angiotensin-II-Type 1 receptor (AGT1R^{1166}) predicts the therapeutic efficacy of ACE inhibition on progression of diabetic nephropathy. Sixty six IDDM patients with diabetic nephropathy were investigated before and during ACE inhibition for a median of 8 (range 3-12) years. No difference in glomerular filtration rate (GFR), albuminuria, blood pressure and haemoglobin A_{1c} was observed between patients with DD-genotype (n=20) and patients with ID or II genotype (n=46) at baseline. In 80% of patients a loop-diuretic was added to captopril (mg/day)(DD: mean 59 (SD 26) and ID +II: 48 (30), NS). Captopril induced nearly the same reduction in mean arterial blood pressure (mm Hg) to mean 102 (SD 8) vs 100 (10) in DD vs ID+II,respectively. Geometric mean albumin excretion (µg/min) remained unchanged in the DD group 567 (antilog SE 1.2), while it decreased from 820 (1.1) before to 419 (1.1) during ACE inhibition in the ID+II group, (p=0.04, comparing changes between groups). The sustained rate of decline in GFR (linear regression of all GFR measurements during ACE inhibition, ml/min/yr) was significantly steeper in DD than in ID+II patients, 4.1 (13.0 to -1.0) vs 2.7 (19.3 to -1.0), respectively (p= 0.03). Patients with the AA genotype of the AGT1R gene polymorphism (n=34) tended to progress at a faster rate than AC+CC patients(n=32), 3.9 (19.3 to -1.0) vs 2.5 (13.0 to -0.7) ml/min/yr, respectively (p= 0.05), while no difference in decline in GFR was observed between the different genotypes of the Ang235 or the Ang174 polymorphisms. The lowest rate of progression was found in patients with ID+II and AC+CC genotype, 2.0 (7.6 to -0.7), (analysis for trend, p= 0.02). Our study suggests that the deletion polymorphism in the ACE gene reduces the longterm beneficial effect of ACE inhibition on albuminuria and the progression of diabetic nephropathy in IDDM patients. The impact on deterioration of kidney function from the Ang235, Ang174 and AGT1R polymorphisms are less important.

OP 10

Coagulation and Lipids

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HYPERGLYCEMIA ACTIVATES BLOOD COAGULATION IN NORMAL SUBJECTS INDEPENDENT OF HYPERINSULINEMIA

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Patients with NIDDM have increased blood coagulability. It was the purpose of this study to investigate in healthy males whether hyperglycemia or hyperinsulinemia may contribute to this defect. Hyperglycemic clamps (glucose ~ 11 mM, insulin ~ 1000 pM) were performed for 48 h in 4 subjects. Hyperinsulinemic-euglycemic clamps (insulin ~ 1000 pM) were performed for 48 h in 5 subjects and served as controls. Plasma factor VIIa (FVIIa, measured with soluble tissue factor), FVIIC and FVIIIC (measured by one stage clotting assays), tissue factor pathway inhibitor antigen (TFPI) and prothrombin fragment F1.2 (reflecting thrombin generation) all rose during hyperglycemic/hyperinsulinemic clamps but remained unchanged during hyperinsulinemic/euglycemic clamps.

	Hyperglycemia		Hyperinsulinemia	
	Baseline	48 hrs	Baseline	48 hrs
FVIIa (mU/ml)	14.3±1.14	80.3±17.7*	33.7±3.21	33.6±3.33
FVIIC(U/ml)	09.0±0.05	1.64±0.16*	0.81±0.08	0.81±0.08
FVIIAg (%)	68.3±2.8	62.1±0.74	72.2±5.02	69.1±3.61
TFPI (ng/ml)	68.0±7.02	105.0±15.95*	74.6±6.85	74.8±4.58
FVIIIC(U/ml	1.11±0.05	1.37±0.02*	1.10±0.07	1.10±0.07
F1.2 (nM)	0.81±0.09	1.25±0.14*	1.22±0.16	1.64±0.46

Comparison of baseline vs 48 hrs: *p<0.05

We conclude that hyperglycemia per se can activate blood coagulation in healthy subjects and may be a factor predisposing chronically hyperglycemic patients for cardiovascular complications.

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SUPPRESSION OF FIBRINOLYSIS AND INCREASED FACTOR VII CONCENTRATIONS WITH DYSGLYCAEMIA.

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Subjects with non-insulin dependent diabetes (NIDDM) or impaired glucose tolerance (IGT) are at increased vascular risk. Tissue plasminogen activator (tPA) Ag, plasminogen activator inhibitor-1 (PAI-1) Ag and factor VII Ag are haemostatic risk factors that cluster with insulin resistance in relation to dysglycaemia. Plasma concentrations were assayed in 248 subjects with increased fasting plasma glucose (fpg) levels (5.5 - 7.7 mmol l^{-1}) recruited into the Early Diabetes Intervention Trial (EDIT). The mean of two oral glucose tolerance tests (OGTT) showed 19% to have NIDDM, 36% IGT and 45% normal glucose tolerance (NGT). tPA Ag levels (mean) correlated with OGTT status (NIDDM 11.2, IGT 10.7, NGT 9.2 ng ml^{-1}, p=0.0014) and were higher than in 164 non-diabetic (fpg <5.5 mmol l^{-1}) controls (7.6 ng ml^{-1}). PAI-1Ag levels were higher than controls (7.7 ng ml^{-1}) and showed a similar OGTT trend (NIDDM 40.1, IGT 38.9, NGT 32.8 ng ml^{-1}, n.s.) as did factor VII Ag (NIDDM 132.8, IGT 126.9, NGT 124.8 ng ml^{-1}, n.s.). Ln PAI-1 Ag, tPA Ag and factor VII Ag were significantly correlated with insulin resistance features including body mass index, triglyceride and Ln fasting RIA insulin. Factor VII Ag levels were higher in females (132.4 *vs* 122.3 ng ml^{-1}, p=0.006) and tPA Ag levels higher in males (10.9 *vs* 9.2 ng ml^{-1}, p=0.0002). PAI-1 Ag levels did not differ with gender. These results indicate that haemostatic risk factors are increased in early dysglycaemic states and may contribute to IGT associated atherothrombotic risk.

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GENETIC AND ENVIRONMENTAL DETERMINANTS OF FACTOR VII ACTIVITY IN HONG KONG CHINESE DIABETIC PATIENTS

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We studied the effects of genetic and environmental influences on factor VII coagulant activity (VIIc) in Chinese diabetic patients (264 NIDDM and 78 IDDM) and 143 normal controls. VIIc was measured by one-stage biological assay. The Arg→Glu mutation at residue 353 of the factor VII gene, which leads to loss of an MspI restriction site (M2 allele) and lower VIIc levels, was detected after MspI digestion of polymerase chain reaction-amplified genomic DNA. In both diabetic and control Chinese subjects the allele frequencies for M1 and M2 were 0.96 and 0.4 respectively, the corresponding reported frequencies in Caucasians being 0.89 and 0.11 respectively. As in Caucasians, VIIc levels were 22% lower in Chinese controls with M1M2 versus M1M1 genotype ($p<0.01$). The corresponding difference was 4% for NIDDM and 24% for IDDM patients respectively ($p=NS$). Despite similar genotypic distributions, NIDDM patients had higher mean VIIc levels than controls and IDDM patients ($p<0.01$); they were also older, and had higher serum creatinine, triglyceride, apo B (all $p<0.01$) and HbA1c ($p<0.01$ and <0.05 respectively), and lower HDL-cholesterol and apo A1 (both $p<0.01$). VIIc levels were similar between IDDM and controls. On multivariate regression analysis, serum triglyceride was the most significant independent determinant of VIIc ($p<0.0001$), contributing to over 25% of the variability in controls and diabetic patients. The contribution of the Arg_{353}→Glu genptype, although significant ($p<0.001$) was only 2.1 % for all subjects and 1% for diabetic patients. Other significant environmental determinants included age, female sex, total cholesterol, serum creatinine, mean albumin excretion rate and HbA1c. VIIc was higher in diabetic patients with macroangiopathy ($p<0.005$) and retinopathy ($p<0.0001$). In conclusion, the M2 allele is apparently less common in Chinese than in Caucasians. Plasma VIIc is determined by both genetic and environmental influences such that in NIDDM patients, the effect of environmental factors, particularly that of hypertriglyceridaemia predominates, almost negating the genetic influence. High VIIc may contribute to the development of macroangiopathic complications and perhaps also retinopathy in patients with NIDDM.

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HYPERTRIGLYCERIDEMIA IS AN INDEPENDENT PREDICTOR OF ELEVATED ENDOTHELIN-1 AND BLOOD PRESSURE LEVELS IN SUBJECTS WITH PLURIMETABOLIC SYNDROME.

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Subjects with plurimetabolic syndrome or syndrome X (sX) show higher risks to develop cardiovascular disease and increased endothelin-1(ET-1) levels than normal subjects. Since sX is a composite of metabolic alterations (hyperinsulinemia, insulin resistance, hypertriglyceridemia, low HDL cholesterol, visceral obesity), it is not completely elucidated the contribution of each component to increase ET-1 levels. To answer this question we studied one hundred forty subjects divided in three groups. Group 1 included patients with impaired glucose tolerance (IGT: n=10) or non-insulin dependent diabetes mellitus (NIDDM: n= 30) with sX and group 2 included patients with IGT (n=8) or NIDDM (n=32) without sX. Groups 1 and 2 were matched for age, sex, fasting and 120 min post OGTT blood glucose (BG) levels, HbA1c, physical activity, duration of diabetes and diabetic complications. Group 3 included 60 normal subjects as control. Triglycerides, insulin levels, insulin resistance index (HOMA), waist/hip ratio and mean blood pressure were significantly higher ($p<0.05$) in group 1 than in groups 2 and 3. In contrast, only BG, HbA1c, insulin levels and HOMA were significantly different between group 2 and 3. ET-1 levels were higher in group 1 than in groups 2 and 3 (9.8±06 vs 8.0±0.5 and 7.0±0.4 pg/ml, $p<0.01$; NS between group 1 and group 2). At single regression analysis, ET-1 levels significantly correlated with triglyceride ($r= 0.35$; $p<0.0001$), insulin levels ($r=0.26$; $p<0.002$), body weight ($r=0.18$; $p<0.003$), HOMA ($r=0.22$; $p<0.009$) and mean blood pressure ($r=0.23$; $p<0.005$). On the contrary, at multiple regression analysis, only triglyceride levels remained independently correlated with ET-1 ($p<0.001$) and mean blood pressure ($p<0.001$). In conclusion, our data seem to suggest a strong role of chronic hypertriglyceridemia as an independent predictor of increased ET-1 levels and hypertension in subjects with syndrome X.

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IMPROVED METABOLIC CONTROL MAKES LDL MORE RESISTANT TO OXIDATION IN SUBJECTS WITH NIDDM.

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Oxidised LDL may play an important role in the development of atherosclerosis because it can induce foam cell formation and exerts cytotoxic and immunogenic effects. Studies on LDL oxidation in NIDDM have yielded controversial results. We studied whether improvement of metabolic control, achieved by insulin therapy, reduced LDL oxidation, assessed by two parameters (Thiobarbituric Acid Reactive Substances (TBARS) and production of Conjugated Dienes after Cu^{2+}-induced oxidation). Twenty subjects with NIDDM and secondary failure to oral antihyperglycaemic agents (age (mean±SD) 58±12 yrs; median time from diagnosis of diabetes 9 (range 1-38) yrs, body mass index 30.2±7.1 kg/m^2; HbA1c 10.5 ±1.0 %) participated. Serum total cholesterol was 6.6±0.9 , HDL 0.9±0.3, LDL 3.8±0.7 and TG (median,range) 3.07 (0.95-7.09) mmol/l. While HbA1c level decreased to 8.0±0.6 % ($p<0.001$) after on average 4 months, lag phase, as a measure of the resistance to oxidation, was prolonged from 60.7±6.2 to 67.5±8.0 min ($p<0.003$). Dienes production rate decreased from 16.9±2.3 to 14.8±1.9 umol/g protein ($p=0.001$). T max (time at which maximum production of dienes is reached) was prolonged from 108±9 to 121±11 min ($p<0.001$). The change in lag phase did not correlate with the decrease in HbA1c, fasting glucose, triglycerides, total cholesterol or LDL cholesterol. Serum TBARS levels did not show any change. We conclude that resistance of LDL to oxidation is improved in subjects with NIDDM when better metabolic control is achieved. This is not reflected by lowering of TBARS levels, which can be explained by the fact that TBARS is an overall index of oxidation and does not measure solely lipid peroxidation.

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LDL SIZE, INSULIN RESISTANCE, AND CORONARY HEART DISEASE IN AMERICAN INDIANS: THE STRONG HEART STUDY

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Small, dense LDL has been associated with the insulin resistance syndrome and coronary heart disease (CHD). The aim of the study was to examine the distribution of LDL size and phenotype within a population-based sample of American Indians to determine relationships with prevalent CHD and to examine associations with hyperinsulinemia and other components of the insulin resistance syndrome. Data are available for 4505 men and women 45-74 years of age who are members of 13 American Indian communities in three geographic areas. Diabetes, CHD, and CHD risk factors were assessed by standardized techniques; LDL size was measured by gradient gel electrophoresis. LDL size was smaller in men compared to women, and in diabetics compared to nondiabetics. In multivariate analysis, LDL size was significantly related to several components of the insulin resistance syndrome, including triglycerides (inversely) and HDL cholesterol (positively). Although univariate relations were positive, LDL size was not significantly related to fasting insulin concentrations or body mass index in the multivariate model. LDL size also showed no relationship to apoE phenotype. When LDL size was compared in those with and without CHD, no significant differences were observed in nondiabetic or diabetic individuals. We conclude that LDL size is most strongly related to lipoprotein components of the insulin resistance syndrome, especially plasma triglycerides. However, in this population with low LDL, it is not related to cardiovascular disease.

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CHOLESTERYL ESTER TRANSFER PROTEIN GENE POLYMORPHISMS IN CHINESE PATIENTS WITH NON-INSULIN-DEPENDENT DIABETES MELLITUS

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We determined the effect of variation at the cholesteryl ester transfer protein (CETP) gene locus on plasma lipid levels in 129 healthy volunteers and 191 Chinese patients with non-insulin-dependent diabetes mellitus (NIDDM). The TaqIA restriction fragment length polymorphism (RFLP) generates two alternative fragments of 7.5kb (A1) and 9.0kb (A2) whereas the TaqIB RFLP produces fragments of 4.4kb (B1) and 5.3kb (B2). There were no significant differences in the allele distribution at these polymorphic loci between the controls (A1=0.86, A2=0.14; B1=0.56, B2=0.44) and the diabetic patients (A1=0.91, A2=0.09; B1=0.65, B2=0.35) and the allele frequencies were similar to those described in Caucasian populations. Diabetic patients with the genotype 1-1 of the TaqIA polymorphism had significantly lower HDL cholesterol (HDL-C) than those with the genotype 1-2 (male: 1.05 ± 0.31 mmol/l vs 1.29 ± 0.45, $p<0.05$; female: 1.14 ± 0.30 vs 1.32 ± 0.42, $p<0.05$). No significant differences were observed in age, body mass index, total cholesterol, triglyceride, LDL cholesterol or HbA1c levels between the two genotypes. A similar association between TaqIA polymorphism and HDL-C was not seen in the controls. Fasting lipid levels between subjects with different genotypes of the TaqIB polymorphism were not significantly different in either the diabetic patients or the controls. In summary our data indicate that there is an association between TaqIA polymorphism of the CETP gene and plasma HDL-C concentration in Chinese patients with NIDDM.

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EFFECTS OF POSTMENOPAUSAL HORMONE REPLACEMENT THERAPY ON SERUM LIPIDS AND LIPOPROTEINS IN NIDDM WOMEN

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Postmenopausal hormone replacement therapy (HRT) is associated with a decrease in coronary disease risk in nondiabetic women. However, the effects of postmenopausal hormone replacement therapy on the common CAD risk factors in women with NIDDM are still unknown. Therefore, the aim of the present study was to evaluate the effects of HRT with transdermal or oral estrogen/progestin on serum lipoproteins in postmenopausal women with NIDDM. Twelve postmenopausal NIDDM women (age 54.4±4.0 years, BMI 31.3±5.3 kg/m^2) receiving oral hypoglycemic medication participated into this cross-over study. All the study subjects received oral HRT (estradiol 2 mg/day plus norethisterone acetate 1 mg/day) for 3 months and transdermal HRT (patches delivering estradiol 50 μg/day) for 3 months. Active treatment phases were separated by a placebo period of 3 months. Blood samples for serum lipoprotein measurements were obtained at the end of each treatment period.

	Baseline	Transdermal HRT	Placebo	Oral HRT
HbA1c,%	7.7±1.4	8.1±1.3	8.2±1.4	7.6±1.0
Cholesterol,mM	6.0±1.0	6.1±1.2	5.8±1.1	5.4±1.0*
Triglycerides,mM	1.8±0.7	1.7±0.7	1.7±0.7	1.8±0.7
HDL-Chol,mM	1.06±0.25	1.06±0.18	1.03±0.21	1.02±0.22
LDL-Chol,mM	4.1±1.0	4.2±1.0	4.0±1.1	3.4±0.8*
ApoB,mg%	110±21	109±16	110±14	109±25
Lp(a), mg%	260±287	203±230	264±269	231±234*

*p<0.05 vs Baseline, Transdermal HRT, and Placebo

We conclude that 1. The route of HRT administration determines the effects on lipoproteins, and 2. postmenopausal HRT with continuous oral administration of estradiol and progestin induces antiatherogenic changes in serum lipoproteins without any deterioration in glycemic control.

OP 11
Clinical Immunology

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THE SARDINIAN SCHOOL CHILDREN - IDDM (SSI) STUDY. GEOGRAPHICAL DISTRIBUTION OF ISLET-RELATED AUTOANTIBODIES IN 7574 HEALTHY SCHOOL CHILDREN.

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In order to identify high and low risk areas of pre-IDDM in Sardinia, 7574 school children (sc) have been recruited in 27 towns (t) of the 4 Sardinian provinces, Cagliari (CA, t=7, sc=2628), Oristano (OR, t=5, sc=1727), Nuoro (NU, t=12, sc=1908) and Sassari (SS, t=3, sc=1311). The sera collected were tested for ICA (7574), GADA (5408) and IA-2icA (5411) respectively. CA showed the highest prevalence of ICA$^+$ for titres $\geq$5 JDFu (5.7%; p<0.01 vs OR=2.8% and NU=3.5%), whereas CA (1.4%) and OR (1.2%) had the highest prevalence of ICA$^+$ for titres >20 JDFu (p<0.05 vs NU=0.5%). OR also had the highest prevalence of GADA$^+$ (2.1%; p<0.05 vs CA=1.1%, NU=0.7% and SS 0.7%) and IA-2icA$^+$ (1.5%; p<0.05 vs SS=0.3%). Combination of autoantibody specificities was always higher in OR than in the 3 other provinces. The prevalence of ICA$^+$ & IA-2icA$^+$ in OR was 0.9% (p<0.01 vs SS=0.1%), GADA+ & IA-2icA$^+$ was 1.0% (p<0.05 vs CA=0.2%, NU=0.0% and SS=0.1%) and ICA$^+$ & GADA$^+$ & IA-2icA$^+$ was 0.7% (p<0.05 vs CA=0.2%, NU=0.0% and SS=0.1%). The identification of OR as an area with the highest prevalence of islet-related autoantibodies is consistent with the EURODIAB data, also showing the highest incidence of IDDM in OR (45 new cases /100,000 inhabitants /0-14 yrs) when compared with CA (38), NU (35) and SS (30). NU and SS, with the lowest incidence of IDDM, had the lowest prevalence of islet-related autoantibodies, either when analysed as individual or combined specificities. Our data showed that, within the island of Sardinia, the geographical pattern of potential pre-IDDM individuals, identified thought islet-related autoantibody determination in a large sc cohort, seems to follow that of the incidence of the disease.

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IA-2 ANTIBODIES IN GENETICALLY HIGH AND LOW RISK SIBLINGS OF CHILDREN WITH IDDM

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To explore the relation between IA-2 antibodies (IA-2A) and genetic risk markers of IDDM, 703 siblings (323 boys) of children with newly diagnosed IDDM were examined. The mean age of the siblings was 9.9 years (range 0.8-19.7 years). IA-2A were tested with a radioligand assay. All siblings with available samples were typed for HLA A, B, C, DR and DQB1 alleles. Data on HLA DQB1 alleles were available for 447 siblings. Thirty-six siblings (5.1%) tested positive for IA-2A in their first sample (cut-off level 1.46 relative units based on the outer fence of 374 healthy control children). 11% of HLA identical siblings had IA-2A compared to 3.1% of haploidentical and 3.5% of non-identical siblings (p=0.0005). 15% of HLA DR3/DR4 heterozygous individuals had IA-2A, compared to 6.6% of DR4/x positive (x=other than DR3), 1.1% of DR3/y positive (y=other than DR4) and 2.3% of siblings negative for DR3 and DR4 (p=0.0002). HLA DR3/DR4 heterozygous siblings had higher IA-2A levels compared to the others (p=0.029). Only one (0.8%) of of those 118 siblings with the protective HLA DR2 allele tested positive for IA-2Acompared to 6% of the other siblings (p=0.021). 21% of siblings with the DQB1*0201/0302 genotype tested positive for IA-2A, compared to 10% of those positive for DQB1*0302/x (x=other than 0201), 4.8% of those positive for DQB1*0201/y (y=other than 0302), and only 1.4% of the remaining siblings (p=0.00002). Siblings heterozygous for DQB1*0201/0302 tended to have higher antibody levels than the other siblings (p=0.069). Two (1.6%) out of 126 siblings positive for the protective DQB1*0602 or 0603 alleles were IA-2A positive compared to 8.1% of the remaining siblings (p=0.011). These results indicate that IA-2A are more frequent in siblings HLA identical with the diabetic proband than in haplo- and non-identical siblings. IA-2A positivity is associated with HLA DR and HLA DQB1 risk alleles, and the frequency of these antibodies increases as a function of enhanced genetic risk for IDDM. Subjects with the strongest genetic risk seem to have higher IA-2A levels than the other siblings. The protective HLA DR and DQB1 alleles are inversely associated with IA-2A, but do not provide a complete protection against these antibodies.

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COMPARISON OF PROTEIN TYROSINE PHOSPHATASES: EXPRESSION, PROCESSING AND ANTIGENIC DETERMINANTS

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To identify protein tyrosine phosphatases (PTPs) present in pancreatic islets, transcripts isolated from αTC-1 (glucagon-secreting) and βTC-1 (insulin-secreting) cell lines were used as templates. A pair of degenerative primers, based on the conserved regions of known PTPs, was used to amplify the transcripts by polymerase chain reaction. A total of 1,620 clones was examined by restriction enzyme analysis and cDNA sequencing. Twenty-one different PTPs were found to be expressed in islet cells: 18 known PTPs and 3 previously unknown PTPs. Some of these PTPs were expressed predominantly in β cells and others in α cells. The degree of relatedness of these PTPs to each other and their differential expression in tissue will be discussed in relationship to their reactivity with autoantibodies in IDDM sera. To analyze in more detail the molecular processing of PTPs, one of them IA-2, was expressed in baculovirus-infected Sf9 insect cells. A 120 kDa IA-2 protein was detected during the early, but not late, phase of the infection. Tunicamycin treatment revealed that the 120 kDa protein was glycosylated, and pulse-chase experiments showed that the it was processed into a 64 kDa doublet and then into several smaller fragments in the 20 to 40 kDa range. Sera from IDDM patients reacted with several of these fragments. The location of the antigenic determinants on these various fragments was analyzed by expressing different portions of IA-2/IA-2β in a reticulocyte transcription/translation system. Immunoprecipitation with IDDM sera revealed that the major antigenic determinants reside in the C-terminus and minor antigenic determinants in the N-terminus of the intracellular domain. It is concluded that many PTPs are expressed in pancreatic islets and that IA-2/IA-2β are the precursors of islet cell antigenic fragments to which autoantibodies in IDDM sera react.

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IDENTIFICATION OF ISLET-RELATED MIMOTOPES BY SCREENING RANDOM PEPTIDE LIBRARIES ON PHAGE WITH TYPE 1 DIABETIC SERA

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Random peptide libraries on phage (RPL) represent a powerful tool for the identification of disease-specific mimotopes reacting with antibodies in human sera. Type 1 diabetes-specific mimotopes could provide a set of novel reagents for diabetes prediction and could also lead to the identification of novel autoantigens or antigens related to possible environmental agents involved in the pathogenesis of the disease. With this aim we screened random nonapeptide libraries of epitopes fused to the major coat protein pVIII of the filamentous bacteriophage F1. Affinity selection with a newly diagnosed Type 1 diabetic serum, immunological screening with 2 other diabetic sera and ELISA re-screening using different human sera (58 newly diagnosed, 23 long-term, 21 polyendocrine, 15 autoimmune organ-specific, 9 autoimmune non-organ specific and 50 normal controls) lead to the selection of 5 phage clones bearing mimotopes (phagotopes) that show a significant difference in the frequency of reactivities between diabetic and normal individuals. Comparing their amino acid sequences with protein bank databases did not show any significant homology with known autoantigens. Among the 5 clones selected, we have characterized phagotope CH1p which we showed to be a mimotope of osteopontin, an islet-related autoantigen expressed by somatostatin cells. In continuing the characterisation of the others, phagotope 195Dyn was represented in 20% of newly diagnosed IDDM sera, but completely negative in normal controls. A rabbit antibody raised against the its encoded synthetic peptide: 1) stained human islets with a classical ICA pattern; and 2) recognized a specific band of approximately 70kD in Western Blot analysis of human pancreatic islet preparations. Absorption studies of ICA positive sera with known IDDM-related autoantigens have indicated that their staining is not completely abolished, thus 195Dyn phagotope may mimic the epitope of a still uncharacterized islet-related autoantigen.

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CHARACTERISTICS OF GAD-REACTIVE T-CELLS IN IDDM AND IN AUTOIMMUNE POLYENDOCRINE DISEASE TYPE 1 (APECED)

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Cellular immunity to GAD is found in IDDM but also in patients with APECED, many of whom do not have IDDM. Characteristics of GAD65-reactive T-cells, such as T-cell proliferation response to GAD65 and IFN-γ secretion of GAD65 stimulated T-cells by EIA were studied. Expression of gut-specific homing receptor α4β7-integrin on GAD65-reactive T-cells was analysed by immunomagnetic depletion of T-cells with high expression of α4β7. T-cell proliferation to GAD (SI>3) was more common in children with newly diagnosed IDDM than in healthy children (15/28 vs 7/27; p=0.05), as well as in patients with APECED than in healthy adults (15/44 vs 3/28; p=0.03). IFN-γ secretion by GAD stimulated T-cells was found in 50% (5/10) of children with IDDM and in 57% (16/28) of patients APECED. T-cell proliferation or IFN-γ response to GAD did not associate with IDDM in APECED (8 of 44 patients had IDDM). Depletion of $\alpha4\beta7^{high}$ T-cells resulted into a significant decrease (mean 70%) in reactivity to GAD in 4 of 7 children with IDDM. In three APECED patients, a decrease of 37% in reactivity to GAD was found only in the patient with IDDM. In contrast, reactivity to tetanus toxoid increased in the majority of the patients studied. Our results indicate that occurrence of GAD-reactive T-cells is a common finding in IDDM but also in patients with APECED without IDDM. Thus, occurrence of IFN-γ secreting GAD-reactive T-cells did not imply clinical IDDM in APECED. Association of α4β7-integrin expression on GAD-reactive T-cells with IDDM suggests that gut-specific homing properties of β-cell reactive T-cells may be important determinant in development of IDDM.

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ALTERED IMMUNE RESPONSE TO INSULIN IN NEWLY DIAGNOSED VERSUS INSULIN TREATED PATIENTS AND CONTROL SUBJECTS

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Insulin dependent diabetes mellitus is the result of a T-cell mediated autoimmune ß-cell destruction,which is accompanied by autoantibodies. We analyzed the cellular and humoral immune response to insulin and insulin peptides in recent onset IDDM patients, IDDM patients treated with insulin, non-diabetic first degree relatives and unrelated control subjects. There were no differences in T-cell reactivity to whole insulin or insulin peptides in general between age matched groups of IDDM patients, relatives or healthy control subjects. In contrast to investigations in NOD mice, no imunodominant or disease specific insulin peptide could be identified. Surprisingly, a positive correlation of T-cell responses to insulin with age was noted ($p<0.005$). This resulted in an inverse relation of IAA and insulin reactive T-cells ($p<0.001$) together with the well described negative correlation of IAA with age. Interestingly, insulin treated patients differed from age matched recent onset IDDM: First, simultaneous immune recognition of insulin with T-cells and IAA was only seen in patients treated for 6 months with insulin, second, insulin treated patients rarely responded to whole insulin; third, they displayed less determinant spreading, and finally, recognition of multiple insulin peptides was not accompanied by crossreactivity to whole insulin. These distinct observations in insulin treated IDDM patients, together with the inverse correlation between humoral and cellular responses to insulin, may result from activation or modulation of different T-cell subsets, and may be of relevance to insulin therapy trials, in which selective activation of non-destructive T-cell subsets may be a key to successful intervention.

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MOLECULAR DISSECTION OF THE INSULIN-LIKE GROWTH FACTOR AXIS IN THE HUMAN THYMUS

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Our previous studies identified immunoreactive (ir) insulin-like growth factor-II (IGF-II) as the dominant polypeptide of the insulin hormone family expressed in the thymic epithelial environment. Preliminary data have revealed a defect of ir IGF-II expression in the thymus of diabetes-prone BB rats. The objective of the present work was to characterize at the molecular level the components of the thymic IGF system, *i.e.* IGF-II gene transcripts, IGF receptors and IGF-binding proteins (IGFBPs). Specific binding of radiolabelled IGF-II was observed on a murine pre-T cell line (RL12-NP), as well as on thymocytes extracted from 4 month-old mice. Only IGF-II, but neither IGF-I nor insulin, could compete with [^{125}I]-IGF-II, with an ED50 around 10 nM. The human Jurkat T-cell line also exhibits a specific binding of IGF-II with an ED50 of 1.5 nM. By affinity cross-linking, the IGF-II receptor protein detected in murine T cells and Jurkat T cells was found to have a molecular weight around 260 KDa. By ribonuclease protection assays, IGF-II gene transcripts were detected in the human thymus, as well as in cultures of human thymic epithelial cells (TEC). Using reverse-transcriptase polymerase chain reaction and specific primers, the promoters of IGF-II expression in the thymus were found to be the same as in other fetal and adult extrahepatic tissues. IGFBP3 to 6 mRNAs were detected by Northern blotting in human TEC with a marked dominance of IGFBP4. With the exception of IGFBP1 and 2, an analogous pattern of expression was observed in the human liver used as a positive control tissue. Interestingly, the human Jurkat T-cell line did not express any IGFBP mRNAs. Altogether, these data confirm that IGF-II is the dominant member of the insulin family synthetized by human TEC, and that there exists a parallel dominance of IGF-II and IGFBP4 in this cell type. Similar investigations are currently performed on the thymus of diabetes-prone and diabetes-resistant BB rats. These observations further support our hypothesis that the thymic IGF axis (in particular IGF-II) is intimately involved in the central T-cell self education to the insulin family.

(Supported by the Juvenile Diabetes Foundation International and the National Fund for Scientific Research of Belgium.)

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DEFECTIVE MONOCYTE-DERIVED DENDRITIC CELLS FROM HIGH-RISK IDDM RELATIVES

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Dendritic cells (DC) play a key role in antigen presentation. Defective DC function in the autologous mixed lymphocyte reaction (MLR) in human insulin-dependent diabetes mellitus (IDDM) has been postulated as a cause of defective regulatory T cell maturation. Therefore, we investigated functional and phenotypic characteristics of monocyte-derived DC generated in the presence of GM-CSF and interleukin-4, from high-risk IDDM relatives and HLA-, sex- and age- matched controls (n=12). Adherent cells were obtained from peripheral blood mononuclear cells (PBMC) by 2-hour adhesion, and cultured for 7 days in medium containing 600 U/ml rhGM-CSF + 400 U/ml rhIL-4. The yield of DC was significantly lower in IDDM relatives ($8.0 \pm 3.3 \times 10^6$ DC from 10^7 PBMC) ($p<0.001$, U test) than in controls ($15.4 \pm 4.8 \times 10^6$ DC from 10^7 PBMC). 90 ± 8.8 % of culture adherent cells from the relatives and 95 ± 3.6 % from controls exhibited the DR+CD14- phenotype. CD1a expression on these cells was significantly lower in IDDM relatives ($72 \pm 20\%$) ($p<0.01$, U test) then in controls ($90 \pm 11\%$). B7-1 and B7-2 expression was also significantly lower in the relatives ($36 \pm 26\%$ and $34 \pm 24\%$) ($p<0.01$, U test) than in the controls ($67 \pm 21\%$ and $62 \pm 22\%$). DC from IDDM relatives displayed significantly lower stimulatory capacity to autologous CD4 cells (27596.2 ± 14691.4 cpm) ($p<0.04$, U test) than DC from controls (42308.6 ± 10893.3 cpm), but not to allogeneic responder cells. In conclusion, DC from high-risk IDDM relatives are generated less efficiently and display defective expression of phenotypic markers and antigen presenting capacity.

OP 12

Endogenous Glucose Production

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BASAL HEPATIC GLUCOSE PRODUCTION IS REGULATED BY THE PORTAL VEIN INSULIN CONCENTRATION

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The ability of portal insulin to regulate hepatic glucose production (HGP) is greatly debated. The aim of our study was to determine if the liver responds to a selective decrease in portal vein insulin. Isotopic (^{3}H-Glucose) and A-V difference methods were used to measure HGP in conscious overnight fasted dogs. A pancreatic clamp (SRIF, basal portal insulin and glucagon) was used to control the endocrine pancreas. A 40 min control period was followed by a 180 min test period. During the latter, portal vein insulin was selectively decreased and while arterial insulin was maintained by turning off the portal insulin infusion and giving insulin peripherally at half the basal portal rate (↓PI, n=5). In ↓PI, a selective decrease of 138 pmol/l in portal vein insulin was achieved while arterial insulin levels did not change (42±7 pmol/l). Portal glucagon remained unchanged (63±8 ng/l) and glucose SA was clamped. In response to the selective decrease in portal vein insulin, net hepatic glucose output (NHGO) increased significantly from 8.1±0.5 (cont) to 30±5.5 by 15 min and eventually reached 14.2±2.3 µmol/kg-min (last 30 min, $p \leq 0.05$). Tracer data confirmed NHGO. Since NHGO increased, arterial plasma glucose increased from 5.9±0.2 to 10.5±0.4 mmol/l. Non-esterified fatty acids (NEFA) dropped from 804±122 (cont) to 420±92 µmol/l (last 30 min, $p \leq 0.05$). Since NEFA levels fell, another group (↓PI+FAT n=5) had intralipid infused during the test period to maintain NEFA levels. In ↓PI+FAT, a selective decrease of 93 pmol/l in portal insulin resulted, while arterial insulin (42±6 pmol/l) and portal glucagon (49±4 ng/l) did not change. NEFA level rose slightly [(470±77 (cont) to 864± 87 µmol/l (last 30 min)]. NHGO again increased quickly from [10.8±2.5 (cont) to 30.2± 6.0 by 15 min and eventually reached 13.4±2.6 µmol/kg-min (last 30 min, $p \leq 0.05$) and arterial plasma glucose increased from 6.0±0.2 to 14.4±0.7 mmol/l. Thus, the liver responds quickly to decreases in portal insulin and NEFA levels play no role in this response. These studies indicate that HGP is acutely regulated by the portal insulin level.

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CAUSAL LINK BETWEEN SUPPRESSION OF LIPOLYSIS VERSUS HEPATIC GLUCOSE OUTPUT SUGGESTS INDIRECT INSULIN ACTION

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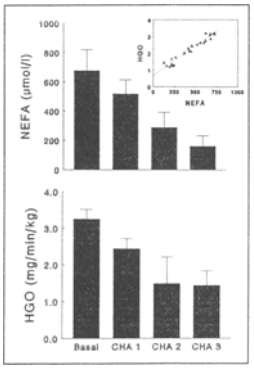
We have previously shown that insulin suppression of hepatic glucose output (HGO) is prevented by the concomitant infusion of Liposyn, supporting the hypothesis that HGO suppression is not a direct effect of insulin on the liver, but rather an indirect effect mediated by suppression of lipolysis in the adipocyte. To examine whether acute suppression of lipolysis independent of insulin increase will suppress HGO, we infused the adenosine analogue N6-cyclohexyladenosine (CHA) into 4 conscious dogs. Following a 2 hour basal period (1.0 mg/min/kg somatostatin, 0.2 mU/min/kg portal insulin, and 1.0 ng/min/kg portal glucagon), CHA was infused in three sequential doses (0.167, 0.500, and 1.667 µg/kg/min) lasting 1 hour each. CHA suppressed NEFA levels in a dose dependent manner (see figure, $p < 05$ ANOVA), causing HGO to fall ($p<.05$ ANOVA) in a nearly identical manner. The correlation between NEFA and HGO was linear with an R value of 0.97



To control for additional effects of CHA, Liposyn (20%, 0.5 ml/min) and heparin (250 U + 25 U/min) were infused with CHA in 2 experiments to maintain NEFA levels above basal. Indeed, Liposyn infusion yielded NEFA levels over basal (891±49 vs. 537±287 µM average of steady-state values vs basal) and prevented the fall in HGO caused by CHA alone (2.2±0.3 basal vs. 1.9±0.1, 2 1±0.1, and 2.1±0.1 at each successive dose, p=n s.). Thus, CHA suppresses lipolysis and HGO similarly, and the suppression of HGO depends on the fall in NEFA levels. However, a decline in glycerol cannot yet be ruled out as contributing to this HGO suppression.

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INSULIN INHIBITS GLUCONEOGENESIS PREDOMINANTLY THROUGH SUPPRESSION OF PHOSPHOENOLPYRUVATE CARBOXYKINASE

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Insulin is known to inhibit endogenous glucose production but the exact mechanism by which it achieves this is not known. It may act at several sites within both the gluconeogenic and glycolytic pathways, or alternatively it has been suggested that the prime mechanism is via the peripheral actions of insulin to inhibit substrate availability. A rat overexpressing an insulin insensitive phosphoenolpyruvate carboxykinase (PEPCK) gene was recently developed (1). This animal model provides a tool for investigating the role of PEPCK in insulin suppression of endogenous glucose production (EGP). After 3 days recovery from surgery, overnight fasted, chronically catheterised, conscious 16 week old rats were studied using the hyperinsulinaemic euglycaemic clamp technique combined with tracer methodology to quantitate glucose turnover. Basal plasma glucose was the same in both groups (5.1±0.2 vs 5.1±0.2 mM in control and transgenic rats respectively), however transgenic rats were hyperinsulinaemic (8.3±0.5 vs 12.6±1.6 μU/ml, $p<0.02$, $n=13$). During the clamp, plasma glucose was not different between the two groups of rats (7.0±0.4 vs 6.1±0.3 mM, p=ns). Insulin levels were significantly increased during insulin infusion but were the same in both groups (30.8±5.7 vs 43.9±6.9 μU/ml, p=ns). As expected, hyperinsulinaemia resulted in significant suppression of EGP by insulin in control rats (44±4 to 24±6 μmol/kg/min, $p<0.05$, $n=10$). However, EGP was not decreased by insulin in transgenic rats (43±5 to 45±6μmol/kg/min). It is concluded that insulin suppresses gluconeogenesis predominantly by inhibition of PEPCK.

1. Rosella G. et al *Molecular Endocrinology* 9: 1396-1404 1995.

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INSULIN REGULATION OF RENAL GLUCOSE METABOLISM IN HUMANS.

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We investigated whether simultaneous renal glucose production (RGP) and utilization (RGU) occur in humans, and whether these processes are responsive to physiologic hyperinsulinemia, using arteriovenous balance measurements combined with tracer technique. After approval by the Institutional Review Board and informed consent, six healthy subjects (3F/3M), mean age 36 yrs, mean BMI 26 kg/m2, were admitted to the Clinical Research Center following an overnight fast for arterialized hand vein and renal vein catheterization under fluoroscopy. Systemic and renal glucose and glycerol kinetics were measured with peripheral infusions of [6-2H2]glucose and [2-13C]glycerol before and after a 2-h period of peripheral insulin (0.125 mU/kg.min) and variable dextrose infusions to maintain arterial glucose concentration and plasma enrichment constant. Renal plasma flow was determined by PAH clearance. Plasma insulin concentration increased from 29±5 to 43±12 pmol/l ($p<0.05$) during the experiment, but there was no change in either arterialized (4.8±0.6 vs 4.7±0.7 mM/l, p=NS) or renal vein (4.7±0.7 vs 4.6±0.7 mM/l, p=NS) plasma glucose concentration. Following insulin infusion endogenous glucose production decreased from 9.9±1.5 to 6.9±1.0 umol/kg.min ($p<0.01$); RGP decreased from 2.4±0.5 to 1.5±0.2 umol/kg.min ($p<0.05$); RGU decreased from 2.6±0.5 to 1.4±0.2 umol/kg.min ($p<0.05$); and renal gluconeogenesis from glycerol decreased from 0.26±0.04 to 0.16±0.06 umol/kg.min ($p<0.05$).

These results indicate that renal glucose production and utilization account for 25% of glucose turnover in postabsorptive humans. Mild physiologic hyperinsulinemia suppresses renal glucose production and utilization by 40%. We conclude that the kidney makes a major contribution to systemic glucose metabolism which is in part regulated by insulin.

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HUMAN KIDNEY SUBSTRATE UTILIZATION AND GLUCONEOGENESIS

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Recent studies indicate that the human kidney may be as important for gluconeogenesis (GN) as the liver. We therefore investigated renal net balance (NB), fractional extraction (Fx), uptake, release and incorporation into glucose of various GN precursors, lactate (N = 17), glutamine (N = 16), glycerol (N = 9) and alanine (N = 9), in relation to their systemic turnover (ST) and systemic GN (SGN) in 43 normal postabsorptive subjects using a combination of isotopic (6-3H glucose, U-14C glutamine, U-14C glycerol, 3-13C lactate and 3-13C alanine) and organ balance (renal vein catheterization) techniques. Our results are shown in Table 1.

Table 1.

	Glucose	Lactate	Glutamine	Glycerol	Alanine
ST (μmol/kg/min)	11.4±0.3	11.8±0.4	5.2±0.3	2.1±0.1	4.6±0.3
SGN (μmol/kg/min)		1.88±0.15	0.46±0.02	0.53±0.09	0.68±0.07
RGN (μmol/kg/min)	2.57±0.19	0.89±0.09	0.33±0.02	0.16±0.02	0.02±0.01
% of SGN		52.9±8.1	72.2±3.1	33.6±4.1	3.3±1.1
% of renal uptake		61.7±6.0	62.6±6	54.6±6.9	10.7±3.5
NB (μmol/min)	-43 ± 8.0	191 ± 23	39±5	37 ± 6	13 ± 10
Fx (%)	2.2 ± 0.2	27.4 ± 1.9	9.7±0.8	53.5 ± 2.7	8.8 ± 1.0
Uptake (μmol/min)	145±11	256 ± 25	55±6	40±6	44±9
% of ST	17.3±1.3	30.4 ± 3.1	14.7±1.1	31.7±4.5	12.7±2.3
Release (μmol/min)	185±12	65±9	16±2	3±1	31±10
% of ST	22.3±1.4	8.1±4.1	4.3±0.6	2.9±1.1	10.2±3.4

Our data indicate that lactate is the major renal GN precursor followed by glutamine and glycerol; renal alanine GN is negligible. The fact that the proportion of lactate, glutamine and glycerol uptake used for GN was comparable (~ 60 %) suggests that substrate availability is rate limiting for renal GN. The kidney uses and produces lactate, glutamine and alanine but only uses glycerol. Since renal uptake accounts for ~ 30 % of systemic lactate and glycerol disposal, the kidney is a major organ for utilization of these substrates.

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CONTROL OF GLUCOSE-6 PHOSPHATASE mRNA AND ACTIVITY IN SMALL INTESTINE OF FASTED AND DIABETIC RATS.

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Glucose-6 phosphatase is the last enzyme of gluconeogenesis and glycogenolysis. In agreement with the concept that only liver and kidney express glucose-6 phosphatase (Glc6Pase), it has been recently reported, using a RT-PCR approach, that Glc6Pase mRNA was not detectable in 9 other tissues. Using the same approach, we demonstrated the presence of Glc6Pase mRNA in duodenum (DUO) and jejunum (JEJ) of normal fed rats, whilst Glc6Pase mRNA was not detected in stomach, ileon, colon, brown adipose tissue, adrenals and bladder. Glc6Pase mRNA and activity were also evidenced in human small intestine. We studied the effect of diabetes and fasting on Glc6Pase mRNA (by Northern blot) and activity (using a highly specific Glc6Pase assay) in small intestine in rats. The Glc6Pase mRNA abundance was increased by 8 times and 6 times ($p<0.01$) in DUO and JEJ in streptozotocin diabetic rats. It was normalized in both tissues upon insulin treatment for 10h. Glc6Pase activity was increased by 300 % in DUO (2.6±0.8 vs 0.83±0.09 U/g wet tissue, mean±S.E.M., n=4, $p<0.05$) and JEJ (1.9±0.3 vs 0.63±0.03 U/g, $p<0.01$) in diabetic rats as compared to normal rats. The Glc6Pase mRNA abundance was increased by 8 and 7 times in DUO and JEJ of 48h-fasted rats. It was normalized in both tissues upon refeeding for 7 hours. Glc6Pase activity was significantly increased in fasted rats (2.5±0.6 and 1.3±0.2 U/g in DUO and JEJ, means±S.E.M., n=5, $p<0.05$ vs normal fed rats). In addition, Glc6Pase mRNA and activity were also expressed in ileon during fasting (1.3±0.4 U/g). These data show that small intestine might possess the capacity to produce endogenous glucose in portal blood. This should have important implications in various metabolic situations.

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ASPECTS OF SYNDROME X DEVELOPING IN RATS WITH GENETICALLY ENGINEERED HEPATIC INSULIN RESISTANCE.

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Central obesity, glucose intolerance, hyperlipidaemia and hypertension form a cluster of disorders (Syndrome X) affecting a large number of individuals. It has been hypothesised that these various abnormalities are all caused by insulin resistance, but definitive proof is lacking. One approach to investigate this hypothesis is to study the development of Syndrome X in animals in which insulin resistance has been produced by genetic engineering. We have made transgenic rats overexpressing an insulin unresponsive PEPCK gene, the rate-limiting enzyme in gluconeogenesis, and shown that they are hyperinsulinaemic (1). The aim of this study is to investigate which aspects of Syndrome X develop in these animals and how a cafeteria (high fat) diet interacts with this genetic defect. Male and female transgenic and control rats aged 26 weeks were studied. Rats were fed either a standard rat chow, or rat chow plus a selection of palatable foods such as potato chips, biscuits and chocolate. Total percent fat measured using DEXA scanning was higher in chow fed (12.0 ± 0.9 vs 17.1 ± 0.9 % $p < 0.05$) and cafeteria fed (34.1 ± 1.4 vs 42.6 ± 1.6% $p < 0.05$) female transgenic rats while in male transgenic animals % body fat was not different in chow fed rats (15.2 ± 0.5 vs 14.4 ± 1.4 % but was higher in cafeteria fed transgenic rats (24.5 ± 1.9 vs 35.3 ± 2.7 % $p < 0.05$). At this age, transgenic rats were not hyperglycaemic but had fasting hyperinsulinaemia (7.6 ± 0.7 vs 11.6 ± 1.4 mU/L $p = 0.017$ $n = 10$). Chow fed transgenic rats had elevated triglyceride (0.6 ± 0.06 vs 1.0 ± 0.1 mmol/l $p = 0.014$) and cholesterol (2.9 ± 0.1 vs 3.7 ± 0.2 $p = 0.006$ $n = 11$) levels. Cafeteria feeding increased insulin and triglycerides but not cholesterol levels in both control and transgenic animals. Blood pressure was not different in chow or cafeteria fed rats. It is concluded that rats with genetically engineered insulin resistance develop several aspects of Syndrome X, strongly supporting the hypothesis that insulin resistance is the initiating defect in this cluster of disorders.

1. Rosella et al *Molecular Endocrinology* 9:1396-1404 1995

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MECHANISM BY WHICH INSULIN INHIBITS NET HEPATIC GLYCOGENOLYSIS IN MAN K. Falk Petersen, D. Laurent, D. L. Rothman, G. W. Cline and G. I. Shulman, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT

^{13}C NMR spectroscopy was used to assess rates of hepatic glycogen synthesis and glycogenolysis under different plasma concentrations of glucose and insulin. Subjects were infused for 6 hrs with insulin (4mU/m^2-min or 40mU/m^2-min), somatostatin (0.1 μg/kg-min) to inhibit endogenous insulin and glucagon release, and 1-^{13}C glucose (20% ^{13}C enriched) to establish one of three conditions: A) Hyperglycemia (10mM)-basal insulin (30pM), n=6; B) Euglycemia (5mM)-hyperinsulinemia (480pM), n=5; C) Hyperglycemia (10mM)-hyperinsulinemia (480pM), n=8. In a fourth group (D) rates of hepatic glycogenolysis and glycogen turnover were monitored during an overnight fast under basal conditions of glucose (5mM) and insulin (30pM), n=5. Hyperglycemia *per se*(A) caused a 92% inhibition of net hepatic glycogenolysis compared to basal conditions (D) (from basal 0.25±0.09 to 0.02±0.03mmol/liter liver-min, $p<0.05$) mainly through inhibition of glycogen phosphorylase flux. In contrast, hyperinsulinemia *per se* (B) caused a 100% inhibition in net hepatic glycogenolysis exclusively through activation of glycogen synthesis (0 to 0.43±0.05mmol/liter liver-min, $p<0.05$). Hyperinsulinemia *per se* had no inhibitory effect on phosphorylase (basal 0.25±0.09 vs. 0.27±0.06mmol/liter liver-min) resulting in extensive glycogen cycling. The glycogenolytic rate (0.27±0.06 mmol/liter liver-min) was ~55% of the glycogen synthetic rate (0.43±0.05mmol/liter liver-min, $p<0.05$) and rates of net hepatic glycogen synthesis were relatively low [0.16±0.01mmol/liter liver-min]. Combined hyperglycemic-hyperinsulinemia (C) resulted in relatively high rates of net hepatic glycogen synthesis (0.36±0.03mmol/liter liver-min) through stimulation of glycogen synthesis (0.40±0.04mmol/liter liver-min) and inhibition of glycogenolysis (0.04±0.02mmol/l liver-min). Conclusions: 1) Insulin and glucose work through different mechanisms to promote hepatic glycogen synthesis, 2) Both substrate and hormonal signals are simultaneously required to cause significant rates of net hepatic glycogen synthesis, 3) Promotion of hepatic glycogen cycling may represent the principal mechanism by which insulin suppresses net hepatic glycogenolysis and glucose production in man.

OP 13

Beta-Cell Development and Function under Normal and Pathological Conditions

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CHARACTERIZATION OF THE BETACELLULIN RECEPTOR IN PANCREATIC AR42J-B20 CELLS

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Betacellulin (BTC) is a member of the EGF family and is shown to exert its mitogenic action via the EGF receptor (Erb-B1). In pancreatic acinar AR42J cells, BTC induces differentiation and converts them into insulin-secreting cells. The effect of BTC is not reproduced by the same concentration of either EGF or TGF-α, suggesting that BTC acts on its own receptor. The present study was conducted to identify the receptor system through which BTC induces differentiation in AR42J-B20 cells. In these cells, two classes of binding sites for ^{125}I-recombinant human BTC with the Kd values of $4.6x10^{-11}$ and $2.9x10^{-10}M$ were observed. When affinity crosslinking was done by using ^{125}I-BTC and disuccinimidyl suberate, followed by immuno-precipitation using antibodies against Erb-B1,2,3, and 4, only Erb-B1 was crosslinked with ^{125}I-BTC. BTC induced tyrosine phosphorylation of the Erb-B1, B2 and B4 whereas EGF induced tyrosine phosphorylation of the Erb-B1 and B2 but not B4. In the presence of activin A, nearly 100 % of AR42J-B20 cells converted to insulin-producing cells by 100 pM BTC. In contrast, only about 20% of the cells became insulin-positive by much higher concentrations of EGF. The results suggest that the differentiation-inducing activity of BTC is exerted via both Erb-B1 and other type of receptor possibly Erb-B4.

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DISTURBED DIFFERENTIATION, MIGRATION AND PROLIFERATION OF PANCREATIC ß- CELLS IN MICE LACKING EGF RECEPTORS

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All pancreatic epithelial cell types, including acini and islets, are believed to differentiate from common ductal precursors. Little is known about the regulators of these processes. Epidermal growth factor receptor (EGF-R) is expressed throughout the developing pancreas and its ligands, particularly transforming growth factor-alpha, are also abundantly present. We have now analyzed the pancreatic phenotype of EGF-R deficient mice (E-), which generally die within the first postnatal week from epithelial immaturity. The knock-out mice were identified from their open-eye phenotype and their normal littermates were used as controls. The E- pancreata were smaller but otherwise appeared normal. Although the DNA content of E- pancreata was 33% lower, the insulin/DNA ratio was 34% higher than in controls. Based on BrdU labelling, proliferation of neonatal ß-cells was only half of that in controls (2.6±0.4 vs. 5.8±0.9 %, $p<0.01$) and the difference persisted even at the age of 7-10 days. A similar proliferation defect was present also in the exocrine pancreas. The most striking feature of the islets in the E- pancreata was that instead of forming circular clusters, the islet cells were mainly located in streak-like structures directly associated with pancreatic ducts (direct duct-association 63±8 % in E- vs. 36±9 % in contr, $p<0.05$). All endocrine islet cells were detected in 6-day cultured explants of E12 embryonal pancreata. However, in E- the most common cell was the glucagon cell (39 % of all endocrine cells) and the proportion of insulin cells was lower than in controls (27±6 vs. 48±8 %, $p=0.05$). PP cells were nearly as abundant as insulin cells in E- (24 % vs. 14 % in controls) and somatostatin cells were equally frequent (8 vs. 10 %). Our findings suggest that the absence of EGF-R mediated signalling leads to a generalized proliferation defect of gastrointestinal epithelia, a moderate delay in ß-cell development, and possibly to disturbed migration of the developing islet cells as they differentiate from their precursors. As a whole the pancreatic islet phenotype is relatively mild, possibly due to an important contribution of signalling through the other three EGF-R (erb-B) tyrosine kinases.

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IMPAIRED B CELL DEVELOPMENT IN THE FETAL GK RAT: IMPACT OF DIABETIC INHERITANCE

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We have previously reported that the lack of insulin secretory response to glucose is a very early defect in the GK rat since it is detectable in vivo as early as fetal age. GK fetuses had an increased plasma glucose concentration, a decreased plasma insulin level and a reduced pancreatic ß-cell mass. The aim of the current study was an attempt to discriminate between gestational effects (such as maternal hyperglycemia) and genetic factors on this defect. For this purpose two crosses were performed: normoglycemic Wistar (W) female with GK male (W/GK) and hyperglycemic GK female with W male (GK/W). Fetuses were named respectively f-W/GK and f-GK/W and they were compared to fetuses issued from W mother and father (f-W/W) and GK mother and father (f-GK/GK). In hybrids f-W/GK plasma glucose and plasma insulin levels were almost normal and their pancreatic ß-cell mass was slighty reduced. Hybrids f-GK/W exhibited higher plasma glucose levels than f-W/W but plasma insulin levels and pancreatic ß-cell mass were normal. In vivo ß-cell reactivity to glucose was studied in the fetuses using an hyperglycemic clamp protocol applied to the mothers during 60min. This techique allowed to obtain a steady-state hyperglycemia in the corresponding fetuses. Under these conditions after 5min hyperglycemia f-W/W increased their plasma insulin level which remained high at 60min compared to basal level before hyperglycemia. By contrast, plasma insulin in the GK fetuses remained at the basal level despite hyperglycemia similar to that in f-W/W. Hybrids f-W/GK and f-GK/W increased their plasma insulin levels in response to hyperglycemia in vivo. The incremental insulin response to glucose over the 60min experiments period were normal (18.4±3.7, 17.2±2.3 and 22.1±3.4ng/ml in f-W/GK, f-GK/W and f-W/W respectively). In summary, our data suggest that hyperglycemia *in utero* does not influence the severity of the decrease of the ß cell mass, neither the lack of the insulin secretory response to glucose. Moreover, they indicate that conjunction of both maternal and paternal genes are necessary in order these defects to appear in the GK model.

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IAPP (AMYLIN) NULL MUTANT MICE; PLASMA LEVELS OF INSULIN AND GLUCOSE, BODY WEIGHT AND PAIN RESPONSES

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Islet amyloid polypeptide (IAPP), also termed amylin, is stored and released together with insulin in response to nutrient stimuli. IAPP-expression is also found in the sensory nervous system. Several biological effects have been reported for IAPP such as inhibititon of insulin release and action, food intake and gastric emptying. To assess the physiological role for IAPP, we have investigated the consequences of a targeted disruption of the IAPP-gene. Until 7 months of age, mice lacking IAPP (*IAPP*-null) had normal basal levels of glucose. However, in IVGTT tests (1g/kg), the glucose elimination rate was found to be increased in *IAPP*-null animals. Thus, at 30 minutes after glucose administration, plasma glucose levels were 10.9±0.4 mmol/l (n=60) in *IAPP*-null animals versus 12.3±0.4 mmol/l (n=65) in controls ($p<0.001$), the difference being more pronounced in males. *IAPP*-null males also presented a simultaneous increase in plasma insulin response to glucose (at 1 minute: 4.2±0.6 nmol/l in *IAPP*-null animals, n=6 versus 1.9±0.3 nmol/l in controls, n=6, $p<0.01$). Moreover, *IAPP*-null males developed a 29% increase in body weight by the age of 18 weeks (44.2±1.7g, n=16 versus 34.2±0.7g in controls, n=25, $p<0.001$). We also report that *IAPP*-null mice (n=12) reacted less to pain stimuli than controls (n=10) showing a 24% reduced licking duration ($p=0.025$) in the late phase of the paw formalin test. The *IAPP*-null phenotype described, relate to two major expression sites for IAPP; the pancreatic β-cells and dorsal root ganglion neurons, and suggests a dual role for IAPP as a regulator of glucose metabolism and insulin release and as a novel sensory neuropeptide.

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REDUCED ISLET AMYLOID POLYPEPTIDE RELEASE IS A CHARACTERISTIC OF IMPAIRED GLUCOSE TOLERANCE AND NIDDM.

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Islet amyloid is a characteristic feature of NIDDM. Its major component is the normal B-cell secretory peptide islet amyloid polypeptide (IAPP) or amylin which is normally coreleased with insulin in a molar ratio of approximately 1:100 or 1%. To determine whether increased release of IAPP may explain the propensity for amyloid deposition in NIDDM, we measured plasma IAPP-like immunoreactivity (IAPP-LI) and immunoreactive insulin (IRI) release in response to an oral glucose load in 78 Japanese-American subjects with normal glucose tolerance (N: n=43), impaired glucose tolerance (I: n=10) and NIDDM (D: n=25) as defined by WHO criteria, who were matched for age (mean±se: 56.0±1.4 y) and body adiposity (BMI: 25.6±0.5 kg/m^2). The incremental increase in IAPP-LI, IRI and glucose (G) at 30 minutes following oral glucose ingestion was used to calculate ΔIAPP-LI/ΔG and ΔIRI/ΔG as measures of B-cell function. As expected, plasma glucose concentrations at both fasting (N: 5.1±0.5, I: 5.5±0.1, D: 6.1±0.3 mM, $p<0.0001$) and 2 hours (N: 6.7±0.1, I: 9.3±0.3, D: 13.2±0.5 mM, $p<0.0001$) were elevated in individuals with IGT and NIDDM. In response to glucose ingestion, plasma IAPP-LI and IRI increased in all subjects but these increments were lower in individuals with reduced glucose tolerance as reflected in the ΔIAPP-LI/ΔG (N: 2.8±0.2, I: 2.0±0.3, D: 1.2±0.2 pM, $p<0.001$) and ΔIRI/ΔG (N: 118±10, I: 90±29, D: 49±6 pM, $p<0.0001$). Moreover, these reductions in the 30 minute incremental IAPP-LI and IRI responses were proportionate such that the molar ratio of IAPP-LI to IRI was not different among the three groups (N: 2.5±0.2, I: 2.6±0.4, D: 2.7±0.4 %, p NS). We conclude that the reduced B-cell function of impaired glucose tolerance and NIDDM includes proportionate reductions in both IRI and IAPP-LI release. Thus, it is unlikely that the development of islet amyloid in NIDDM is the result of increased release of IAPP-LI.

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PROLONGED EXPOSURE OF PANCREATIC B-CELLS TO FREE FATTY ACIDS IN VIVO INCREASES GLUCOSE-INDUCED INSULIN SECRETION AND B-CELL MASS IN RATS

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Free fatty acids (FFA) exert divergent short and long term effects on insulin secretion. To investigate the mechanisms of long term effects of FFA on glucose-induced insulin secretion, intralipid and heparin (hyperlipidemic rats, HL rats) or saline (control rats, C rats) was infused to normal rats for 48h. At the end of infusion, intra gastric glucose tolerance test (500 mg/kg) was performed. Insulin secretion calculated as the insulinogenic index (ΔI/ΔG), was dramatically increased in HL rats compared to C rats (C rats: 2.17±0.4; HL rats: 14±2, $P<0.001$). B-cell responsiveness to glucose was related at least in part to an increase in the B-cell mass which was ~1.5 fold higher in HL rats than in C rats, as measured by an histomorphometric method (C rats: 4.6±0.4, HL rats: 7.1±1.3 mg, $P<0.05$). These data contrast with the rather deleterious effect of FFA on B-cell function in vitro and may therefore reflect the role of extrapancreatic factors, especially nervous factors controlling insulin secretion. To test this hypothesis we recorded parasympathetic and sympathetic firing rate at the end of infusion at the level of the thoracic branch of the vagus nerve and the superior cervical ganglion, respectively. We found a decrease in both parasympathetic (C: 2.20±0.3; HL: 0.60±0.07 spikes/s, $P<0.001$) and sympathetic nervous activity (C: 2.80±0.30; HL: 0.24±0.03 spikes/s, $P<0.001$) in HL compared to control rats. This decrease was more marked in sympathetic nerve. In conclusion, prolonged exposure of pancreatic B-cell to high FFA levels in vivo results in an increase in both B-cell mass and insulin response to glucose, which can be related in part to alterations of autonomic nervous system activity, especially a sharp decrease in the sympathetic one.

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CCAAT/ENHANCER-BINDING PROTEIN BETA (C/EBPβ) IS INDUCED BY HYPERGLYCEMIA AND INHIBITS TRANSCRIPTION OF THE RAT INSULIN I GENE THROUGH INTERACTION WITH E2A TRANSCRIPTION FACTORS

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A reduction of insulin gene transcription by sustained high glucose levels is due in part to a loss of the stimulating transcription factors IDX-1 and RIPE3b1 as has been shown in the β-cell lines HIT-T15 (1), β-TC6, INS-1 and in the rodent diabetes model of partial pancreatectomy (2). We recently reported the inhibitory actions of the stress induced transcription factor C/EBPβ on insulin gene transcription (3). Here we examine the regulation of C/EBPβ in response to sustained high glucose levels in β-cell lines as well as during the time course of development of diabetes in the Zucker diabetic fatty (ZDF) *(fa/fa)* rat. C/EBPβ expression was asessed by Western blotting in HIT-T15 and INS-1 cells , exposed to high glucose levels (11.1, 25mM) for 25 weeks (long term) or 96 hours (short term). Expression in pancreatic tissue sections of ZDF rats *(fa/fa)* at the age of 6 and 12 weeks, Lean Littermates *(fa/+)* (ZLC) or Wistar Rats (WR) were examined by fluorescence immunocytochemistry and image quantitation. Interaction of C/EBPβ with E47 on the rat insulin I promoter was examined by Electrophoretic Mobility Shift, GST-pulldown, and immunoprecipitation assays. In β-cells exposed to high glucose for long term, C/EBPβ expression is irreversibly upregulated whereas a reversible upregulation is observed during short term exposure. A similar upregulation of C/EBPβ concomitant with the loss of insulin expression is seen in ZDF rats during the development of diabetes at 6 and 12 weeks of age, as opposed to ZLC and WR controls. GST-pull down and immunoprecipitation from INS-1 cells with GST-C/EBPβ and antibody to E47 provide evidence for a direct interaction of these two factors. Our *in-vitro* and *in-vivo* findings suggest that C/EBPβ may contribute to the glucotoxic reduction of insulin gene transcription via inhibitory interactions with the E47 transcription factor on the Far- and Nir-elements of the rat insulin I gene promoter. (1) Sharma A, et al. (1995) Mol Endo 9: 1127-1134; (2) Zangen DH, et al. (1996) Diabetes: in press; (3) Lu M, Habener JF (1996) Proc Internat Congress Endocrinol: OR19-6

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THE GLP-1 RECEPTOR ANTAGONIST, GLP-1 (9-36AMIDE), IS A PRIMARY PRODUCT OF THE INTESTINAL L-CELL.

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The initial metabolism of exogenous GLP-1 consists of deletion of its 2 N-terminal amino acid residues catalyzed by the enzyme dipeptidyl peptidase IV (DPP IV). The product GLP-1 (9-36amide) acts as a GLP-1 receptor antagonist with sufficient potency to influence GLP-1 activity and occurs in equal or higher concentrations than GLP-1 in the circulation. We studied the molecular nature of newly released GLP-1 using isolated perfused porcine ileum (n = 7), stimulated by either intraluminal glucose or intraarterial bombesin infusion, both robust stimuli for GLP-1 secretion. The stimuli were repeated in the presence of specific DPP-IV inhibitors (diprotin A or pyrrolidide derivatives) infused a) intravascularly and b) intraluminally. GLP-1 was analysed by differential radioimmunoassay techniques, capable of measuring intact or intact plus degraded GLP-1 and by analytical HPLC. Newly released GLP-1 consisted of 54 ± 10 % intact GLP-1 and 44 ± 9 % GLP-1 9-36 amide (no other metabolites identified). Addition of DPP-IV inhibitors , regardless of route, completly prevented cleavage of GLP-1. All stored GLP-1 (extracted from ileum before and after experiments) was intact. Measurable degradation neither occurred in perfusion medium sampled from the arterial nor the venous line. We conclude that large amounts of GLP-1 9-36 are formed at the stage of L-cell exocytosis. Being a primary secretory product, specific actions of GLP-1 (9-36amide) should now be sought.

OP 14

Hypoglycemia and Protein Metabolism

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EFFECT OF RECENT, ANTECEDENT HYPOGLYCAEMIA AND RESPONSE OF CORTISOL PER SE', ON RESPONSES TO SUBSEQUENT HYPOGLYCAEMIA IN HUMANS.

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Cortisol response to antecedent hypoglycaemia (hypo) mediates some of the suppressive effects of hypo on responses to subsequent hypo, but the relative roles of antecedent response of cortisol "per sè" and hypo are not known. To establish the effect antecedent response of cortisol independent of hypo, 5 normal volunteers were studied on 4 occasions at 1 month intervals (Studies 1-4) with the hyperinsulinaemic-hypo clamp (plasma glucose, PG, decreased stepwise from 5.0 to 2.2 mmol/l). On the day prior to study, either euglycaemia was maintained (S1); or 2 episodes of insulin-induced hypo (PG 2.7 mmol/l, 13.00-15.00 and 21.00-23.00 h) (S2); or S2 + blockade of cortisol response (oral metyrapone, S3), or S3 + cortisol replacement to mimic cortisol response of S2 (S4) were performed. As compared to S2, lack of response of cortisol to antecedent hypo in S3 resulted in greater adrenaline (4.51±0.64 vs 3.1±0.45 nmol/l) and glucagon (150±18 vs 118±15 pg/ml) ($p<0.05$), but not growth hormone and cortisol responses, although all responses remained more suppressed than in S1 ($p<0.05$). In S3, autonomic (but not neuroglycopenic) symptom score was greater than in S2 (10.5±2.5 vs 6.4±1.9, $p<0.05$) and no longer different from S1 (p=NS). However, deterioration of cognitive function (CF) was not affected in S3 vs S2, and was consistently less evident than in S1 ($p<0.05$). In S4 responses were no different from those of S2 (p=NS). Conclusions: responses of cortisol to antecedent hypo largely mediate suppression of adrenaline and glucagon and autonomic symptoms. In contrast, responses of growth hormone and cortisol and neuroglycopenic symptoms, and deterioration of CF are mediated by antecedent hypo "per sè" independent of cortisol responses.

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SYMPTOMS, COUNTER-REGULATORY HORMONE RESPONSES AND COGNITIVE FUNCTION DURING HYPOGLYCAEMIA IN NIDDM

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To determine the effects of glycaemic control on responses to hypoglycaemia in NIDDM, we have measured hormones, symptoms and cognitive function during stepped hypoglycaemia (nadir 2.4 mmol l^{-1}) in 7 poorly controlled patients (mean HbA_1c 11.3 ± 0.5 %) before and after improving control with insulin treatment, and in 7 non-diabetic control subjects. In poorly controlled NIDDM, counter-regulatory hormone responses were brisker than in health (AUC for adrenaline 690 ± 107 vs. 391 ± 57 nmol(180 min)l^{-1}, p= 0.027), and started at higher plasma glucose (PG) levels (adrenaline 4.4 ± 0.2 vs. 3.7 ± 0.2 mmol l^{-1}, p= 0.023; growth hormone 4.2 ± 0.2 vs. 3.4 ± 0.1 mmol l^{-1}, p= 0.018). Following insulin treatment, significantly improved glycaemic control (mean HbA_1c 8.1 ± 0.4 %, p <0.001) was achieved without severe hypoglycaemia. Hormonal responses were significantly reduced (AUC adrenaline 306 ± 93 vs. 690 ± 107 nmol(180 min)l^{-1}, p= 0.036; AUC cortisol 50822 ± 10050 vs. 85211 ± 7646 nmol(180 min)l^{-1}, p= 0.010) and started at much lower PG (adrenaline 3.5 ± 0.3 vs. 4.4 ± 0.2 mmol l^{-1}, p= 0.005; growth hormone 3.2 ± 0.2 vs. 4.2 ± 0.2 mmol l^{-1}, p <0.001; cortisol 3.7 ± 0.2 vs. 2.9 ± 0.1 mmol l^{-1}, p= 0.004). PG at which hypoglycaemic symptoms developed fell from 3.6 ± 0.2 to 3.0 ± 0.2 mmol l^{-1} (p= 0.019). In contrast, PG at which cognitive impairment occurred did not change significantly (3.1 ± 0.1 vs. 2.8 ± 0.1 mmol l^{-1}, p= 0.125). We conclude that:(1) counter-regulatory responses but not cognitive impairment begin at normoglycaemia in poorly controlled NIDDM; (2) glucose thresholds for responses to hypoglycaemia are determined by recent antecedent glycaemic experience; (3) intensified therapy lowers PG threshold for hypoglycaemic symptoms to levels associated with cognitive impairment; (4) severe hypoglycaemia is not an inevitable association with this change but patients are likely to be at increased risk of asymptomatic hypoglycaemia.

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Hyperglucagonemia Accelerates Protein Catabolism in Type 1 Diabetes Mellitus. Charlton MR and Nair KS. Mayo Clinic and Foundation and the University of Vermont. USA

Background: Hyperglucagonemia in the presence of insulin deficiency increases leucine oxidation in nondiabetic subjects. Patients with type I diabetes have both high glucagon levels and increased rates of leucine oxidation. **Hypothesis:** We hypothesized that increased glucagon levels contribute to the increased leucine oxidation seen during insulin deficiency. **Methods:** To test this hypothesis we used a primed continuous infusion of L[1-13C] leucine to measure leucine oxidation in 6 insulin deprived, C-peptide negative type I diabetic patients during a) insulin deprivation and suppressed endogenous glucagon levels (INS DEP + SRIH) and b) insulin deprivation and suppressed endogenous glucagon levels with high level of glucagon replacement (INS DEP + SRIH + GGON). Paired t-tests were used to compare hormone levels and leucine kinetics between protocols. **Results:**

	INS DEP + SRIH		INS DEP + SRIH + GGON	
	baseline	**plateau**	**baseline**	**plateau**
insulin	< 35	< 35	< 35	< 35
glucagon	105 +/- 14	84 +/- 12*	110 +/-11	225 +/- 38†
leu oxidation	40 +/- 2.5	32 +/- 2.7*	36 +/- 1.6	48+/- 3.0 †

all results expressed +/- SEM, *denotes lower ($p<0.02$) vs. baseline, †denotes higher ($p<0.009$) than either baseline or INS DEP + SRIH plateau.

Leucine oxidation was significantly higher during the plateau period of the INS DEP + SRIH + GGON (when glucagon levels were high) studies than either baseline period or the plateau period of the INS DEP + SRIH studies (when glucagon levels were lower). The mean values of other hormones (GH, cortisol, IGF-1, epi- and norepinephrine) did not differ between studies. Leucine oxidation was seen, by multiple regression analysis, to correlate with glucagon levels (R^2=0.85). **Conclusions:** While insulin deficiency increases protein breakdown, hyperglucagonemia is primarily responsible for increased leucine oxidation. The protein catabolic state in insulin-deprived type I diabetic patients is contributed to not only by insulin deficiency but also by elevated glucagon levels.

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EFFECTS OF HYPER- AND HYPOOSMOLALITY ON WHOLE BODY PROTEIN METABOLISM IN MAN

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Diabetic subjects frequently experience changes of extracellular osmolality. Cell volume alterations due to alterations of osmolality have recently been recognized to represent a new principle of metabolic control. To investigate the effects of acute hyper- and hypoosmolality on whole body protein metabolism, leucine kinetics were determined in 10 postabsorptive healthy male subjects 3 times in randomized order: hyperosmolality was induced by fluid restriction and additional NaCl 2-5% iv (w/v); hypoosomolality by i.v. administration of a synthetic analog of vasopressin (Minirin®), liberal water drinking and infusion of NaCl (0.4%); the control study consisted of water ad libitum. Whole body protein kinetics measured using the 1-^{13}C-leucine infusion technique, demonstrated that leucine flux-a parameter of protein breakdown-decreased under hypoosmolal conditions compared to controls (from 1.91±0.05 to 1.78± 0.06 µmol/kg/min, $p<0.02$). Leucine oxidation-a parameter of irreversible catabolism-decreased in the hypoosmolality group (from 0.34±0.01 to 0.27±0.01µmol/kg/min, -19±6% $p<0.005$ vs controls) and remained unchanged in the hyperosmolality group (+4±8%) and in controls (+9±7%). Plasma concentrations of insulin and glucose were lower, and plasma glycerol and ketone bodies higher under hypoosmolal conditions than in controls ($p<0.05$ or less). Indirect calorimetry demonstrated that utilization of fat as percentage of nonprotein energy expenditure was increased (54±9% vs. 34±5%) and that of protein and glucose decreased during hypoosmolality ($p<0.05$ or less vs controls). During hyperinsulinaemic euglycemic clamping glucose metabolic clearance rate was decreased during hypoosmolal conditions (4.39±0.35 vs 3.26±0.17 ml/kg/min $p<0.05$ vs controls). It is concluded that acute hypoosmolality exerts a protein sparing effect with increased utilization of fat and decreased insulin sensitivity of peripheral glucose metabolism; these alterations resemble those observed during prolonged starvation.

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LEPTIN TREATMENT DOES NOT PREVENT THE DEVELOPMENT OF OBESITY IN BROWN FAT DEFICIENT TRANSGENIC MICE

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Transgenic mice (TG) in which the uncoupling protein promoter drives expression of diphtheria toxin A-chain in brown adipose tissue (BAT) have primary BAT deficieny and subsequently develop severe obesity, insulin resistance and late-onset hyperphagia. The aim of the present study was to investigate whether leptin treatment of BAT deficient mice could prevent the development of the obese phenotype. Starting at the age of 6 weeks, when body weight, food intake, plasma leptin, insulin and blood glucose of TG and controls were not significantly different, leptin (5 µg/g) or saline were injected i. p. once daily. During the following 6 weeks, leptin-treated controls lost 0.5 g of weight, while saline-treated controls gained 2.9 g. Saline-treated TG gained 13.5 g and leptin-treated TG gained 13.4 g body weight. Body lipid was 2.36 g compared to 0.65 g in leptin-treated controls ($p<0.01$), and markedly elevated in both TG groups after saline (11.3 g) or leptin treatment (10.7 g). This was accompanied by a 46% decrease in plasma leptin in leptin-treated controls, while both groups of TG revealed a ~9 fold increase in plasma leptin. In leptin-treated controls, total food intake was reduced by 10.6 % ($p<0.05$) while significant hyperphagia was observed in TG both under saline (+27.3%) and leptin treatment (+29.6%). Compared to controls, glucose, insulin, triglycerides and cholesterol levels were significantly elevated both in saline and leptin treated TG, but as for the data mentioned above, none of the measured parameters were significantly different between TG treated with either saline or leptin. In conclusion, chronic leptin administration cannot prevent the development of marked obesity, hyperphagia and abnormal glucose and lipid metabolism in BAT deficient mice. These data suggest that intact BAT function is of critical importance for leptin's role in the regulation of energy homeostasis. (Supported by DFG Ha 2093/2-1).

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ACUTE HYPERGLYCEMIA WITHOUT HYPERINSULINEMIA CAUSES SYMPATHOEXCITATION AND VASODILATION.

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Chronic hyperglycemia plays a role in the development of autonomic neuropathy and macrovascular disease in diabetes. To learn more about the acute effects of hyperglycemia we measured muscle sympathetic nerve activity (MSNA, microneurography), forearm vascular resistance (FVR, air plethysmography) in 7 normal humans (age: 28±1 yrs; mean±se) before and during 60 min hyperglycemic clamp (12.5 ± 0.6 mmol L^{-1}). Octreotide (250 ug/hr), insulin (4 mU m^{-2} min^{-1}), and 20% dextrose infusions to maintain euglycemia (5.4 ± 0.2 mmol L^{-1}) and fasting plasma insulin levels (67±29 pmol L^{-1}) were started at least 90 min before the clamp. Subsequent studies with equal volume infusions of 20% mannitol and 0.2% saline to control for the effects of hyperosmolarity and volume of dextrose infusion were performed in each subject. Mean arterial and central venous pressures did not vary during any of the sessions. Results for MSNA (bursts/min) and FVR (units) are shown in the table below.

	Hyperglycemia		Mannitol		0.2% Saline	
	MSNA	FVR	MSNA	FVR	MSNA	FVR
Baseline	15±2	66±6	19±3	49±10	18±2	42±7
60 min	25±3*	45±7*	22±1	35±8*	22±3	43±8

*$p<0.01$ versus baseline

These results indicate that acute hyperglycemia causes sympathoexcitation and peripheral vasodilation even without changes in plasma insulin concentration. The increase in MSNA appears to be independent of both osmolar and volume effect but the vascular action may be partially mediated by increased osmolarity.

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INSULIN AND GLUCOSAMINE INCREASE O-LINKED GLYCOSYLATION OF SKELETAL MUSCLE PROTEINS IN VIVO

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O-linked N-acetylglucosamine (O-GlcNAc) is an abundant post-translational modification of serine/threonine residues of nuclear and cytoplasmic proteins. We determined whether insulin, or coinfusion of glucosamine (GlcN) with insulin alters O-linked glycosylation. Three groups of conscious fasted rats received 6-hour infusions of either saline (BAS), insulin (18 mU/kg·min) and saline (INS) or insulin and GlcN (30 μmol/kg·min, GLCN) during maintenance of normoglycemia. Insulin increased whole body glucose uptake from 49±5 to 239±8 μmol/kg·min, p<0.001), glycogen in abdominis muscle from 138±11 to 370±26 mmol/kg dry, p<0.001), the amount of cytosolic N- and O-linked glycosylation by 56 % from 362±30 to 564±45 dpm/μg protein·100 min (p<0.02), and O-linked glycosylation from 221±16 to 339±27 dpm/μg·100 min (p<0.02). Glycogen content was positively correlated with the amount of total (r=0.90, p<0.005) and O-linked glycosylation in the insulin infused animals. Coinfusion of GlcN with insulin increased muscle UDP-GlcNAc 3-4-fold (196±43 nmol/g) compared to insulin (56±10, p<0.05) or saline (66±11, p<0.05) infused animals. GlcN also decreased glucose uptake over 6 hours by 30 % to 168±8 μmol/kg·min (p<0.001 for GLCN vs INS), and muscle glycogen to 292±24 mmol/kg dry (p<0.05 for GLCN vs INS). Both total (635±60 dpm/μg·100 min, p<0.002) and O-linked (375±36 dpm/μg·100 min, p<0.002) cytosolic glycosylation were significantly higher in the GLCN (635±60 dpm/μg) than the BAS rats (p<0.002). As in the INS rats, muscle glycogen and O-linked glycosylation were positively correlated in GLCN rats (r=0.54, p<0.05). Variation in total and O-linked glycosylation in the GlcN infused rats was due both to glucosamine (p<0.02) and variation in the glycogen content (p<0.005).

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EFFECT OF LIVER TRANSPLANTATION (LTx) ON INSULIN RESISTANCE IN CIRRHOTIC-DIABETIC PATIENTS.

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Liver cirrhosis is frequently complicated by overt diabetes mellitus and the most important pathogenic mechanism is considered to be insulin resistance. The aim of this study was to assess the effect of successful LTx on glucose homeostasis and on insulin action in 19 cirrhotic-diabetic. 14/19 subjects (74%) withdrew insulin therapy within 10±2 ms and the remaining 5 patients (26%) reduced the insulin regimen by 28% and 60% respectively 12 and 18 ms after Tx. 7 cirrhotic-diabetic (CIR: Child Pugh B, age=53±2 ys, BMI=27±2 kg/m^2, plasma glucose=156±13 mg/dl), 5 transplanted patients still on insulin treatment (DM-LTx: age=54±2 ys, BMI=27±1 kg/m^2, PG=114±9 mg/dl, Insulin Dose=28±6 U/day, Tx age=8±2 ms, prednisone=5±3 mg/day, cyclosporin A=4.4±0.7 mg/[kg·day]) and 4 transplanted patients who discontinued insulin treatment 12±4 ms following the transplant (LTx: age=56±3 ys, BMI=24±2 kg/m^2, PG=100±8 mg/dl, Tx age=33±12 ms, CyA=3.4±0.4 mg/[kg·day] but no prednisone) were studied by means of the euglycemic-hyperinsulinemic (40 mU/[m^2·min]) clamp to measure glucose uptake (GU) in combination with bolus-continuous infusion of [3-^{3}H]glucose to measure endogenous glucose production (EGP). 6 subjects with chronic uveitis on the same immunosuppressive therapy of transplanted patients and 6 normal healthy subjects (NOR) served as controls. CIR showed hyperinsulinemia (29±5 μU/ml; p=0.01), insulin resistance (GU: 2.3±0.2 mg/[kg·min]; p=0.01), increased EGP at both fasting (3.2±0.3; p=0.01) and clamp (1.0±0.2 mg/[kg·min]; p=0.01) conditions, when compared to NOR. DM-LTx normalized EGP (2.1±0.3 mg/[kg·min]; p=0.002 vs CIR and p=0.87 vs NOR), reduced insulin levels (8±1 μU/ml; p=0.01 vs CIR and p=0.91 vs NOR) and improved, but did not normalize insulin resistance (GU: 3.5±0.3 mg/[kg·min]; p=0.03 vs CIR and p=0.04 vs NOR). Finally, LTx normalized insulin levels, EGP and moreover insulin sensitivity when compared to NOR (GU: 6.2±0.4 vs 7.5±0.9 mg/[kg·min]; p=0.41). In summary, liver transplant normalizes 1) insulin levels, 2) endogenous glucose production and 3) peripheral insulin sensitivity. In conclusion liver transplant is capable to cure diabetes mellitus in 74% of cirrhotic-diabetic patients mainly acting via a marked improvement of hepatic and peripheral insulin action.

OP 15

Cardiovascular Risk Factors and Atherosclerosis

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FACTORS ASSOCIATED WITH FIRST CORONARY EVENTS IN PATIENTS WITH NON-INSULIN-TREATED DIABETES IN A COMMUNITY SETTING

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Coronary heart disease is a major source of morbidity and mortality in patients with non-insulin-dependent diabetes mellitus (NIDDM). Identifying factors associated with the occurrence of coronary heart disease can provide directions for preventive strategies. Since most patients with NIDDM are treated in general practice, studies concerning this setting are needed. We have studied 728 patients with NIDDM treated in general practice without previous clinically manifest cardio-vascular disease. Mean age at baseline: 64.3 years (25-93); male: 336; median duration of clinical disease: 1.5 years (0-34). Median follow-up: 24 months (1-79). New coronary events were defined as objectively-proven angina pectoris, myocardial infarction, coronary artery bypass grafting, percutaneous transluminal angioplasty, and sudden unexpected death. Association between events and baseline variables were tested using the log rank test. Continuous variables were dichotomised using median or accepted cut-off points. Variables tested: sex, age ≤65 years, duration of disease >1.5 years, smoking, alcohol use, plasma creatinine >90 μmol/l; glycemic control: HbA1c>7.0%; lipids: total cholesterol >6.5 mmol/l, LDL-cholesterol >4.5 mmol/l, triglycerides >2.0 mmol/l, low HDL; blood pressure: systolic hypertension (>150 mm Hg), diastolic hypertension (>90 mm Hg), or high pulse pressure (PP) (>60 mm Hg); obesity (Body Mass Index >27); high fasting C-peptide (>1.10 nmol/l); use of sulphonurea or biguanides. Significant factors were entered into the Cox model to test for independence. A total of 34 events were observed. Younger age (p=0.01), low HDL (p=0.001), systolic hypertension (p=0.006), and high PP (0.0009) were associated with events, with creatinine>90 (p=0.058) of bordeline significance. Low HDL, high PP, and creatinine>90 were independently associated with events (p=0.001, p=0.004, and p=0.042). Substituting systolic hypertension for high PP yielded similar results. First coronary events in non-hospital-treated patients with NIDDM initially not on insulin are independently associated with low HDL, elevated systolic pressure or pulse pressure, and higher plasma creatinine. Preventive intervention strategies aimed at these factors are warranted to assess their benefit.

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DETERMINANTS OF SEVERITY AND EXTENT OF CORONARY DISEASE IN TYPE 2 DIABETIC AND NONDIABETIC PATIENTS

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To elucidate whether the determinants of the severity and extent of coronary artery disease (CAD) differ in Type 2 diabetic CAD patients (DM) compared with nondiabetic CAD patients (non-DM), we used quantitative coronary angiography (QCA) to grade the severity (tightness of stenoses) and extent (proportion of the coronary tree involved in stenoses) in 55 DM patients (48 men and 7 women) and in 55 non-DM patients matched for sex, age, and body mass index. We also calculated per-patient global atheroma burden indices, which incorporate both severity and extent. We used stepwise multivariate linear regression analysis to select determinants of the angiographic characteristics in each group from a large number of potential risk factors, including demographics, lifestyle variables, glucose and insulin levels, lipoproteins separated by ultracentrifugation, major apolipoproteins, and apoE phenotype. In the DM group, the models selected accounted for 19%, 2%, and 11%, and in the non-DM group, 39%, 32%, and 35% of the variability in severity, extent, and atheroma burden, respectively. For the DM group, severity was associated with duration of diabetes (p=0.073) and IDL-cholesterol (C) (p=0.080); extent had no predictors; and atheroma burden had an inverse relation to HDL-C (p=0.017). The following predictors were found in the non-DM group: for severity, duration of CAD (p<0.001), smoking (p=0.002), and LDL-C (p=0.008); for extent, apoE phenotype (4/3 associated with milder CAD than 3/3, p=0.001) LDL-C (p=0.004), and apoA-II (inverse, p=0.011); and for atheroma burden, respective predictors were apoE phenotype (p=0.003), apoA-II (inverse, p=0.010), LDL-C (p=0.020), and HDL_2 triglycerides (inverse, p=0.040). We conclude that determinants of CAD severity and extent are different in Type 2 diabetes compared with nondiabetics and that conventional risk factors have greater predictive power in nondiabetics than in diabetic patients.

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VISCERAL ADIPOSITY, BLOOD PRESSURE, AND GLUCOSE ARE INDEPENDENT RISK FACTORS FOR CORONARY HEART DISEASE

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The "insulin resistance syndrome" is associated with increased risk for coronary heart disease (CHD) and non-insulin-dependent diabetes mellitus (NIDDM). Baseline risk factors were examined in relationship to the development of CHD (clinical history or ECG consistent with Minnesota Code categories 1.1, 1.2, or 7.1) over 10 yr of followup of 174 Japanese-American men without CHD at baseline, 54 of whom also had NIDDM at baseline (clinical history or WHO criteria for a 75-g oral glucose tolerance test). Fasting plasma was collected at baseline to measure insulin, C-peptide, glucose, cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides (TG). Computed tomographic scans of the thorax, abdomen, and thigh were done to assess body fat distribution. Body mass index (BMI) was calculated from weight (kg)/height^2 (m^2). There were 51 new cases of CHD. NIDDM and hypertension (systolic blood pressure [BP] ≥140 and/or diastolic BP ≥90 mm Hg) at baseline were significantly related to CHD. Univariate logistic regression analysis showed these baseline variables to be associated with CHD, adjusting for NIDDM at baseline (odds ratio for 1 S.D. increase, 95% C.I.): intra-abdominal fat (IAF, 1.52, 1.06-2.16), glucose (1.82, 1.12-2.95), HDL (0.66, 0.45-0.97), TG (1.49, 1.03-2.16), systolic BP (1.72, 1.20-2.47) and diastolic BP (1.97, 1.33-2.91). Notably, insulin, C-peptide, cholesterol, and LDL-cholesterol were not significantly associated with CHD. IAF, BP, and glucose remained independent risk factors in multivariate age- and BMI-adjusted logistic regression analysis of models that included IAF, NIDDM, insulin, C-peptide, glucose, hypertension, BP, HDL, and TG. Thus the association between IAF and CHD is very strong and independent of lipids and hyperinsulinemia. Both BP (hypertension) and glucose (diabetes) were also independently associated with CHD risk. Conclusion: These findings are consistent with the hypothesis that visceral adiposity and not insulin resistance (hyperinsulinemia) is central to the development of the metabolic syndrome associated with CHD.

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PREDICTORS OF CORONARY ARTERY DISEASE (CAD) AND CEREBROVASCULAR DISEASE (CVD) IN NON-INSULIN DEPENDENT DIABETES MELLITUS (NIDDM)

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Classical risk factors do not fully explain the increased risk CAD and CVD in NIDDM. The aim of this study was to assess the risk of future CAD and CVD in relation to classical risk factors and other possible factors such as hyperglycemia and Lipoprotein(a) [Lp(a)]. From 1968-82, glucose, cholesterol (chol), triglycerides (TG) and Lp(a) were obtained in 449 Olmsted County residents with NIDDM and were free of CAD or CVD at baseline. Lp(a) was measured by a semi-quantitative method based on electrophoretic patterns. This cohort was followed for the incidence of CAD and CVD events using medical index (ICD9) codes. The cohort consisted of 47% men , 53% women and 45.7% smokers. The prevalence of hypertension was 37.4%. Mean age was 57 years, mean body mass index (BMI) 29.2, mean chol 6.34 mmol/L, mean TG 2.40 mmol/L and mean glucose 9.2 mmol/L at baseline. Mean duration of diabetes was 4 years. The frequency of distribution of Lp(a) scores 0, 1, 2, 3 (0=absent, 1= mild, 2= moderate, 3=maximally increased) were 55.9%, 26.1%, 16.5% and 1.6% at base line respectively. Mean follow up was 11.6 years. There were 216 CAD events and 115 CVD events. On multivariate analysis significant predictors of CAD were age (p=0.0001, HR 1.04), baseline glucose levels (p=0.0004, HR 1.63), TG (p=0.0038, HR 1.49) and smoking (p=0.0078, HR 1.45). Duration of diabetes, gender, hypertension, BMI and Chol did not predict CAD events. Significant predictors of CVD were age (p=0.0001, HR 1.07), baseline glucose levels (p=0.0264, HR 1.69), hypertension (p=0.0018, HR 1.89) and smoking (p=0.0296, HR 1.57). Duration of diabetes, gender, BMI, Chol and TG did not predict CVD events. Increments in Lp(a) levels were not associated with increased risk for developing either CAD or CVD. Conclusion: In NIDDM, baseline glucose levels, TG levels, smoking status and age were the only significant predictors of developing CAD, while baseline hypertension, glucose levels, smoking status and age were the only significant predictors of developing CVD. We did not find an association between baseline Lp(a) measurements and future risk of developing CAD or CVD.

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INCREASED RISK FOR HEART DISEASE BY HIGH HOMOCYSTEINE LEVEL IS RELATED TO GLUCOSE TOLERANCE: THE HOORN STUDY

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High serum total homocysteine is an important risk factor for cardiovascular disease. To investigate the relationship between high serum total homocysteine and ischaemic heart disease independent of glucose tolerance we studied an age-, sex- and glucose tolerance stratified sample (n=631) of a 50-74 year old general Caucasian population. Glucose tolerance was assessed by means of two oral glucose tolerance tests, except in subjects with non-insulin-dependent diabetes mellitus (NIDDM) who were treated with insulin and/or hypoglycaemic agents. Cardiovascular history and an ECG were obtained from 288 subjects with normal glucose tolerance (NGT), 170 with impaired glucose tolerance (IGT), 173 with NIDDM. Any ischaemic heart disease was defined as a history of angina pectoris, myocardial infarction, coronary artery bypass grafting, (a) Minnesota Code(s) indicative of possible ischaemic heart disease and/or a Cardiac Infarction Injury Score > 20. High serum total homocysteine was defined as > 14 μmol/l. After stratification of the three glucose tolerance categories, we performed a logistic regression analysis. After adjustment for age, sex, hypertension, smoking and serum total cholesterol, the odds ratios (95% CIs) for any ischaemic heart disease for high serum total homocysteine were 0.96 (0.47–1.96) in NGT, 1.49 (0.66–3.35) in IGT and 2.72 (1.18–6.28) in NIDDM. We conclude that a high serum total homocysteine level is a stronger risk factor for ischaemic heart disease in non-insulin-dependent diabetes mellitus independent of classical risk factors, compared with normal and impaired glucose tolerance.

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IMPACT OF RAMIPRIL ON LEFT VENTRICULAR HYPERTROPHY IN NORMOTENSIVE NON-ALBUMINURIC NIDDM PATIENTS.

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NIDDM patients have raised cardiovascular morbidity and mortality even in the absence of albuminuria and hypertension. Left ventricular hypertrophy (LVH) which is an ominous prognostic sign and an independent risk factor for cardiac events is often present in NIDDM patients. Therefore the aim of our six months randomized, double blind, parallel study was to compare the effects of Ramipril (R) 5 mg/day (n = 16, 10 males, 60 (9) years) with placebo (P) (n=15, 8 males, 55 (10) years) on left ventricular mass index (LVMI, echocardiography, Vingmed CFM725) and 24-h ambulatory blood pressure (ABP, A&D TM2420) in normotensive (repeated clinic blood pressure < 140/90 mm Hg), non-albuminuric (albuminuria < 100 mg/24-h) NIDDM patients with LVH (LVMI > 131 g/m^2 in males and > 100 g/m^2 in females). ABP was almost identical at baseline, mean (SE), 132/76 (3/1) vs 133/74 (5/2) mm Hg and remained stable during treatment 134/76 (3/1) vs 136/74 (6/2) mm Hg in the R and P group, respectively. LVMI was comparable at baseline, mean (SE), 137.1 (7.0) vs 129.6 (3.7) g/m^2 in the R and P group, respectively, and decreased significantly more in the R group as compared with the P group, mean (SE) 17.6 (3) vs 5.7 (4.6) g/m^2, respectively, mean difference (95% CI) 11.9 (0.7 to 23.1) g/m^2, (p = 0.027). In conclusion: Ramipril induces regression of LVH in normotensive, non-albuminuric NIDDM patients, independent of systemic blood pressure.

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MICROALBUMINURIA AND CAROTID WALL THICKNESS IN NONDIABETIC AND DIABETIC SUBJECTS: THE IRAS STUDY

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Microalbuminuria (MAB) is associated with cardiovascular mortality and prevalent atherosclerotic vascular disease. Recent studies using ultrasound have shown that carotid atherosclerosis, expressed as an increased intima-media thickness (IMT), correlates with coronary disease. It is not known whether MAB is related to an early stage of atherosclerosis manifested as asymptomatic intimal thickening of arterial wall. Therefore, we investigated the relationship between MAB and IMT of the common carotid artery (CCA) in 991 nondiabetic (nonDM) and 450 NIDDM subjects aged 40-69 years and participating in the Insulin Resistance Atherosclerosis Study (IRAS). Altogether 14% of nonDM and 28% of NIDDM subjects had MAB and 31% of nonDM and 51% of NIDDM subjects had hypertension. Subjects with MAB had greater IMT of the CCA compared to those without MAB (nonDM: 0.84±0.01 vs. 0.79±0.01 mm, p=0.010; NIDDM: 0.89±0.02 vs. 0.86±0.01 mm, p=0.152; combined: 0.86±0.01 vs. 0.82±0.01 mm, p = 0.005). The relationship between MAB and IMT was independent of age, gender, ethnicity, clinic, lipid and lipoprotein levels, and smoking. Further adjustment for systolic blood pressure in the multiple linear regression analysis decreased the difference in IMT of the CCA between subjects with and without MAB to borderline significant level (combined: 0.86±0.01 vs. 0.83±0.01 mm, p=0.056). In conclusion, MAB was associated with increased IMT of the CCA. This relationship was partly mediated by systolic blood pressure. Our results suggest that MAB is related to atherosclerosis already at an early stage of the disease process.

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RISK FACTORS FOR STROKE IN NON-INSULIN DEPENDENT DIABETIC SUBJECTS.

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Newly diagnosed, white Caucasian, subjects with NIDDM, were studied after three months' diet to determine risk factors for subsequent stroke (fatal or non-fatal). Of 3776 subjects, 99 had a stroke within 10 years. Cox proportional hazards models, adjusting for age and gender, assessed the effect of each of body mass index, waist-hip ratio, systolic and diastolic blood pressure (BP), hypertension (systolic BP>160mmHg, diastolic BP>90mmHg, or on antihypertensive therapy), fasting plasma glucose, haemoglobin A_{1c}, total, LDL and HDL cholesterol, triglyceride, insulin, exercise and smoking. Systolic BP (p=0.009) and hypertension (p=0.001) were significantly associated with risk of stroke. In a stepwise procedure only hypertension was included in the model in addition to age and gender. The estimated hazard ratios (95% confidence interval) for subjects aged 50–54, 55–59 or ≥60 relative to <50 years were 1.37 (0.62, 3.00), 3.22(1.68, 6.19) and 4.78(2.56, 8.92) respectively. The estimated hazard ratio for men relative to women was 1.63 (1.08, 2.47) and for subjects with hypertension relative to those without was 2.47 (1.64, 3.74). When systolic BP was included instead of hypertension, the hazard ratios for the middle and upper tertiles, relative to the lower tertile were 1.96(1.01, 3.81) and 2.99(1.57, 5.69) respectively. In summary, in non-insulin dependent diabetic subjects, hypertension was the major modifiable risk factor for stroke.

OP 16

Recent Progress in Diabetic Neuropathy

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INHIBITION OF PROTEIN KINASE C CORRECTS NERVE CONDUCTION AND BLOOD FLOW DEFICITS IN DIABETIC RATS.

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Increased activation of the protein kinase C (PKC) / diacylglycerol (DAG) system has been implicated in the aetiology of vascular complications in experimental diabetes. The aim was to test whether this mechanism was important for peripheral nerve function in streptozotocin-diabetic rats by examining the effects of a specific PKC inhibitor, WAY151003 (2,6-Diamino-*N*-([1-(1-oxotridecyl)-2-piperidinyl]methyl) hexanamide), which is active at the regulatory domain, and the effects of cremophor EL, a lipid formulation that complexes DAG to prevent PKC activation. Treatment was for 2 wk following 6 wk of untreated diabetes. An approximately 20% deficit ($p < 0.001$) in sciatic motor nerve conduction velocity (NCV) in diabetic rats was corrected by 92.4 ± 5.0% ($p < 0.001$) by WAY151003 (3 mg/kg) and 81.3 ± 6.3% ($p < 0.001$) by cremophor EL (100 mg/kg) treatment. Similarly, a 19.2 ± 2.4% deficit in sensory saphenous NCV was 72.3 ± 7.6% ($p < 0.001$) and 66.1 ± 9.3% ($p < 0.001$) corrected by WAY151003 and cremophor EL respectively. Sciatic nutritive endoneurial blood flow was 50.4 ± 3.5% ($p < 0.001$) reduced by untreated diabetes. This was completely ($p < 0.001$) corrected by WAY151003 treatment and 69.4 ± 7.2% ($p < 0.001$) corrected by cremophor EL. PKC inhibitor effects on motor NCV and blood flow were completely abolished ($p < 0.001$) by co-treatment with the nitric oxide synthase inhibitor N^G-nitro-L-arginine (10 mg/kg). PKC inhibition did not alter motor or sensory NCV and blood flow in non-diabetic rats. Thus, the data implicate the PKC-DAG mechanism in the aetiology of impaired neurovascular function in diabetic rats, perhaps by causing a deleterious effect in the nitric oxide system of vasa nervorum.

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A DOUBLE BLIND PLACEBO TRIAL OF THE EFFECT OF AN ACE INHIBITOR, TRANDALOPRIL ON DIABETIC POLYNEUROPATHY.

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There is accumulating evidence that vascular factors are important in the pathogenesis of diabetic neuropathy. One animal study and two open label studies have shown benefit with Angiotensin converting enzyme (ACE) inhibitor. A double blind placebo controlled trial was undertaken in 41 diabetic patients who received either Trandalopril (T) 2mg od (n=21) or Placebo (P) (n=20) over 12 months. All patients were well matched for age and degree of glycaemic control (HbA1c (%), T(10.1± 0.43) and P(10.7 ± 0.29)). Baseline Peroneal motor nerve conduction velocity (PMNCV-m/s)(T(38.5 ± 0.98), P(37.42±1.1)), Sural nerve amplitude (SNAP-μv)(T (8.2 ± 1.40), P(6.1 ± 1.4)), Neuropathy symptom score (NSS) (T(3.6 ± 0.5), P(3.3 ± 0.5)), Neuropathy deficit score (NDS) (T(3.1 ± 0.28), P(3.8 ± 0.2)), Vibration perception threshold (VPT-volts)(T(20.9 ± 2.5), P(22.8 ± 2.5)), 30:15 ratio (T(1.08 ± 0.02), P(1.07 ± 0.01)) and Heart rate variation during deep breathing (HRV)(T(11.33 ± 1.3), P(9.2 ± 1.1)) did not differ significantly between the two groups. After 12 months of treatment a trend for improvement was observed. PMNCV improved by +0.91 ± 0.52 in T and decreased by -0.85 ± 0.62 in P (P<0.04). SNAP also showed a trend for improvement in T (+1.21 ± 0.78) compared to P (-0.48 ± 0.73) (p= 0.12). Clinical evaluation of the NSS showed no significant difference between T (-0.24 ± 0.64) and P (-1.2 ± 0.66) (P= 0.3). The NDS also did not change significantly between T (+0.7 ± 0.42) and P (+0.7 ± 0.33) (P= 1.0). VPT decreased in T (-1.26 ± 1.29) and increased in P (+0.32 ± 1.2) (P= 0.66) favouring active treatment. Autonomic function evaluated by the 30:15 ratio decreased by the same amount in T (-0.04 ± 0.02) and P (-0.04 ± 0.02 (p= 0.92) and the HRV increased more in T (+1.43 ± 1.13) than P (+0.7 ± 0.92) (p= 0.62). This short term study has shown a beneficial effect of ACE inhibition in diabetic patients with mild to moderate neuropathy with a positive trend for improvement in parameters assessing peripheral and autonomic neuropathy but not symptoms or signs. The results support the potential role of ACE inhibition in the treatment of diabetic polyneuropathy.

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A MULTICENTRE DOUBLE-BLIND TRIAL OF GAMOLENIC ACID IN DIABETIC PERIPHERAL SENSORIMOTOR NEUROPATHY

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Two trials have previously reported improved electrophysiological function in patients with diabetic neuropathy after treatment with gamolenic acid (GLA). This study aimed to compare the effect of gamolenic acid and placebo in neuropathic patients. 291 patients (7 centres) were randomised double blind to GLA (n=146) or placebo (n=145) for twelve months, subsequently all patients received GLA single blind for a further twelve months. At entry all subjects had clinical signs of peripheral neuropathy with either abnormal electrophysiological (peroneal nerve mcv $< 42 ms^{-1}$) function and/or thermal perception. Twenty eight variables were assessed: 4 thermal threshold, 12 electrophysiological and 12 clinical assessments. After 12 months 23 out of 28 assessments had improved on GLA (10 at $2p < 0.05$). All 28 variables on placebo had worsened (19 at $2p < 0.05$). Differences between GLA and placebo were in favour of GLA ($2p < 0.05$) in 21 of the 28 assessments. During the second year, those previously on GLA significantly further improved in 8 variables ($2p < 0.05$), those previously on placebo showed a trend to improvement in 18 assessments. GLA did not affect glycemic control and side effects (mainly GI) were minor. GLA appears to improve nerve function in established diabetic neuropathy and is well tolerated.

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BENEFICIAL EFFECT OF DIETARY OMEGA 3 FATTY ACIDS ON Na,K-ATPase AND NERVE FUNCTION IN DIABETIC NEUROPATHY.

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Omega 3 fatty acids from fish oil may help in the amelioration of cardiovascular disease. However, there are very few studies about their preventive effects during diabetes-induced neuropathy. The objective of this study was to assess the preventive ability of a fish oil rich diet (rich in omega 3 long chain fatty acid) on diabetes induced abnormalities in Na,K-ATPase activity, isoenzyme expression, fatty acid content in sciatic nerve membranes, nerve conduction velocity (NCV) and morphological lesions. Three groups of 15 rats were studied. In two groups diabetes was induced by streptozotocin (STZ). Diabetic animals were fed standard rat chow supplemented with either fish oil (MaxEPA) (DM) or olive oil (DO) at a daily dose of 0.5g/kg by gavage during 8 weeks. Non-diabetic control animal were fed standard rat chow supplemented with olive oil (CO). The results show that in olive oil treated diabetic rats compared to controls, membrane phospholipids fatty acid composition was altered, Na,K-ATPase $\alpha 1$ and $\alpha 3$ isoforms activity ($\alpha 1$: CO ; 2,41±0.23 µmol Pi/h/mg Prot vs DO ; 1,09±0,07 $p<0,05$/ $\alpha 3$: CO ; 1,18±0,19 vs DO ; 0,11±0,03 $p<0,05$), expression and conduction velocity of sciatic nerve were decreased (CO ; 48,33±1,17 m/s vs DO ; 34,35±0,55 m/s, $p<0,05$). Morphological abnormalities in sciatic nerves were also observed. In diabetic rats fish oil restored Na,K-ATPase activity (CO ; 4,8±0,7 vs DO ; 2,18±0,1 $p<0,05$ and DO vs DM ; 3,56±0,16 $p<0,05$), $\alpha 1$ and $\alpha 3$ isoforms expression and activity ($\alpha 1$: DM ; 1,72±0,18 vs DO $p<0,05$/ $\alpha 3$: 0,97±0,1 vs DO $p<0,05$), partially conduction velocity (DM ; 39,1±0,96 m/s vs DO, $p<0,05$) and some morphological lesions. These results suggest that fish oil treatment could normalize fatty acid composition in membrane phospholipids, and thus has beneficial effects on diabetes-induced alterations in sciatic nerve.

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LONG-TERM EFFECTS OF ACETYL-L-CARNITINE ON MYOCARDIAL SYMPATHETIC INNERVATION IN DIABETIC PATIENTS

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L-carnitine and its derivative, acetyl-L-carnitine (ALC) may have beneficial effects on cardiac performance and nerve function in diabetes mellitus. To investigate the effects of ALC on myocardial sympathetic innervation and autonomic nervous function (ANF), we studied 19 patients (placebo group: n = 6, age 56 ± 5 years, HbA_{1c} 9.0 ± 0.5 %; ALC group: n = 13, age 57 ± 2 years, HbA_{1c} 9.4 ± 0.6 %, mean ± SEM) in a randomized placebo-controlled double-blind one-year trial. The dose of ALC was 1500-3000 mg/day. A dual-tracer isotope single-photon emission tomography (SPET) was performed before and after the one-year treatment to assess myocardial sympathetic innervation (^{123}I-metaiodobenzylguanidine, MIBG) in relation to perfusion (^{99m}Tc-methoxyisobutylisonitrile) at rest. Autonomic nervous function tests and power spectral analysis of heart rate variability were also performed. The two groups did not differ with respect to their ANF results or plasma catecholamine levels. In patients with placebo, global myocardial MIBG uptake (100 ± 16 % vs. 77 ± 6 %, p = 0.03 before vs. after treatment) deteriorated during the one-year trial, whereas in ALC-treated patients MIBG uptake (100 ± 10 % vs. 102 ± 6 %, p = NS) remained constant. Similarly, resting myocardial perfusion was lower at the one-year examination in the placebo group, but remained unchanged in the the ALC group. No changes were observed in myocardial MIBG/perfusion ratio or ANF results in either group. We conclude that long-term treatment with ALC might prevent the progressive loss of myocardial sympathetic innervation in diabetic patients. MIBG-SPET is a sensitive and reproducible method in assessing the development of myocardial sympathetic denervation.

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Insulin affects Cardiac Sympatho -Vagal Balance: a clamp study.

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The effects of insulin on cardiac neural regulation were studied in 15 subjects aged 30(5.1) (M(SD)), 8 normals aged 31.5(5.7) and 7 obese aged 29(3.9) who underwent a euglycaemic clamp with a three step insulin infusion-rate: a) 20 mU/min/m2, b) 40 mU/min/m2, c) 80 mU/min/m2 . Power Spectral Analysis (PSA) of Heart Rate (HR) quantified cardiac Sympatho-Vagal balance throughout the study. While there was no significant variation (before and after insulin infusion) in HR and HR Variance, a linear correlation was found between progressive insulin infusion rate and the percentage increase of Low (LF) - High (HF) Frequency ratios of HR spectral components ($b = 30.4$, $r = 0.30$, $p < 0.02$). This correlation was significantly higher in the obese group ($b = 52.13$, $r = 0.45$, $p < 0.001$) but it was not statistically significant in the normal group ($b = 21.09$, $r = 0.21$, p = n.s.). Specular correlation was found between HF values and steps of insulin infusion rate in all subjects ($b = -8.64$, $r = -0.26$, $p < 0.04$) and to a greater extent in the obese group ($b = -13.6$, $r = -0.37$, $p < 0.007$) while statistical significance was not reached in the normal group ($b = -4.8$, $r = -0.14$, p = n.s.). However no significant increases in plasma Noradrenalin and Adrenalin concentrations were found in all subjects nor in both groups before and after any step of insulin infusion rate. As the LF and HF components of PSA are commonly thought to be related, mainly to the sympathetic and entirely to the vagal influences respectively on the cardiac autonomic drive, we found evidence that insulin per se affects the Sympatho-Vagal cardiac balance leading to a vagal withdrawal as well as to a relative sympathetic predominance related to the insulin infusion rate . These effects have been shown to predominate in obese rather than in normal subjects . Specular data on hypoglicaemic clamp in the same subjects are available but have not been discussed here.

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INCREASED AUTONOMIC DYSFUNCTION IN IDDM PATIENTS AT HIGH RISK OF CARDIOVASCULAR MORTALITY: A POPULATION STUDY.

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BACKGROUND: IDDM patients with macroalbuminuria have a high cardiovascular mortality and microalbuminuria is a strong predictor for the development of macroalbuminuria. In IDDM patients, impaired autonomic function is related to sudden cardiac death. Thus, the aim of the present study was to investigate if patients with IDDM and raised urinary albumin excretion rate (AER) had autonomic dysfunction more frequently compared to controls. *STUDY POPULATION AND METHODS:* In an epidemiological study of IDDM patients we identified all with raised AER and divided the material into three groups: 1. *Microalbuminuria*: AER=20-200 μg/min (n=74),2. *Macroalbuminuria*: AER>200 μg/min (n=53), and 3. *Raised AER*: >20 μg/min (n=127). From the same material three control groups with normal AER, matched to the above groups according to sex, age, duration of diabetes and HbA1c (n=148, 106 and 254, respectively) were identified. AER was based upon three overnight urine collections and autonomic function was determined by beat-to-beat variation during deep breathing, where ≤ 10 beats/min was defined as definite abnormal (Ewings criteria). *RESULTS:* In the microalbuminuria group vs. control group we found 35(47%) vs. 59(40%) with autonomic dysfunction (Kruskal-Wallis chi-square=163.56, p<0.0001), in the macroalbuminuria group vs. control group 39(74%) vs. 54(51%) (Kruskal-Wallis chi-square=119.37, p<0.0001) and in the group with raised AER vs. control group 74(58%) vs. 108(43%) (Kruskal-Wallis chi-square=290.02, p<0.0001). *CONCLUSION:* We conclude, that IDDM patients at high risk of cardiovascular mortality have increased autonomic dysfunction, which may contribute to cardiovascular death.

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DECREASED MOTOR NERVE CONDUCTION VELOCITY (MNCV) IN DIABETIC RATS IS LINKED TO CYTOSOLIC REDUCTIVE STRESS

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Aim. To assess the role of sorbitol pathway-induced reductive stress (a decrease in cytosolic free NAD/NADH) in mediating impaired MNCV in diabetic rats. The effects of sorbinil (50 mg/kg bwt day in the chow), an aldose reductase inhibitor (ARI), and CP-166,572 (200 mg/kg bwt/day in the drinking water), an inhibitor of sorbitol dehydrogenase (SDI), were assessed on MNCV in sciatic peroneal (SP) and distal tibial nerves (DT) 4 months after induction of diabetes (D) by iv injection of 50 mg/kg bwt of streptozotocin in male Sprague-Dawley rats (bwt ~300 g). On the day of sacrifice rats were anesthetized with thiopental and sciatic nerves were quickly sampled for measurement of lactate, pyruvate, malate, acetoacetate, and β-hydroxybutyrate for estimating cytosolic and mitochondrial free NAD/NADH and cytosolic NADP/NADPH. D rats gained less weight and had plasma glucose levels of 33-40 mM vs ~7 mM for controls (C). MNCV in D was ~11 % slower (p<0.005) than in C in both SP (65.3±4.2 m/sec in C, n=12, vs 59.0±5.0 in D, n=8) and DT (59.2±5.2 in C vs 52.3±4.1 in D). SDI and ARI prevented the decreased MNCV in both SP (68.4±7.1 for SDI, n=11, and 65.1±4.8 for ARI, n=10) and DT (58.2±5.2 for SDI and 57.1±6.6 for ARI). Cytosolic free NAD/NADH was decreased 46 % (p<0.001) in D (272±95) vs C (508±132). SDI and ARI prevented this decrease (478±195 for SDI and 438±142 for ARI). Cytosolic free NADP/NADPH was decreased 44 % (p<0.005) in D (0.0090±0.0032) vs C (0.0161±0.0048); this decrease was prevented by SDI and ARI. Mitochondrial free NAD/NADH was decreased 37% (p<0.02) in D (16.4±4.6) vs C (26.1±7.9); it was not normalized by SDI (16.2±3.5) or by ARI (17.1±5.0). In conclusion, in rats with diabetes of 4 months duration, decreases in MNCV and in cytosolic NAD/NADH and NADP/NADPH (but not the decrease in mitochondrial NAD/NADH) were prevented by ARI and by SDI. These observations suggest a potentially important role for sorbitol pathway-induced reductive stress in mediating decreased MNCV in diabetic rats.

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Inflammatory Mediators and Beta-Cell Destruction

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ROLE OF TGFβ SECRETED FROM A CD4+ SUPPRESSOR T CELL CLONE IN THE PREVENTION OF IDDM.

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We have previously shown that a CD4+ suppressor T cell clone (NY4.2) isolated from islet- infiltrating lymphocytes of NOD mice prevented the development of autoimmune IDDM. This investigation was initiated to determine the mechanisms involved in the prevention of IDDM by the CD4+ suppressor T cell clone NY4.2. First, we examined whether the suppressive activity of NY4.2 T cells results from the cytokines secreted from the cells. Measurement of several cytokines from the supernatant of NY4.2 T cell culture using ELISA revealed that NY4.2 T cells secrete substantial amounts of TGFβ, IL-10, and IFN-γ , but not IL-2 or IL-4. Next, we examined which of the cytokines, TGFβ, IL-10 or IFN-γ are involved in the suppression of immune responses. We treated the supernatant from the culture of NY4.2 cells with antibody against TGFβ, IL-10 and IFN-γ and the immunosuppressive activity of the supernatant was measured. Most of the immunosuppressive activity of the supernatant was abrogated after treatment with anti-TGFβ antibody, but not by treatment with anti-IL-10 or anti-IFN-γ antibodies. Finally we examined whether TGFβ suppression resulted in a loss of suppressive activity by transducing TGFβ -expressing NY4.2 cells into TGFβ-suppressed NY4.2 cells using a retroviral vector which expresses antisense TGFβ genes. We found that these transduced NY4.2 cells no longer suppress cell-mediated autoimmune IDDM in NOD mice. On the basis of these observations, we conclude that TGFβ secreted from CD4+ NY4.2 T cells plays a major role in the prevention of IDDM in NOD mice.

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MODULATION OF CYTOKINE-INDUCED APOPTOSIS IN NEONATAL RAT ISLETS. N.John J.Mabley,S. Thomas, A-M Dunger * D. Schröder *S. Schmidt, * M. Di Matteo, and I.Green, Univ. of Sussex, Brighton UK and *Univ. of Greifswald, Germany.

We have previously shown that treatment of rat islets with the cytokine combination IL-1ß, TNF and IFN inhibited ß cell function and decreased cell number. The aim of this study was to determine if cytokines caused programmed cell death by apoptosis in islet beta cells and whether pretreatment with growth factors could prevent cytokine-induced chromatin condensation. Islets from rats aged 8-16 days were cultured for at least 2 days prior to growth factor pretreatment (TGFß (3.3 x 10^{-11}M), IGF-1 (10^{-8}M) or insulin (4nM) (24h) followed by cytokine treatment (IL-1ß, TNF (both 100pM) and IFN 2U/ml) for 24-48h. Dissociated single cells were harvested and analysed; intact islets were dispersed into single cells. Cell suspensions were mixed with acridine orange (10μg/ml) and apoptotic nuclei were counted by fluorescence microscopy. This methodology was validated using additional features of electron microscopy and DNA laddering in both cytokine and free radical treated cells. An increased number of apoptotic nuclei 11.6% was observed following 24-48h combined cytokine treatment versus 2.7% in control cells, [nuclei viewed 855 and 703 respectively; 7 experiments P<0.001]. This increase was partially blocked by 24h pretreatment with growth factors (apoptotic cells cytokine treated 15.5%; TGF+ cyt 7%; IGF-1+ cyt 6.9%; INS +cyt 5.3%. Confirmation that beta cell nuclei were showing chromatin condensation was made by use of a beta-cell specific antibody in conjunction with acridine cytochemical analysis. We conclude that cytokines accelerate programmed cell death in beta cells and that growth factors maintain cell viability.

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DESTRUCTION OF BETA CELLS IS ASSOCIATED WITH APOPTOSIS IN NON-OBESE DIABETIC (NOD) MICE

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To clarify how beta cell is injured in autoimmune diabetes, we studied apoptosis of beta cells in cryostat sections of pancreas from non-obese diabetic (NOD) mice by an in situ apoptosis detection methods. After DNA unveiling preparation, slides were incubated with terminal deoxynucleotidyl transferase and digoxigenin labeled nucleotides. The catalytically added nucleotides were visualized with a peroxidase labeled anti-digoxigenin antibody. Female NOD mice (4-35 wk-old) were split into three groups according to age and existence of diabetes: non-diabetic mice (group I; 4-10 wk and group II; 11-35 wk), and diabetic mice (group III, 11-35 wk). The diabetic mice were sacrificed the day after the plasma glucose level exceeded 14 mM. The apoptotic cells were detected within islets of non-diabetic mice of group II, and these cells were also stained with anti-insulin antibody. No apoptosis was observed in islets of both non-diabetic young mice (group I) and diabetic mice (group III). The absence of apoptosis in diabetic mice suggest that the apoptotic beta cells are rapidly removed. Apoptotic lymphocytes were detected among infiltrating lymphocytes in all three groups. We conclude that apoptosis plays an important role in the beta cell injury in autoimmune diabetes.

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NF-κB ACTIVITY IS REQUIRED FOR TNF-α-INDUCED APOPTOSIS OF NIT-1 INSULINOMA CELLS.

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TNF-α is a potential mediator of β cell destruction in IDDM. We have investigated the cytotoxic effects of TNF-α in NIT-1 insulinoma cells. Apoptosis was induced in NIT-1 cells after treatment with murine TNF-α (1-1000U/ml) for 24 hrs, and was potentiated by IFN-γ. Human TNF-α also induced apoptosis in NIT-1 cells, implying the involvement of the p55 TNF-R in delivering the apoptotic signal. TNF-α-induced apoptosis is generally considered to involve activation of the Caspase (formerly ICE) family of cysteine proteases, but not the TNF-inducible transcription factor NF-κB. NIT-1 cells transfected with crmA, a cowpox virus-encoded inhibitor of ICE, were protected from TNF-α-induced apoptosis. Unexpectedly, NIT-1 cells stably transfected with a dominant negative inhibitor of NF-κB (Δsp), which lacked TNF-inducible NF-κB activity in a gel mobility shift assay, were also protected from TNF-α-induced cell death. Δsp-expressing NIT-1 cells were deficient in ICE mRNA compared with parental cells, consistent with a role for NF-κB in ICE regulation, and potentially explaining their protection from TNF-mediated apoptosis. Inducible nitric oxide synthase (iNOS) is a gene known to be regulated by NF-κB. NO does not, however, appear to play a role in TNF-α-induced apoptosis of NIT-1 cells, as treatment with 0.5mM N^G-Methyl-$_L$-Arginine, a competitive inhibitor of iNOS, did not protect cells against TNF-α-induced death. Our results show that both NF-κB and ICE activity are required for TNF-induced apoptosis of NIT-1 insulinoma cells, and these molecules are therefore potential targets for prevention of cytokine-mediated beta cell destruction in IDDM.

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ERK1/2 AND p38 MITOGEN ACTIVATED PROTEIN KINASE (MAPK) INHIBITION BLOCKS IL-1 INDUCED RAT ISLET NITRIC OXIDE (NO) SYNTHESIS.

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Interleukin-1ß (IL-1) is selectively cytotoxic to rat pancreatic ß-cells by inhibiting glucose oxidation, causing DNA damage and inducing apoptosis. Nitric oxide (NO) is a necessary, but not sufficient mediator of these effects. We have previously reported that IL-1 causes phosphorylation of Elk-1, activation transcription factor (ATF) 2 and heat shock protein (Hsp) and activates the extracellular signal-regulated kinase (ERK) 1/2 and the p38 MAPK in rat islets and rat insulinoma (RIN) cells. Here we show that mitogen activated protein kinases (MAPK) are involved in IL-1 signalling of NO dependent and independent effects in ß-cells. Specific ERK1/2 and p38 MAPK inhibition using the PD and VK compounds blocked Elk-1 and Hsp25 phosphorylation, respectively, demonstrated by phosphotransferase activity assay of lysates of IL-1 exposed rat islets, and when combined completely prevented IL-1 induced phosphorylation of Elk-1, ATF2 and Hsp25, indicating that ERK1/2 and p38, but not c-Jun NH2 terminal kinase (JNK) are responsible for the phosphorylation of these substrates. The ERK1/2 and p38 MAPK inhibitors individually reduced, but in combination blocked IL-1 mediated islet NO synthesis determined as nitrite by the Griess method. p38 MAPK, but not ERK1/2 inhibition prevented IL-1 mediated inhibition of glucose-stimulated insulin release. We conclude that ERK1/2 and p38 MAPK activation is necessary, but not sufficient for IL-1 mediated ß-cell NO synthesis and that p38 MAPK is involved in signalling of NO independent effects of IL-1 ß-cells.

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ROLE OF MACROPHAGE-DERIVED SOLUBLE MEDIATORS IN THE PATHOGENESIS OF EMC VIRUS-INDUCED DIABETES IN MICE.

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We have previously shown that the major population of infiltrating cells, in mice, at an early stage of encephalomyocarditis (EMC) virus infection are macrophages. The inactivation of macrophages prior to viral infection resulted in the prevention of diabetes, whereas activation of macrophages prior to viral infection resulted in the enhancement of β-cell destruction. This investigation was initiated to determine whether macrophage-produced soluble mediators play a role in the destruction of pancreatic β-cells in mice infected with a low dose of diabetogenic EMC-D virus. When we examined the expression of the soluble mediators interleukin -1β (IL-1β), tumour necrosis factor- α (TNF-α) and inducible nitric oxide synthase (iNOS) in the pancreatic islets, we found that these mediators were clearly expressed at an early stage of insulitis and this expression was evident until the development of diabetes. We confirmed the expression of these mediators by *in situ* hybridization using digoxygenin-labelled RNA probes or immunohistochemistry in the pancreatic islets. Mice treated with antibody against IL-1β and TNF-α, or the iNOS inhibitor aminoguanidine, exhibited a significant decrease in the incidence of diabetes. Mice treated with a combination of anti-IL-1β antibody, anti-TNF-α antibody and aminoguanidine exhibited a greater decrease in the incidence of disease when compared to mice treated with one of the antibodies, or aminoguanidine. On the basis of these observations, we conclude that macrophage-produced, soluble mediators play an important role in the destruction of pancreatic β-cells, resulting in the development of diabetes in mice infected with a low dose of EMC-D virus.

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CHANGES IN PROTEIN EXPRESSION DURING DISEASE OCCURENCE IN ISLET SYNGRAFTS IN BB-DP RATS AND DURING REJECTION OF BB-DP ISLET ALLOGRAFTS.

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IL-1 is cytotoxic to rat ß-cels, and increased expression of IL-1 mRNA is found in the islets of Langerhans during development of diabetes in BB-DP rats and NOD mice. IL-1 has been proposed to induce a race between protective and deleterious proteins in the ß-cells.

In this study we investigated *1)* changes in protein-expression during IL-1 stimulation of islets in vitro, *2)* if these changes are also induced in vivo during disease occurrence in islet syngrafts in BB-DP rats, *3)* and during islet allograft rejection. Two-hundred neonatal BB-DP rat islets were grafted under the kidney capsule of either 30 days old BB-DP rats killed at onset of diabetes or of 30 days old Wistar Kyoto (WK) rats, killed 12 days after grafting. The excised islet grafts or in vitro IL-1 exposed neonatal BB-DP rat islets were labeled with [^{35}S]-methionine, and processed for two dimensional gel electrophoresis. Fluorographs of the gels were analyzed by computer. Significant changes in protein expression were defined as $p<0.01$ (Student's t-test).

Interleukin-1 was found to change level of expression of 82 proteins (22 up- and 60 down-regulated) in neonatal BB-DP rat islets in vitro. Of these 35 (5 up- and 30 down-regulated) also changed level during disease occurence in syngeneic islet grafts from diabetic BB-DP rats, and 33 (5 up- and 28 down-regualted) during rejection of BB-DP islets grafted to WK rats. Changes in the level of expression of 17 (4 up- and 13 down-regulated) of the 82 proteins altered by IL-1 in vitro were found only in syngeneic islet grafts in diabetic BB-DP rats, and changes in the expression level of 11 (3 up- and 8 down-regulated) were found only in BB-DP islet allografts in WK recipients.The identity these proteins are unknown and under study.

Identification of these proteins may be important in understanding the mechanisms of islet destruction and be new possible immunological markers for the development of IDDM, and islet graft rejection.

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GALECTIN-3, A LECTIN INVOLVED IN CYTOKINE-MEDIATED BETA-CELL DESTRUCTION AND IDDM?

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Beta cells are exquisitly sensitive to the toxic effect of cytokines, especially interleukin-1 beta (IL-1). Understanding of the molecular background for this may identify new intervention strategies in IDDM.

Analysis of control and IL-1 exposed isolated rat islets by 2D-gel electrophoresis revealed up-regulation of 52 protein-spots, three of which were identified by mass-spectrometry as three different phosphorylation stages of galectin-3 (gal-3), a 27 kd lectin involved in the development of the endocrine pancreas, inhibition of apoptosis and stimulation of monocyte IL-1 and superoxide radical formation. Chromosome mapping localized the gene for gal-3 to human chromosome 14q21, thus a potential candidate gene for IDDM11. The gal-3 gene was cloned from rat islets and stably expressed in the rat insulinoma cell-line RIN-5AH under a CMV promoter. Two-D-gel analysis of the stable transfectants showed high expression of two different phosphorylation stages of the gal-3 protein. Functional analysis of the transfectants induced a significantly increased proliferation rate of the RIN-5AH cells and high the gal-3 expression level had a protective effect on the cytokine induced (IL-1, TNFa and IFNg) cytotoxicity otherwise seen in non-transfected RIN-5AH cells.

In conclusion: A 2D-gel identified, IL-1 upregulated islet protein demonstrated to be gal-3, was cloned, expressed and partially characterized. Our in vitro studies suggest that intracellular expression of this protein have protective effects agains the cytotoxic effect of cytokines. Further analysis will establish its potential role in the pathogenesis of IDDM in vitro and in vivo.

OP 18
Endothelial Function

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BASAL INSULIN LEVELS MODULATE ENDOTHELIAL FUNCTION BY REGULATING FREE FATTY ACID LEVELS.

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Euglycemic hyperinsulinemia causes endothelium dependent vasodilation (EDV) and enhances vasodilation to the endothelium dependent vasodilator Methacholine Chloride (MCh). It is unclear whether basal insulin contributes to EDV. We studied leg blood flow (LBF) responses to graded intrafemoral artery infusions of the endothelium dependent vasodilator Mch in 8 lean subjects, age 35±2, body mass index 22±1, mean blood pressure (86±3 mmHg) and cholesterol (175±13 mg/dl). Each subject was studied during systemic infusion of saline (SAL) and after 2 hours of systemic somatostatin infusion (SRIF) designed to lower basal insulin levels. During SRIF, basal insulin levels decreased from 11±1 to 5±1 mU/L ($p<0.05$), basal glucose level was 95±2 and was clamped during SRIF infusion at 94±6 mg/dl (p=ns). Free fatty acid levels (FFA) rose from 474±22 to 1042±116 mmol/L ($p<0.01$), and LBF rose from 0.211±0.018 to 0.268±0.023 L/min ($p<0.05$). In response to MCh doses of 5.0, 10.0 ,and 15.0 mg/min, LBF increased by 79±19,120±31 and 165±25% during SAL and by 24±8, 54±15, and 83±15% during SRIF ($p<0.02$, SAL vs SRIF, ANOVA). To examine the role of the SRIF induced rise in FFA on EDV, circulating FFA levels were raised in a separate group of 7 normal subjects by infusion of Intralipid+heparin to achieve FFA levels similar to that in the SRIF infused group(1275±224 mmol/L) and EDV measured by graded intra-arterial infusion of Mch. Compared to basal, EDV was impaired by 50%($p<0.01$), thus to a similar degree as in the SRIF infused group. These data suggest that 1) circulating FFA elevations cause endothelial dysfunction and 2) basal insulin action to regulate lipolysis is an important indirect modulator of EDV. Given insulin's impaired ability to suppress lipolysis and the elevated FFA levels observed in insulin resistant subjects, it is reasonable to suggest that elevated FFAs are responsible at least in part for the impaired EDV observed in these patients and thus may contribute to their increased risk of macrovascular disease.

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ROLE OF CHRONIC HYPERGLYCEMIA AND OXIDIZED LDL IN ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH IDDM

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Chronic hyperglycemia and oxidized LDL have been implicated as risk factors for impaired vascular function in IDDM. We determined the concentration of autoantibodies against oxidized LDL (ratio between antibodies to native vs oxidized antibodies, oxLDLab) and blood flow responses to intrabrachial infusions of acetylcholine, sodium nitroprusside and L-NMMA in 23 male patients with IDDM (age 33±1 yrs, body mass index 24.3±0.6 kg/m^2, HbA_{1c} 8.5±0.3 %) and in 33 matched normal males. Total (4.7±0.2 vs 4.4±0.2), LDL (2.8±0.1 vs 2.8±0.1) HDL (1.3±0.1 vs 1.3±0.1) cholesterol (mmol/l, IDDM vs normal subjects) concentrations were comparable between the groups. OxLDLab were 41 % higher in patients with IDDM than in the normal subjects (1.74±0.21 vs 1.23±0.07, $p<0.01$). Within the group of IDDM patients, HbA_{1c} but not oxLDLab or LDL cholesterol, was inversely correlated with forearm blood flow response to acetylcholine ($r=-0.51$, $p<0.02$) but not to sodium nitroprusside ($r=0.06$, NS). These data implicate that oxidative modification of LDL is increased in normolipidemic patients with IDDM compared to normal subjects. Despite this, chronic hyperglycemia, but not oxLDLab, is an independent determinant of impaired endothelium-dependent vasodilation in patients with IDDM. These changes could contribute to the increased cardiovascular risk in IDDM.

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RETINAL AND GLOMERULAR ENDOTHELIAL CELL MONOLAYER PERMEABILITY IS INCRESED BY HIGH GLUCOSE

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Vascular permeability was shown to be increased in tissues targets of diabetic complications, including the retina and the kidney. This study was aimed at assessing whether the diabetic milieu is capable of impairing retinal and glomerular endothelial cell barrier function directly, independent of other factors affecting permeability. Bovine retinal endothelial cells (BREC) and human glomerular endothelial cells (HGEC) were cultured for 10 days under high glucose (HG) vs. iso-osmolar mannitol (M) and normal glucose (NG) conditions on permeable PET membranes inserted in 35-mm culture dishes. To assess the time-course or HG-induced changes, BREC were exposed to the above conditions also for 30 days (through 3 passages) and 5 hours (i.e. during the assessment of permeability). At confluence, monolayer permeability was measured by a radiotracer technique using ^{125}I-labelled horseradish peroxidase (HRP), bovine serum albumin (BSA), IgG or fibrinogen (FBG, to exclude an abnormal leakage of monolayers), which were added to the upper chamber at time 0. Medium was sampled from both chambers at 1, 2, 3 and 5 hours and percent leakage was then calculated. BREC grown for 10 days under HG, but not M, showed increased leakage of HRP (+35%), BSA (+44%), and IgG (+60%), but not FBG, compared with NG control cells ($p<0.001$). The increases vs. NG in HRP and BSA (but not IgG) leakage were even more pronounced in BREC grown in HG for 30 days, whereas no change was evident in BREC exposed to HG for 5 hours. HGEC cultured in HG for 10 days also showed significant increases vs. NG ($p<0.001$) in the transendothelial passage of HRP (11.7±0.5 vs. 8.1±0.9), BSA (7.9±1.2 vs. 5.4±0.7), and IgG (5.8±1.2 vs. 4.1±0.5). DNA synthesis and cell number did not change in response to HG, thus ruling out the possibility that the increased permeability observed under these conditions was dependent on reduced cell density. These results show that the diabetic milieu directly impairs retinal and glomerular endothelial cell barrier function.

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ALTERATIONS OF GLYCOSYLATION BY GLYCATED PRODUCTS IN RETINAL MICROVASCULAR CELLS.

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Diabetic retinopathy is a complication characterized by alterations of specific cell-cell interactions in the retinal capillaries. Glycation, as a result of reactions between sugars and proteins, produces advanced glycosylation end products (AGEs), known to accumulate in basement membranes and to affect the retinal cell proliferation in vitro. However, at the cellular level, little is known concerning the effects of the diabetic environment on the structure and/or biosynthesis of the glycoconjugates, making up the glycocalix, a key-structure for cellular interactions. So, we decided to study the **effects of AGEs on the glycoprotein profiles of retinal cells.** Endothelial cells (BREC) and pericytes from bovine retinal microvessels were separately cultured in the presence of glycated albumin (AGE-BSA). The cell homogenates were analyzed by lectin affino-blotting. The total protein content was not affected by the glycated products. However, the lectin analysis of the glycoprotein profiles showed specific alterations of the glycosylation pattern for the BREC cultured with AGE-BSA. A significant decrease of α(2,3) sialylation, β–galactosylation and α-fucosylation was observed mainly for a 210 kDa glycoprotein, localized in a membrane-rich fraction. Similar alterations were obtained by metabolic labelling of endothelial cell cultures with labelled sugars ([^{3}H]ManNac, [^{3}H]Fuc, [^{14}C]Gal). The alterations appeared AGE dose-dependent. In contrast, The 210 kDa glycoprotein was only weakly modified in pericytes. The enzymatic glycosylation was then studied through glycosidase and glycosyltransferase assessments : a similar trend was observed in various enzymatic activities. Our results show for the first time that **glycated products can affect carbohydrate structures resulting from enzymatic glycosylation.** They suggest that post-translational protein modifications can be further modulated by a subsequent change such as glycation. The resulting altered glycoforms might exhibit modified biological properties.

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MODULATION BY HIGH GLUCOSE AND TNF-α OF APOPTOSIS ASSOCIATED MOLECULES IN CULTURED ENDOTHELIAL CELLS

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Using human umbilical vein endothelial cells (HUVECs) we have previously shown 30 mM glucose and TNF-α to induce apoptosis and to upregulate protooncogene p53 expression, probably involved in the development of vascular complications in diabetes mellitus. In this study we evaluated the effects of high (30 mM) glucose and TNF-α on "p53-dependent genes" p21, an inhibitor of cyclin dependent kinases, and thrombospondin (TSP), a mitogen for smooth muscle cells and a "bridging molecule" between phagocytes and apoptotic cells. Exposure of HUVECs to 30 mM glucose (13±1 days) significantly increased p21 (133±29% of control, $p<0.01$;n=15) and TSP (120±23% of control, $p<0.05$;n=10) mRNA expression versus control cells in 5 mM glucose.Determination of p53 and p21 in the identical individual isolate after 30mM glucose long term culture exhibited a more pronounced stimulation of p21 (+30%) compared to p53 gene expression with a range between 93 and 176%, suggesting at least in part p53-independent upregulation by hyperglycemia of p21. High glucose long term culture of HUVECs (13±1 days, n=7) previously to TNF-α (1300U/ml/24 h) exposure further stimulated cytokine induced p21 mRNA upregulation from 133±33% to 167±31% of control ($p<0.05$ each). Both, increase in p21 and observation of reduced (-95%) hyperphosphorylation of the retinoblastoma protein (pRb), analyzed by Western blotting, seem to mediate the antimitogenic effects of TNF-α in HUVECs. Parallel cell cycle studies indicate more pronounced susceptibility of HUVECs to TNF-α induced apoptosis in G1- than in S-phase. The extent to which p21, TSP and pRb affect induction of apoptosis or growth arrest, remains to be elucidated. These data, however, suggest hyperglycemia and TNF-α to locally exhaust the replicative potential of endothelial cells due to apoptosis and growth arrest, and thus could be prerequisite for vascular lesions.

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ENDOTHELIAL CELL DYSFUNCTION IN MICROALBUMINURIC IDDM PATIENTS; FURTHER IMPAIRMENT WITH ADDITIONAL COMPLICATIONS

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Haemodynamic abnormalities have been described in microalbuminuric IDDM patients, however the mechanisms involved are unclear. The aim of this study was to investigate the skin perfusion responses to acetylcholine (ACh) (an endothelium-dependent vasodilator) and sodium nitroprusside (SNP) (a smooth muscle relaxant) in microalbuminuric IDDM patients (albumin excretion rate of 20-200 μg min^{-1} on at least 2 out of 3 occasions). In 12 microalbuminuric IDDM patients (age 41.1 ± 3.3 years (mean ± sem); 4 female) and 12 age and sex matched controls forearm skin blood flow responses to iontophoretically applied 1% ACh and 0.01% SNP were recorded by laser Doppler perfusion imaging. ACh significantly increased forearm skin perfusion ($P<0.001$, ANOVA), however the vasodilatation was significantly lower in the IDDM patients with microalbuminuria compared with the controls ($P<0.001$), e.g. the maximum vasodilatation to ACh was 1.25 ± 0.11 V in IDDM patients *vs* 1.86 ± 0.11 V in the controls. Skin perfusion increased following SNP ($P<0.001$) but was not significantly different between the 2 groups, e.g. at the fifth measurement point the vasodilatation to SNP was 0.28 ± 0.06 V in IDDM patients *vs* 0.32 ± 0.07 V in healthy volunteers. The response to ACh was further impaired in microalbuminuric patients with (pre)proliferative retinopathy and/or peripheral neuropathy (n=7) compared to IDDM patients with microalbuminuria and background retinopathy (n=5) ($P<0.05$). Diabetes duration was also significantly longer in microalbuminuric patients with (pre)proliferative retinopathy and/or peripheral neuropathy (32.2 ± 2.5 years) compared to IDDM patients with microalbuminuria and background retinopathy (19.2 ± 3.7 years ($P<0.011$), however the maximum response to ACh did not significantly correlate with duration of diabetes. These data suggest that skin microvascular endothelial cell function but not smooth muscle cell function may be abnormal in IDDM patients with microalbuminuria. The level of endothelial cell dysfunction may be related to the degree of diabetic microangiopathy and/or diabetes duration.

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THE EFFECT OF EXERCISE ON THE ENDOTHELIAL FUNCTION OF THE MICRO- AND MACRO- CIRCULATION IN IDDM.

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Exercise is beneficial in diabetes and can reduce cardiovascular disease. We have examined the effect of regular, intense exercise on the endothelial function in both the micro- and macro- circulation in IDDM. For this we studied four groups of patients matched for age, sex and Body Mass Index: 1. Non-diabetic control regular exercisers (CE) (n=12, 9 Males, mean age 33 years) , 2. IDDM exercisers (DE) (n=15, 13 M, age 34 yrs, DM duration 16 yrs) 3. sedentary controls (CS) (n=17, 10 M, age 34 yrs) and 4. sedentary diabetic patients (DS) (n=4, 3 M, age 32 yrs, DM dur. 21 yrs). Autonomic neuropathy was present in 5 DE and 0 DS patients, retinopathy in 4 DE and 4 DS and macro-proteinuria in none. There was no difference in the weekly energy expenditure from exercise, evaluated by the Harvard Alumni Health Questionnaire, between CE (6497 ± 1374 kcal/week) (mean ± sd) and DE (6437 ± 3036) but both groups were higher compared to CS (281 ± 501) and DS (150 ± 300, p <0.0001). Similar results were found in maximal oxygen uptake (VO_2 max) (CE 55.3 ± 8.0 ml/kg/min, DE 52.4 ± 10.7, CS 38.4 ± 6.0, DS 34.8 ± 10.0, p <0.0001). The endothelial function in the microcirculation was assessed by measuring the changes in the erythrocyte flux after the iontophoresis of acetylcholine and in the macro-circulation by assessing the changes in the brachial artery diameter induced by reactive hyperemia. When all diabetic patients were considered as one group the micro-circulation endothelial function was reduced compared to the controls (91 ± 49 % flux increase vs 122 ± 41, p <0.05) but no difference existed between DE (95 ± 51) and DS (73 ± 42) and between CE (117 ± 44) and CS (125 ± 40). Similarly, in the macro-circulation a reduced response in the diabetic patients (7.0 ± 4.5 % diameter increase vs 11.2 ± 6.6, p< 0.05) while no difference existed between DE (6.7 ± 4.6) and DS (8.0 ± 4.7) and between CE (12.0 ± 5.8) and CS (11.5 ± 7.3). We conclude that regular exercise does not prevent the diabetes-induced impairment of the endothelial function.

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INSULIN INDUCED VENODILATATION IS FURTHER IMPAIRED IN TYPE II DIABETES MELLITUS WITH HYPERCHOLESTEROLEMIA.

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We have shown that insulin induces venodilatation via the endothelial dependent NO-cGMP pathway and that this effect is impaired in NIDDM, a disease associated with endothelial dysfunction. As hypercholesterolemia increases the risk of cardiovascular diseases in NIDDM, we have studied the venodilatory response to insulin in NIDDM subjects with cholesterol > and ≤ 200 mg/dl. Sixteen healthy caucasians, 19-67 years old (Group 1), nine normotensive NIDDM 42-77 years old, with cholesterol 251 ± 22 mg/dl, and LDL cholesterol 147 ± 23 mg/dl (Group 2) were compared to nine matched NIDDM with cholesterol 173 ± 12 mg/dl, and LDL cholesterol 107 ± 12 mg/dl (Group 3). Cephalic vein of the left hand was visualised 1cm distal to the cannula tip using an Acuson 128XP ultrasonograph with 7.5 Mhz linear array transducer. Norepinephrine was infused at 12.5, 25, 50 and 100 ng/min and this was followed by a coinfusion of norepinephrine at 100 ng/min with regular insulin at 8, 16, 24, and 32 μu/min, each dose for 5 mins. A blood pressure cuff was inflated at the arm to 40 mm of Hg 3 minutes into each infusion and the venous diameter was measured between 4 to 5 minutes using the M-mode image.

	Baseline	$N_{12.5}$	N_{25}	N_{50}	N_{100}	Mean	SEM	I_8	I_{16}	I_{24}	I_{32}	Mean	SEM
Group 1	100 (2.8)	80	70	57	47	72	2.9	71	77	90	95	87	2.0
Group 2	100 (2.5)	67	52	57	57	67	3.0	45	55	64	57	64*	2.4
Group 3	100 (3.5)	76	66	61	55	72	2.7	65	60	64	68	72*+	2.4

Data expressed as percentage of baseline diameter (mm); *p<0.05 when compared to Group 1. +p<0.05 in comparison to Group 2. Analysis by twanova. These data show that hypercholesterolemia further impairs the venodilatory response to insulin in NIDDM subjects. Coexistence of these disease states have a synergistic effect on endothelial dysfunction and this correlates clinically with the increase in vascular risk seen in such conditions.

OP 19

Better Care for People with Diabetes

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Measurements of glycated haemoglobin and microalbuminuria in West-Germany from 1988 to 1994

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The number of measurements of glycated haemoglobin and microalbuminuria is an indicator of process quality of care in people with diabetes. In Germany the number of all determinations of glycated haemoglobin and microalbuminuria is available through a nationwide account and information system. This system summarises all determinations in ambulatory care for patients, who are members of the compulsory health insurances in West-Germany (57.5 million persons). Based upon epidemiological data the prevalence of diabetes can be assumed to be 5 % (2.875 million persons with diabetes). The number of measurements has increased significantly since 1988 (Tab.1). This increase cannot be attributed to an increase in the number of insured persons nor in the number of all medical acts (indicated by EKG).

Tab. 1. Number of insured persons, EKG and determinations of $HbA_{1(c)}$ and microalbuminuria from 1988 to 1994.

	1988	1989	1990	1991	1992	1993	1994
Insured persons (mill.)	55.1	54.7	55.9	56.8	57.2	57.5	
Diabetic patients (mill.)	2.76	2.74	2.80	2.84	2.86	2.875	
$HbA_{1(c)}$ (mill.)	1.0	1.3	1.6	1.9	2.2	2.5	2.6
Microalbuminuria (thousand)	0	2.6	8.4	33.9	72.2	77.3	125.5
EKG (mill.)	16.0	16.0	15.6	16.1	16.4	17.0	17.2

Guidelines state that glycated haemoglobin should be determined quarterly and microalbuminuria annually in persons with diabetes. During six years the number of $HbA_{1(c)}$ measurements increased by 160 %. It would be interesting to compare our data with population-based data of other countries, but to our knowledge such data are not yet available. If physicians adhered fully to guidelines a maximum of 11.5 million measurements of glycated haemoglobin and at least 8.6 million measurements of microalbuminuria would be necessary on an annual basis in West-Germany. Authors of guidelines for better care of persons with diabetes should be aware of the tremendous financial consequences of their application.

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SPECIFICITIES OF ORGANISATION OF DIABETES HEALTH CARE DURING WAR TIME

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The Model of diabetes health care relies on the network of diabetes centres and continuous flow of information from highly specialised centres to primary health care and the flow of rationally referred patients from primary to tertiary health care. The model has shown its advantages during the war in Croatia. The register was the basis for planning the supplies of oral agents, insulin, and self- monitoring equipment in the war-struck regions. In 1991 there were 111.096 diabetic persons registered, 16% of them requiring insulin, 46% oral agents and 36% treated by basic principles of treatment only (education, exercise, diet). All patients were provided with a 60-day supply of insulin or oral agents. As destruction increased there was need to modify the existing system of information exchange which remained only on telephone communication. Use was made of existing PC compatible equipment and the computer network created to prepare for accepting and distributing of patients evacuated from destroyed hospitals or dialysis centres, and to learn of movements of larger groups of displaced persons. Written instructions were issued to patients on how to prepare themselves for long stays in shelters, disinfection of water, lack of drugs and food. For assessing glycaemic control, a simple method was developed for transporting blood samples in specially prepared tubes and transporting them at room temperature to the nearest lab for haemoglobin A1C determination. Except for diabetic gangrene, probably due to poor hygienic conditions in war areas, there were no more cases of acute diabetic complications. This could be attributed to good education of diabetic patients within the model of diabetes health care as a basic principle of treatment, especially in the regions far from specialised centres.

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LIONS DIABETES MINNESOTA: A MODEL FOR COMMUNITY-BASED INTERVENTIONS IN PRIMARY CARE.

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In 1993 the Lions Clubs of Minnesota developed a community-based approach to public and professional education in diabetes. The purpose of this study was to determine to what extent this regional diabetes program changed diabetes practices. Lions Diabetes Minnesota (LDM) was created to reach all of the rural communities of Minnesota with a 3 part program: (1) to screen for diabetes in a high risk population; (2) to provide public education in the prevention of diabetes and its complications; and, (3) to assess care practices in order to provide professional education to improve care. A total of 1351 individuals came to the public screening program, of whom 629 were in treatment for diabetes. The remaining 722 had at least one risk factor for type II diabetes. Through the screening program and follow-up, 72 individuals were diagnosed with diabetes. The subsequent public education program (for 1846 individuals) focused on management of weight, exercise and life style to prevent diabetes. These individuals came from 30 clinics which agreed to participate in an assessment of current diabetes care. On the basis of 731 chart audits at these sites it was estimated that 70% of the patients were at a level of glycemic control (HbA1c >1.5% above normal) that would contribute to the development of microvascular complications and <15% of the patients were appropriately evaluated for complications. The follow-up professional education program at these sites focused on appropriate classification of diabetes, therapeutic goals, treatment options and surveillance for complications. It was attended by 684 physicians, nurses and dietitians. The overall goal was to improve glycemic control and surveillance for microvascular and macrovascular complications. One year follow-up showed a significant ($p<.001$) decrease in HbA1c (-1.4%) and a significant ($p<.01$) increase in surveillance for eye disease (23 to 34%) and foot disease (16 to 33%). A survey of the sites revealed: 77% increase in SMBG, 66% increase in HbA1c to 3-4/year, 77% increase in patient education and nutrition services and 55% increase in the rate of screening for microalbumin. LDM was able to identify persons at risk for diabetes, diagnose undetected cases and provide education which resulted in improved glycemic control and complications surveillance.

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SHARED CARE FOR NIDDM PATIENTS IN GENERAL PRACTICE: SUSTAINED GOOD GLYCAEMIC CONTROL AFTER TWO YEARS.

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A prospective non-randomized cohort study was designed to assess the intermediate term (2 years) effect of structured NIDDM care with (study group=SG; 22 GPs) and without (control group=CG; 6 GPs) support from a 'diabetes service' on glycaemic control and well-being. The 'diabetes service', supervised by a diabetologist, included a registration system and consultation facilities of a dietitian and diabetes nurse educator. GPs of the SG received protocolized glucose lowering therapy advises. This included home blood glucose monitoring and insulin therapy. In the SG 350 known NIDDM patients above 40 years of age (206 women; mean age 65.3±SD 11.9 and diabetes duration 5.9±5.4 years) have been followed for at least 2 years. The CG consisted of 68 patients (28 women; age 64.6±10.3 and diabetes duration 6.3±6.4 years). Mean HbA1c (reference value 4.3-6.1%) fell from 7.4±1.6 to 7.0±1.3% after 1 and 2 year of follow-up in SG, but rose from 7.4 to 7.6 in CG ($p=0.004$). The part with poor HbA1c (>8.5%) decreased from 21.4 to 11.7% in SG but increased from 23.5 to 27.9% in CG ($p=0.008$). Good control (HbA1c <7.0%) was achieved in 54.3% (at entry 43.4%) in SG and in 44.1% (at entry 54.4%) in CG ($p=0.013$). Insulin therapy was initiated in 29.9% (SG) and 8.8% (CG) ($p=0.000$). The incidence rate of severe hypoglycaemia was low 0.019 /patient year in SG and not reported in CG. General well-being, as assessed with annual self-report questionaires, did not change during intensified therapy. In conclusion, implementation of structured care, including protocolized therapeutic advice, results in safe and sustained good glycaemic control in the majority of NIDDM patients in primary care.

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CAN PRIMARY CARE BASED REVIEWS KEEP INDIVIDUALS WITH DIABETES OUT OF HOSPITAL?

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It has been suggested that increases in routine diabetes review in primary care could reduce hospital admission rates for individuals with diabetes and that interventions should target those at highest risk of admission. However recent trials of intense primary care interventions in high risk groups have been unable to reduce overall admission rates. This observational study explored the relationship between routine diabetes review in primary care and risk of diabetes related hospital admission in groups with and without comorbidity. A historical cohort study of 1092 individuals with diabetes, from randomly selected general practices, collected information on service contacts and clinical, social and demographic variables using patient records and postal questionnaires. Logistic regression modelling was used to establish risk factors for admission during a five year period. The main predictors of admission were the number of non-diabetic drugs regularly prescribed (odds ratio=1.4 per drug on repeat prescription, $p<0.0001$) and type of diabetes treatment (odds ratio=1.6 for insulin, 0.9 for tablets, $p=0.0001$). Only in the subgroup without comorbidity (defined as those prescribed no regular treatment other than diabetes treatment) was general practice review associated with a lower risk of admission (odds ratio=0.30, $p=0.02$). Structured care for diabetes in primary care may in the long term reduce diabetes related hospital admissions. However interventions to increase frequency of routine review which target high risk groups which have high levels of comorbidity, are unlikely to reduce admission rates.

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THE COST OF DIABETIC FOOT ULCERS IN A DEVELOPING COUNTRY

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The prevalance of diabetic foot ulcers is around 7 % and of amputations due to foot ulcers is 3 %. The total direct economic burden of diabetic foot problem in the USA is 150 million USD annually and the mean cost of a diabetic patient who undergoes lower extremity amputation is 24700 USD. The present study was done to disclose the main features and calculate the avarage cost of 60 randomly chosen inpatients treated for diabetic foot ulcers. 18 patients were female (30%) and 42 were male (70%), with mean age of 58.9+10.1 (31-84), diabetes duration of 13.5+7.6 years(0.3-30). Mean HbA1c was 9.4+2 (4.6-15). 14 patients previously had ulcers (23.3%) and 11 had (18.3%) previous amputations. All of the 14 patients previously had and were treated for ulcers, had received programmed education for foot care and prevention of foot ulcers. 54 patients had neuropathy as the predominant etiologic factor for foot ulcers (90%) , 4 (6.7%) had both ischemia and neuropathy and 2 (3.3%) had ischemia solely. 36 patients (60%) had grade 3 or more severe ulcers according to Wagner classification. Ulcers were localized on the toes in 27 patients, 9 (15%) of them being on the plantar surface of great toe, 19 (31.7%) on the metatarsal head, 9 (15%) on the heel, 3 (5%) on volar surface of the foot, 2 (3.3%) on the wrist. Swab culture of the ulcer yielded single microorganism in 33 (55%) patients and multiple organisms in the rest. 21(35%) patients received single antibiotic, 39 (45%) had combined antimicrobial therapy. 22 patients (36.7 %) treated conservatively whereas 7 (11.7%) underwent grafting, 11 (18.3%) transmetatarsal amputation, 15 (25%) wedge resection of the toes, 4 (6.7%) below knee amputation, 1 (1.7%) above knee amputation. Mean duration of hospitalisation was 5.45+ 4.22 weeks, grade of ulcer being the only factor affecting this. The mean total cost of a hospitalized patient was found to be 7000 USD. In our country where avarage income per family is below 200 USD per month and some 60% of total population is not covered by any kind of social security, diabetic foot is even a more severe economic and social problem than in the USA and European countries. This urgently mandates planning and implementation of diabetic foot clinics all over the country.

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THE USE AND THE COSTS OF ALL MEDICATIONS OF DRUG-TREATED DIABETIC PATIENTS IN FINLAND

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The aim of the study was to investigate the total use and costs of all kinds of medications used by persons with diabetes (PWD). The persons having purchased medications for diabetes were identified from the Central Drug Registry which codes and registers all prescriptions of medicines with a unique personal identification number. The use of all kinds of medicines by PWD was identified. The medications used in hospitals and sold over the counter were not included. There was a total of 125193 persons (2.25% of the population) having purchased diabetes medicines in 1995, out of whom only 73.7% used their right for reimbursement. The total cost of drugs used by PWD was 130.3 million US dollars (USD), or 1040.5 USD/PWD (vs. 264.5 USD/ inhabitant). 50000 had used insulin for 31.3 million USD and 91000 oral hypoglycemic drugs (OHD) for 20.4 million USD. Antidiabetic drugs totalled to 51.7 million USD (40% of total costs). 13000 had purchased both insulin and OHD. The costs of other medications was 78.6 million USD (60%). The cost of all cardiovascular drugs was 33.3 million USD. 19.6% of PWD used digitalis vs. 2.3% of population at large. Use of cholesterol reducing drugs was 5.3% vs. 3.1%, that of ACE inhibitors 25.1% vs. 9.7% respectively. The given figures are crude. The study has an age and sex matched control population, but the results are in preparation. Our preliminary results show that use and costs of medications by PWD are remarkably higher than those of population at large. The final results will give exact figures of the extra usage.

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POTENTIAL COST-EFFECTIVENESS OF PRIMARY PREVENTION OF INSULIN-DEPENDENT DIABETES MELLITUS.

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We analyzed the potential cost-effectiveness of primary prevention of IDDM, using a simulation model of IDDM . We used hazard rates for diabetes from the Diabetes Prevention Trial for Type 1 Diabetes (DPT-1) of 22.5%/year for the parenteral insulin protocol (P), and 6%/year for the oral insulin protocol (O). Screening costs ($1751/case found) and intervention costs ($3172/yr (P) and $1360/yr (O)) were estimated from DPT-1. Modeled patients receive intensive treatment if IDDM develops ($4545/yr). Incremental costs and benefits are in present value dollars (3% discount rate). Life years are adjusted for quality of life (QALYs) using utilities for blindness (0.69), renal failure (0.61), and amputation (0.8). We assume IDDM reduces quality of life by 5%. The perspective of the analysis is from a single payer responsible for all direct medical costs. The model predicts that 99% (P) and 97% (O) progress to IDDM. The predicted lifetime risks of visual acuity <20/100, renal failure, and amputation are 42%, 16%, and 5% (P) and 31%, 11% and 3.5% (O). Primary prevention that reduces the risk of progressing to IDDM by 50% delays the mean age at onset of diabetes from 12.5 years to 17 years (P), and from 22.9 to 28.8 years (O). Complications occur in later in life; cumulative incidence is reduced to 38%, 14%, and 4.8% (P), and 22%, 8%, and 2.4% (O). Reduced treatment costs for clinical IDDM, reduced lifetime risk of severe hypoglycemia, and reduced complications partly offset the costs of screening and preventive treatment. We estimate that parenteral insulin would cost ~$22,000/QALY gained , and oral insulin ~$5,800/QALY gained. Savings of ~$4,700/person treated are predicted if subcutaneous insulin alone is effective in the parenteral insulin trial. The cost/QALY gained is stable if the HbA1c assumed under intensive care is 7.2%-8.7%, assuming that the incremental costs of intensive care are proportional to the HbA1c achieved. Primary prevention is a reasonable goal to pursue from a health and economic perspective.

OP 20

Insulin Action in Vitro

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IRS-2 IS THE MAIN DOCKING PROTEIN FOR PI 3-KINASE IN ADIPOCYTES FROM NIDDM PATIENTS.

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IRS proteins are substrates for the insulin receptor tyrosine kinase and act as docking proteins. The aim of this study was to investigate the role of IRS proteins in the insulin signaling pathway in adipocytes from healthy subjects or NIDDM patients. We examined insulin-stimulated glucose transport, protein content of key proteins involved in insulin action and performed immunoprecipitation, immunoblotting and PI-3 kinase assays using specific antibodies to IRS-1 and 4PS/IRS-2. Glucose transport activity in response to insulin was reduced by 40-60% in NIDDM patients, IRS-1 protein content was reduced by 50-90% while 4PS/IRS-2 protein content was unaltered. In contrast, other key proteins such as the insulin receptor, PI 3-kinase (p85 and p110), SHC or MAP kinases were unchanged in NIDDM. Following insulin stimulation 4PS/IRS-2 was rapidly tyrosine phosphorylated, associated with the p85 subunit of PI 3-kinase and stimulated the activation of PI 3-kinase in NIDDM subjects. In contrast, in anti IRS-1 immunoprecipitates from NIDDM adipocytes no clear tyrosine phosphorylated band corresponding to IRS-1 or an associated PI 3-kinase activity was detected. Conclusion: IRS-1 is the dominant tyrosine phosphorylated protein which binds p85 and accounts for most of the phosphotyrosine associated PI 3-kinase activity in adipocytes from normal human subjects. In contrast, in cells from NIDDM subjects, which are deficient in IRS-1, 4PS/IRS-2 becomes the principal docking protein.

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PI3-KINASE ACTIVATION ALONE IS NOT SUFFICIENT FOR THE MEDIATION OF INSULIN ACTION

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Two naturally-occuring mutations (Pro1178Leu and Arg1174Gln) in the insulin receptor tyrosine kinase domain, found in patients with severe insulin resistance, retain the ability to phosphorylate IRS-1 yet fail to mediate insulin-stimulated glycogen synthesis or mitogenesis. Further studies were undertaken to examine the molecular basis for the defective signalling associated with these mutant receptors. The mutant receptors failed to stimulate any GLUT4-GFP translocation to the plasma membrane in microinjected 3T3 fibroblasts and were unable to stimulate activation of a reporter plasmid consisting of the fos promoter upstream of luciferase ($p < 0.001$ vs wild type receptor). Although at low concentrations of insulin these receptors were impaired in their tyrosine phosphorylation of IRS-1, at 100nM insulin cells expressing the 1174 and 1178 mutant receptors showed significantly enhanced tyrosine phosphorylation of IRS-1, association of the p85α subunit of PI3-kinase with IRS-1, and PI3-kinase activity in anti-IRS-1 immunoprecipitates compared to parental CHO cells. In contrast, at 100nM insulin, no significant enhancement of the tyrosine phosphorylation of Shc, GTP loading of ras, or map kinase activation was seen. These findings indicate that PI3-kinase activation alone may be insufficient to mediate the metabolic and mitogenic effects of insulin and suggest that additonal signalling events involving the shc-ras-map kinase pathway may be required for the full mediation of insulin's metabolic and mitogenic effects. Additionally, these data provide support for the notion that insulin's activation of ras is dependent on shc, rather than IRS-1, phosphorylation.

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DIFFERENTIAL ROLES OF REGULATORY SUBUNITS OF PI 3-KINASE IN GLUCOSE HOMEOSTASIS IN THE P85α PI 3-KINASE KNOCKOUT MICE

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The aim of this study is to investigate the role of phosphatidylinositol 3-kinase (PI3K) in glucose homeostasis by targeted disruption of a regulatory subunit of PI3K gene (p85α) in mice. Alternative isoforms of the p85α gene, p55α and p50α, were normally expressed in this knockout mice. Total PI3K activity associated with anti-phosphotyrosine antibody after an insulin injection was normal in the liver and 70% of the normal in the skeletal muscle. A 50kDa protein (p50) recognized by anti-p85 antibody, which was presumably p50α, played a compensatory role for p85α deficiency in insulin-stimulated PI3K activation by binding to IRS-1 and p110 catalytic subunit of PI3K. Mice lacking p85α showed lowered set point of glucose levels associated with decreased insulin levels. Steady state plasma glucose levels after somatostatin, insulin, and glucose infusion were 125±34 and 254±15 mg/dl in knockouts and the wild-type ($P<0.01$), although steady state plasma insulin levels were indistinguishable. Studies on glucose metabolism intermediates in the liver strongly suggested that glucose output was rather increased in response to enhanced glucose utilization by the peripheral tissues. In fact, 2-deoxy-glucose uptake into the soleus muscle was increased by 40% in knockouts compared with the wild-type, and 3-*o*-methyl-glucose uptake into the adipocyte was 2-3 fold increased with the physiological concentrations of insulin in knockouts. These results demonstrated that increased glucose utilization in the peripheral tissues, rather than primary reduction of glucose output by the liver, was the main cause of hypoglycemia and increased insulin sensitivity. Although PI3K activity associated with IRS-1 was essentially normal, efficient targeting of PI3K activity to the GLUT4 vesicle via p50/p110 complex may have resulted in efficient glucose transport in the p85α knockout mice. Thus, PI3K isoforms may play differential roles in glucose metabolism in the peripheral tissues, and p85α deficient mice should give important insight into the molecular mechanism linking PI3K activation and GLUT4 translocation.

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DECREASED IRS-1 PHOSPHORYLATION AND PI 3-KINASE ACTIVITY IN MUSCLE FROM NIDDM PATIENTS.

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We examined the effect of physiological hyperinsulinemia on IRS-1 tyrosine phosphorylation and PI3-kinase activity in skeletal muscle from six lean to moderately obese NIDDM and six healthy subjects. A rise in serum insulin levels from ~60 to ~650 pmol/l increased IRS-1 tyrosine phosphorylation 6-fold over basal levels in control muscle ($P<0.01$), whereas no significant increase was noted in NIDDM muscle. The reduced IRS-1 phosphorylation in the NIDDM muscle was not related to changes in IRS-1 protein content, since IRS-1 protein expression was similar between control and NIDDM subjects (16.0±1.7 vs. 22.9±4.0 arbitrary units/mg for control and NIDDM, respectively, N.S.). Physiological hyperinsulinemia increased PI 3-kinase activity in control muscle 2-fold, ($P<0.01$), whereas no increase in insulin-stimulated PI 3-kinase activity was noted in the NIDDM muscle. Furthermore, in vitro insulin-stimulated (600 pmol/l) 3-O-methylglucose transport was 40% lower in isolated muscle from NIDDM subjects ($P<0.05$). The present findings couple both reduced insulin-stimulated IRS-1 tyrosine phosphorylation and PI 3-kinase activity to the impaired insulin-stimulated glucose transport in skeletal muscle from lean to moderately obese NIDDM subjects.

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HYPERINSULINEMIA POTENTIATES ACTIVATION OF p21Ras BY GROWTH FACTORS.

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Detrimental role of hyperinsulinemia has been suggested by numerous epidemiological studies correlating insulin resistance and/or hyperinsulinemia with atherosclerosis and hypertension. Biochemical evidence of the cause-and-effect relationship remains, however, elusive. We have recently discovered that insulin promotes phosphorylation and activation of the farnesyltransferase, FTase, an enzyme crucial for preparing the Ras signaling pathway for subsequent activation. FTase catalyzes farnesylation of p21Ras, a step necessary for anchoring p21Ras at the plasma membrane where it can be activated by various growth factors. Insulin in a dose- and time-dependent manner activated FTase in fibroblasts, adipocytes, vascular smooth muscle cells, and endothelial cells. In 3T3-L1 fibroblasts, this action of insulin on FTase activity led to a 3 fold increase in the size of the cellular pool of farnesylated p21Ras, which upon farnesylation is translocated to the plasma membrane. The effect of insulin was inhibited by the α-hydroxyfarnesyl phosphonic acid (HXPA) (1 μM), an inhibitor of FTase. The half-life of the farnesylated p21Ras at the plasma membrane appears to be six hours. Insulin and other growth factors stimulate GTP loading only of the membrane-associated p21Ras. An augmentation in the plasma membrane-associated farnesylated p21Ras by hyperinsulinemia resulted in 5-8 fold increases in p21Ras•GTP loading in response to EGF, IGF-1, or PDGF. This potentiation effect of insulin was also inhibited by the α-HXPA. We conclude that hyperinsulinemia increases the cellular pool of the plasma membrane-associated and farnesylated p21Ras available for activation by other growth factors. This increase creates a new background sufficient to elicit an exaggerated response to various circulating growth factors.

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PROTEIN KINASE Cs βII, δ and ζ MEDIATE INSULIN-INDUCED GLUCOSE TRANSPORT IN CULTURED SKELETAL MUSCLE.

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The purpose of this study was to clarify the role of protein kinase C (PKC) in the mediation of insulin-induced glucose transport in skeletal muscle. Studies were performed on primary cultures prepared from skeletal muscle of 1-2 day old rat pups. Glucose uptake was determined by measurements of the uptake of [^{3}H]-2-deoxy-glucose (2DG), and identification and translocation of glucose transporters and PKC isoforms from the cytosolic compartment to the plasma membrane were determined by western blotting. Skeletal muscle expresses GLUTs 1, 3 and 4, and PKC isoforms βII, δ, and ζ, as well as α and θ. Insulin causes a significant increase in 2DG uptake within 20-30 min and this is associated with translocation of GLUTs 3 and 4. Insulin also translocates PKC isoforms βII, δ, and ζ, but not α and θ. PKC activation by phorbol esters (TPA) increases 2DG uptake, and is associated with translocation of GLUTs 1 and 3, but not GLUT4. TPA translocates PKCδ but not the other PKC isoenzymes. PKC inhibitors (staurosporine, chelerythrine) increase basal 2DG uptake and block effects of both TPA and insulin. Inhibition of PI3 kinase by wortmannin (WM) does not alter basal 2DG uptake and inhibits effects of both insulin and TPA. In addition, WM blocks the insulin-induced translocation of GLUTs 3 and 4 as well as TPA-induced translocation of GLUTs 1 and 3, and prevents insulin-induced translocation of PKCs βII and ζ but not TPA-induced translocation of PKCδ. Thus, WM appears to inhibit both PI3 kinase activated by the insulin receptor cascade, as well as GLUT 1 and 3 translocation induced by translocation of PKCδ. We conclude that insulin-induced translocation of GLUTs3 and 4 is mediated via PKCs βII and ζ which are activated by PI3 kinase probably through the intracellular release of diacyl glycerol. (Supported by funds from the Sorrel Foundation, Harvett-Aviv Research Fund and the Ben and Effie Raber Research Fund. SRS is the incumbent of the Louis Fisher Chair in Cellular Pathology.)

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IS PHOSPHOLIPASE Cγ INVOLVED IN THE METABOLIC EFFECTS OF INSULIN IN 3T3-L1 ADIPOCYTES?

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The role of phospholipase C in insulin action has been debated for a long time. Early studies used metabolic labelling to examine phospholipid levels following insulin treatment. However, these studies would not reveal transient changes or localized increases. Previously, we have identified a novel autophosphorylation site in the B isoform insulin receptor (IR) which is the isoform present in insulin target tissues. We also found that this site allows the B isoform IR to interact with phospholipase Cγ (PLCγ). The present study was designed to investigate whether PLCγ might be involved in the metabolic actions of insulin in 3T3-L1 adipocytes. PLC hydrolyzes phosphatidylinositol-bisphosphate to diacylglycerol (DAG) and inositol triphosphate (IP3). Insulin caused a biphasic increase in DAG levels in 3T3-L1 adipocytes with a transient peak (180%) at 30 sec. followed by a second larger increase (230%) at 30 min. While we did not measure IP3 levels directly, insulin did not cause an elevation in intracellular Ca^{2+} levels measured by single cell FURA2 imaging. Microinjection of PLCγ SH2 domains blocked GLUT4 translocation in an immunofluorescent localization assay suggesting a role in stimulation of glucose uptake. A specific PLC inhibitor U73122 and its weakly active analogue U73343 were tested for their effect on insulin action in 3T3-L1 adipocytes. U73122, but not U73343, inhibits insulin-stimulated glucose transport in primary adipocytes. This inhibitor also blocks insulin-stimulated GLUT4 translocation and MAPKinase activation in a dose-dependent manner but has no effect on IR tyrosine kinase activity, IRS1-associated PI-3Kinase activity or p70-S6Kinase activation. Based on this data, we hypothesize that the B isoform IR initiates an alternative signaling pathway to glucose uptake through a transient interaction with PLCγ.

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SKELETAL MUSCLE GLYCOGENESIS AFTER EXERCISE IN TRANSGENIC MICE OVEREXPRESSING GLUT1 GLUCOSE TRANSPORTERS IN MUSCLE

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We have shown that skeletal muscle glycogen concentration in transgenic mice overexpressing GLUT1 is 10-fold higher than their littermates. This marked elevation of muscle glycogen concentration is associated with an increase in glucose transport activity. To examine the effect of increased glucose transport activity on the rate of muscle glycogenesis, both GLUT1 transgenic mice and their littermates underwent 3 h of swimming. Gastrocnemius muscle glycogen concentration (n=6) was determined before exercise, at 0, 5 and 24 hr post-exercise, during which food and water were provided. Exercise resulted in a 90% reduction of muscle glycogen in both controls (11.2±1.4 to 2.1±1.3 umol/g) and transgenic mice (99.3±4.7 to 11.8 umol/g). During recovery, the glycogen concentrations were 38.2±7.3 (5h post) and 40.5±2.8 (24h post) in controls and were 57.5±7.4 (5h post) and 152.1±15.7 (24h post) umol/g in transgenic mice respectively ($p<0.01$). Plasma insulin was lower in transgenics than in controls at all time points post exercise, while plasma glucose was lower only at 0 hr after exercise. There is no difference in glycogen synthase activity between control and transgenic mice ($p>0.05$). CONCLUSIONS: These results demonstrate that the rate of glycogenesis after exercise is increased in transgenic mice and the level of muscle glycogen is determined by muscle glucose transport activity.

OP 21

Insulin Secretion in Vitro: Secretagogue Recognition and Signal Transduction

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MITOCHONDRIAL ACTIVATION DIRECTLY TRIGGERS THE SECRETION OF INSULIN IN RAT PANCREATIC β-CELLS.

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The mitochondria of the β-cells are crucial in the generation of signals coupling glucose recognition to insulin secretion. The production of reducing equivalents by substrates in the mitochondria causes hyperpolarization of the mitochondrial membrane potential ($\Delta\psi_m$) permitting the rise in mitochondrial Ca^{2+} ($[Ca^{2+}]_m$) to the concentration range for activation of the NADH-generating dehydrogenases. The mitochondrial Ca^{2+} uptake is mediated by a uniporter and driven by the $\Delta\psi_m$. To elucidate the mechanism underlying the metabolism-secretion coupling in the β-cell we have monitored 1) the $\Delta\psi_m$ by Rhodamine-123 fluorescence, 2) the $[Ca^{2+}]_m$ by transient transfection of the Ca^{2+}-sensitive photoprotein aequorin targeted to the mitochondria, and 3) the insulin secretion by radioimmunoassay. Part of these experiments were performed in permeabilized rat islet cells with Staphylococcus α-toxin, permitting the study of cell-impermeant metabolic substrates such as the Krebs cycle intermediate succinate under conditions of clamped, saturating [ATP] (10 mmol/L) and stimulatory cytosolic $[Ca^{2+}]$ (800 nmol/L). In intact cells, 5 mmol/L of the cell-permeant methyl-ester form of succinate (met-Suc) caused a hyperpolarization of the $\Delta\psi_m$ and a transient peak of $[Ca^{2+}]_m$. These changes in mitochondrial metabolism were accompanied by a 4-fold stimulation of insulin secretion in perifused islet cells. In permeabilized cells kept in an intracellular-type buffer containing permissive $[Ca^{2+}]$ (800 nmol/L), 5 mmol/L succinate induced a rapid hyperpolarization of the $\Delta\psi_m$ and a 4-fold stimulation of insulin secretion in perifused cells. These results provide evidence that activation of mitochondrial metabolism in rat pancreatic islet cells generates factors other than Ca^{2+} and ATP which are capable of triggering insulin secretion.

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ATP-DEPENDENCE OF EXOCYTOSIS IN MOUSE PANCREATIC β-CELLS

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Cytoplasmic ATP, via closure of the ATP-sensitive K-channels, plays a central regulatory role in glucose-stimulated insulin secretion. However, there is also evidence that ATP interacts with the insulin secretory process at more distal stages. Here we have explored the effects of cytoplasmic ATP on Ca^{2+}-induced exocytosis in individual mouse pancreatic β-cells by a combination of the patch-clamp technique, capacitance measurements, microfluorimetry of the cytoplasmic Ca^{2+}-concentration ($[Ca^{2+}]_i$) and flash photolysis of caged ATP and Ca^{2+}. The standard whole-cell configuration was used and all intracellular media were supplemented with 0.1 mM cAMP. The steady-state rate of capacitance increase observed at a $[Ca^{2+}]_i$ of 2 μM amounted to 20±4 fF/s in the presence of 3 mM ATP. Replacement of ATP with the stable analogue AMP-PCP resulted in 80% reduction of exocytosis. The latter effects could be mimicked by inclusion of 5 mM ADP in the pipette solution in the continued presence of 3 mM ATP. In experiments utilizing photorelease of caged ATP, exocytosis elicited by 170 nM $[Ca^{2+}]_i$ increased (after a delay of 11 s), from an initial 1.7±0.7 fF/s to 5.1±0.8 fF/s after appliciation of ATP ($P<0.001$; n=12).In the presence of intracellular ATP, elevation of $[Ca^{2+}]_i$ by photorelease from Ca/NP-EGTA produced a biphasic stimulation of exocytosis consisting of a rapid (<200 ms) first phase and a protracted (≥10 s) second phase. Whereas the initial phase (limited to 100 fF which corresponds to the release of 50 secretory granules) was resistant to replacement with AMP-PCP and NEM (an inhibitor of NSF), the late phase was abolished by either of these maneouvres. We interpret the biphasic stimulation of exocytosis as the release of granules from two different pools: a readily releasable pool, situated in the close vicintiy of the release site, and a reserve pool located further away from the membrane.Whereas the replenishment of the readily releasable pool requires 10 s and ATP-hydrolysis, exocytosis of granules already sitting in the readily releasable pool is rapid and proceed in an ATP-independent fashion. The small exocytotic response obtained after suppression of granule mobilization finally suggests that the pool of release-competent granules in the β-cell is <1% of the total population of granules.

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MUTIPLE EXOCYTOTIC PATHWAYS REVEALED BY AMPEROMETRIC AND CAPACITANCE MEASUREMENT IN PANCREATIC β-CELLS.

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Precise time- and Ca^{2+}-dependence of exocytosis from single cultured mouse β-cells were investigated using membrane capacitance measurement and amperometric detection of vesicular contents. Serotonin was pre-loaded into large dense-core vesicles for the amperometry. Exocytosis was induced with rapid elevation of cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_i$) using caged-Ca^{2+} compounds. We revealed two major components in increase of membrane capacitance, reflecting exocytosis, only the slow one of which was associated with serotonin secretion, indicating that the slow component represent exocytosis of the large dense-core vesicles. Consistent with this idea, the fast (~100 ms) and slow (100ms~) exocytoses induced the characteristic endocytoses that often followed exocytoses of small-clear and large dense-core vesicles, respectively. The two exocytotic pathways exhibited similar Ca^{2+} dependencies; the half-maximal rates of secretion were achieved at 18-26 μM in $[Ca^{2+}]_i$, suggesting physiological involvement of both pathways. Interestingly, the slow exocytosis was further decomposed into two phases. Serotonin secretion was associated with both of the two phases in 30% of cells, while only with the slower phase in the rest of the cells. These suggest the presence of two types of large dense-core vesicles with distinct time constants of exocytosis. Our data for β-cells provide a new theoretical basis for mechanisms by which the biphasic insulin secretion takes place, and emphasize the existence of multiple exocytotic pathways with divergent fusion kinetics in endocrine cells

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GLUCOKINASE OVEREXPRESSION INDUCES GLUCOSE REGULATION OF INSULIN PRODUCTION AND STIMULATION OF MITOGEN ACTIVATED PROTEIN KINASE.

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Glucokinase (GK) is suggested to play a crucial role in the glucose sensing ability of pancreatic islets. Distal events to glucose phosphorylation have still to be elucidated. In the present study we evaluate the role of GK in mediating the glucose sensing ability of the rat insulin promoter-1 (RIP) activity in RIN1046-38 (RIN-38) cells, and make a first attempt to figure out whether this effect is mediated by activation of mitogen activated protein kinase (MAPK). Beta-cell type GK gene as well as the RIP-CAT (the reporter gene chloramphenicol acetyl-transferase) and the control CMV-CAT and CMV- luciferase (cytomegalovirus promoter, not affected by glucose) were introduced by adenoviral infection as a gene transfer tool. We found out that RIP activity in RIN-38 cells of passage 19 is glucose regulated, insulin promoter activity is 2-3 fold higher in 20mM glucose as compared to 0.2mM glucose (21.89±0.8 vs.7.88±0.5 $p<10^{-4}_{M.W.}$). The promoter activity gradually decreased with time in culture and its glucose regulation was lost together with specific dramatic decrease in GK gene expression and enzymatic activity. RIN-38 cells of passage 30 and 52 exhibited RIP activity levels which were 2 and 5 fold lower, respectively, as compared to cells at passage 19. Overexpression of GK in RIN-38 increased total glucose phosphorylating activity 4 fold (3.3±0.78 to 11.55±1.1 u/mg prot.) GK activity increased 5 fold (1.67±0.094 to 8.25±1.8 u/mg prot.), glucose regulation of insulin promoter activity increased 2-3 fold. 20mM glucose caused a several fold increase in endogenous activated MAPK levels in RIN-38 cells of passage 30 (by immunobloting with antibody which recognizes activated MAPK, Promega) overexpression of GK further increased the activated MAPK levels. **In conclusion,** GK plays an important role in induction of glucose sensing ability of insulin production. The glucose signaling pathway may be mediated via activation of MAPK, the effect of specific inhibitors of MAPK activity on glucose regulation of insulin promoter activity should supply important information on the signaling pathway leading to glucose regulation of insulin production and secretion in pancreatic islets.

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CHARACTERIZATION OF THE DISTAL REGION OF THE β-CELL PROMOTER OF THE RAT GLUCOKINASE GENE

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Previous studies on the promoter of the β-cell specific transcription unit of the rat glucokinase gene have shown the involvement of at least two distinct promoter regions in the transcriptional regulation. Whereas the proximal promoter region up to nucleotide -300 bp, contains *cis*-elements which are necessary for transcription in β-cells, the distal promoter region, at least up to nucleotide -609 bp, allows maximal transcriptional activity in HIT M2.2.2 cells, but seems to exhibit no cell type specific properties.

In order to characterize the distal promoter region in more detail, we performed 5'- and 3'-deletion ana-lysis and *in vitro* as well as *in vivo* DNA-protein binding studies to delineate *cis*-elements contributing to transcriptional control. Deletional analysis revealed that both positive and negative acting *cis*-elements contribute to the action of the distal promoter region. Positive acting elements have been mapped between -277 bp/-206 bp and -609 bp/-63 bp. Due to the combination of 5'- and 3'-deletion studies

the localization of the negative acting *cis*-element could be determined between -373 bp and -359 bp. This region contains a perfect N-box motif, which has been described as a binding site for the negative acting factor HES-1. The minimal -609/-n fragment which exhibits a positive effect on reporter gene expression was -609/-463. This fragment also conferred positive transcriptional activity in an orientation dependent manner when fused to a heterologous minimal promoter. The positive action of this fragment was observed in insulin-producing HIT M2.2.2 and non-insulin-producing BHK 21 cells. DNase I footprinting analysis of the region -609/-463 bp revealed the same protected region between nucleotides -562 to -516, when using nuclear extracts prepared from HIT M2.2.2 and BHK 21. This protected region which is highly enriched in AT-bases, is also involved in protein binding *in vivo*. By performing a MAR-assay we could show that the AT-rich region of the distal promoter part does not act as a Matrix Attachment Region. This region contains putative *cis*-elements, which show high homology to consensus motifs reported for the transcriptional factors Pit 1, NFMR 1, HINFA, HNF 1, HNF 3, BRN 2 and HSFA.

Site directed mutagenesis of the putative cis-elements within the distal promter part and DNA/protein interaction studies are in progress to study the contribution of the above mentioned transcription factors in the transcriptional control of the rat glucokinase gene in insulin-producing cells.

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GLUCOSE CAUSES A COORDINATED INDUCTION OF GLYCOLYTIC AND LIPOGENIC GENES IN ß(INS-1) CELLS

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Long term exposure ot the ß-cell to elevated glucose causes a high rate of insulin secretion at low glucose, ß-cell hypertrophy and hyperplasia. These actions of glucose are expected to be associated with changes in the expression level of a number of genes which must be identified. The exposure of ß(INS) cells from 5 to 25mM glucose for 3 days induced phosphofructokinase-1(isoforms C, M and L), glyceraldehyde 3-phosphate dehydrogenase, L-pyruvate kinase, acetyl-CoA carboxylase and fatty acid acid synthase proteins, whereas pyruvate dehydrogenase (subunits E1alfa and E3) malic enzyme, pyruvate carboxylase, ATP citrate lyase and carnitine palmitoyl-transferase I enzymatic activities remained constant. Similar patterns were observed at the mRNA level. These changes in metabolic genes expression were associated with an increased ß-cell metabolic activity (indicated by the reduction of the artificial electron acceptor MTT) at low glucose and a marked glycogen deposition which was readily mobilized upon lowering of the ambient glucose. Chronically elevated concentrations of citrate, malate and malonyl-CoA with an associated sustained inhibition of fatty acid oxidation and exaggerated fatty acid esterification as well as triglyceride deposition also ocured. The data provide a possible mechanism for the ß-cell hypersensitivity to glucose (also named "glucose toxicity") caused by the chronic exposure of the ß-cell to elevated glucose. It is proposed that a coordinated induction of metabolic genes by glucose causes pleiotropic alterations in ß-cell metabolism (glycogen and triglyceride deposition, glycolysis, Kreb's cycle activity, anaplerosis, fatty acid oxidation and esterification) resulting in the exaggerated production at low glucose of metabolic coupling factors regulating insulin secretion.

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DIFFERENTIAL ROLES OF THE RHO SUBFAMILY OF GTP-BINDING PROTEINS IN GLUCOSE- AND CALCIUM-INDUCED INSULIN SECRETION.
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We utilized clostridial toxins (with known specificities for modification of the rho subfamily of G-proteins) to ascertain the contribution of candidate GTP-binding proteins in physiologic insulin secretion from β cells. Exposure of normal rat islets or HIT-T15 cells to *Clostridium difficile* toxin A or B resulted in the glucosylation of Cdc42, rac and rho, accompanied by inhibition of glucose (>70%)- or potassium (>60%)-induced insulin secretion. *Clostridium sordellii* lethal toxin (LT) specifically glucosylated rac2 and rap1, but, like toxins A or B, attenuated glucose (-57%)- or potassium (-58%) induced insulin secretion. In contrast, *Clostridial* C3-exoenzyme (which fully ADP-ribosylated rho) did not affect either glucose or potassium-induced secretion. These data suggest that Cdc42, rac, and/or rap (but not rho) may be needed for glucose- or calcium-induced secretion. These toxin effects appear to be specific for relatively late steps in stimulus-secretion coupling, since no differences in metabolic viability were demonstrable between control or toxin -treated cells. Also, toxin-treatment did not alter glucose- or potassium-mediated rises in $[Ca^{2+}]_i$, suggesting that these G-proteins are involved in steps distal to elevations in $[Ca^{2+}]_i$. In intact cells, the carboxyl methylation of Cdc42 was stimulated by glucose, whereas that of rap and rac was regulated by glucose or potassium. Together, these findings provide direct evidence, for the first time, that rho subfamily of G-proteins play key, differential regulatory roles in insulin secretion. Furthermore, they suggest that Cdc42 may be required for early steps in glucose stimulation of insulin release (probably phospholipase activation), whereas rap and rac may be required for a later, calcium-dependent step(s) in insulin exocytosis.

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EFFECT OF BOTULINUM NEUROTOXIN C1 ON EXOCYTOSIS OF INSULIN AND SNARE-PROTEIN CLEAVAGE IN PANCREATIC β-CELLS

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The botulinum neurotoxin C1 cleaves the membrane proteins syntaxin 1,2 and 3 and thereby inhibits neurotransmission and neuroendocrine secretion. We investigated the effect of botulinum neurotoxin C1 (BoNT C1) on syntaxin 1 (*syx*) cleavage and insulin secretion/exocytosis in pancreatic β-cells to gain insight into the function of syntaxin. As the toxin cannot enter intact β-cells, cells were first permeabilized with streptolysin-O (SLO). Under those conditions recombinant BoNT C1 (3-100 nM) cleaves *syx* 1 in HIT-T15, INS-1 and primary islet cells. The levels of SNAP-25 or VAMP, known targets of BoNT A/E and tetanus toxin, remain unchanged. The cleavage of *syx* by BoNT C1 is accompanied by a progressive loss of Ca^{2+}-stimulated exocytosis (10 μM Ca^{2+}) in all three cell types without alterations in basal levels of insulin release (0.1 μM Ca^{2+}). GTPγS-induced exocytosis, which is calcium-independent, was not reduced by BoNT C1 in INS-1 or primary islet cells and was toxin-sensitive only in HIT-T15 cells. In contrast, BoNT E inhibited Ca^{2+} and GTPγS-stimulated exocytosis in all three cell types. To test whether BoNT C1 cleavage of *syx* interferes with different stimuli of insulin release in intact cells, we transiently expressed the light chain of BoNT C1 (BoNT C1 LC) together with human preproinsulin in hamster HIT-T15 cells and measured human C-peptide release as a marker of insulin release from transfected cells. Under those conditions BoNT C1 LC abolished secretion evoked by KCl, forskolin, glucose and forskolin or by the phorbol ester PMA. *Syx* cleavage and light chain expression was verified by transient cotransfection of BoNT C1 LC together with the green fluorescent protein GFP(S65T) and subsequent fluorescent activated cell sorting of the transfected cells. In conclusion are data demonstrate that (i) syntaxin is required for Ca^{2+}-, but not for GTPγS-dependent exocytosis of insulin and that (ii) β-cell impermeable neurotoxins can successfully be expressed in insulin-secreting cells, which will provide a valuable tool for further investigation of secretory mechanisms.

OP 22

Low Birthweight, Gestational Diabetes, Pregnancy

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LOW BIRTHWEIGHT AND GLUCOSE INTOLERANCE: A ROLE FOR THE SYMPATHETIC NERVOUS SYSTEM?

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We have examined the hypothesis that persisting sympathetic nervous system (SNS) activity determined *in utero* could be one of the processes explaining the link between reduced fetal growth and raised blood pressure and glucose intolerance in adult life. We tested this by examining the relation between birth size and resting pulse rate, an index of SNS activity, in 449 men and women born in Preston, Lancashire, aged 46-54 years whose birth records have survived. Subjects were visited at home and resting heart rate recorded with an automated recorder. Resting pulse ranged from 44-108 (mean 73) beats per minute. It rose with increasing body mass index ($r=0.14$, $p=0.003$) and waist to hip ratio (adjusted for sex $r=0.10$, $p=0.003$) and correlated significantly with systolic and diastolic blood pressures ($p=0.001$), fasting glucose ($p=0.02$), split proinsulin ($p=0.001$) and triglyceride concentrations ($p=0.02$). Pulse rate fell progressively from 76/min among subjects who weighed 2.5kg or less at birth to 71/min among those who weighed 3.3kg or more ($p=0.01$). The association was independent of current body mass index, smoking habit, alcohol consumption and social class. These findings suggest that elevated SNS activity established *in utero* may be a mechanism linking small size at birth with raised blood pressure and glucose intolerance in adult life.

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LOW BIRTHWEIGHT, GLUCOSE INTOLERANCE AND FAMILY HISTORY OF DIABETES IN SWEDISH MIDDLE-AGED MEN.

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Low birthweight has been suggested as a potential risk factor for non-insulin-dependent diabetes (NIDDM). This could be due to the effect of fetal malnutrition. However, the impact of other possible factors influencing birthweight and glucose tolerance, such as family history of diabetes, particularly diabetes in the mother, and socio-economic status, have not been elucidated in detail. The aim of this study was to investigate the association between low birthweight and glucose intolerance taking into account family history of diabetes and socio-economic status. We performed a cross-sectional study of 3129 Swedish men in the age 35-56 years of whom 52% had a strong family history of diabetes (at least one first or two second degree relatives with diabetes). Information on birthweight and fathers' socio-economic status were obtained by questionnaire from 2237 (71%) of these men. Oral glucose tolerance testing detected 102 cases of impaired glucose tolerance (IGT) and 35 cases of NIDDM. After adjustment for family history of diabetes and body mass index the relative risk of NIDDM associated with low (≤3000 g) birthweight was 3.8 (95% confidence interval (CI) =1.7-8.4). Correspondingly, the relative risk of IGT and high 2-h glucose levels within the normal interval (5.8-7.7 mM) was 1.5 (95% CI=0.9-2.6) and 1.6 (95% CI=1.1-2.2). Men with low birthweight (≤3500g) and family history of diabetes had 8.1 times the risk of having NIDDM (95% CI=2.4-27.3). For IGT and high 2-h glucose levels within the normal interval, corresponding estimates were 2.8 (95% CI=1.5-5.1) and 2.0 (95% CI=1.4-2.8). Controlling for fathers' socio-economic status and diabetes in the mother left the results virtually the same as those already presented. We conclude that there is an association between low birthweight and glucose intolerance when family history of diabetes, diabetes in the mother and fathers socio-economic status are taken into account. Furthermore, family history of diabetes and low birthweight synergistically increase the risk of NIDDM.

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ASSOCIATION OF LOW BIRTH WEIGHT WITH REDUCED BETA-CELL FUNCTION

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Reduced growth in fetal and neonatal life has been linked with an increased risk of developing impaired glucose tolerance and type 2 diabetes in adult life. The pathophysiological basis of the association between impaired intrauterine growth and type 2 diabetes has been uncertain. The aim of this study was to define the relationship between birth weight and Beta-cell function in the first degree relatives of type 2 diabetic subjects. The study was cross sectional, and involved 101 Caucasian adults of known birth weight from 47 families which had at least one member with type 2 diabetes. The subjects were of mean age 43 ± 7 years (± 1 SD). Glucose tolerance was measured with the 5 mg.kg ideal body weight/min continuous infusion glucose tolerance test. Beta-cell function and insulin sensitivity were calculated from the fasting glucose and insulin concentrations with Homeostasis Model Assessment. Beta-cell function was standardised to allow for the confounding effects of age and obesity. Birth weight was expressed as a centile taking into account gestational age, gender, birth order, and maternal height. Twenty-seven of the subjects had type 2 diabetes, 32 had impaired glucose tolerance, and 42 were normoglycaemic. Birth weight correlated with the Beta-cell function in the complete cohort (r_s=0.29, p=0.005), in the type 2 diabetic subjects (r_s=0.50, p=0.023) and in the 74 nondiabetic subjects (r_s=0.29, p=0.013). There was no significant correlation between birth weight and insulin sensitivity. The diabetic (n=27) and nondiabetic (n=74) subjects had similar median (interquartile range) centile birth weight: 50% (19%-91%) and 53% (30%-75%) respectively. However the diabetic subjects had significantly lower Beta-cell function: 69% (48%-83%) vs 97% (86%-120%) p<0.001. The cause of the association between low birth weight and reduced Beta-cell function in adult life is uncertain. Nutritional factors determining fetal and infant growth may influence the size or vascularity of the adult pancreatic Beta-cell complement. Alternatively, lower birth weight may result from the phenotypic expression of genetic Beta-cell defects associated with reduced fetal insulin secretion and reduced anabolic effect in utero. The marked impairment of Beta-cell function in the type 2 diabetic subjects could not be accounted for by low birth weight alone, and additional genetic and environmental factors are likely to be necessary for the development of type 2 diabetes.

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RELATIONSHIP OF MATERNAL BIRTH WEIGHT TO ORAL GLUCOSE TOLERANCE, INSULIN RESISTANCE AND NEONATAL WEIGHT IN NON-DIABETIC MOTHERS.

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According to the hypothesis of *'thrifty phenotype'* a low birth weight is associated to the appearance of non-insulin-dependent diabetes mellitus, as well as of other clinical correlates of the insulin resistance syndrome in the adult life. Among known determinants of birth weight there is the status of glucose tolerance during the pregnancy either in mothers with gestational diabetes or in non diabetic women. Whether maternal birth weight could be in some way related to the weight of their neonates as well as to both glucose tolerance and insulin response after an oral glucose load, are the questions raised by this study. Both glucose and insulin plasma levels were evaluated basely, at 60 min and at 120 min after an oral 100 g-glucose load in a group of 96 non-diabetic pregnant women, between the 24th and the 28th gestational week. Neonatal weight was, on average, significantly higher in the group of the highest quartile of maternal birth weight (n=23; 3530±701(SD)g), as compared to the mean value of the women of the lowest quartile (n=29; 3103±661g; p=.03). This difference remained significant even after adjusting for gestational age, neonatal sex, maternal height or maternal caloric intake (p=.039). No significant differences were observed in both maternal and insulin area under the curve across the quartiles of maternal birth weight. The only further variable significantly associated to maternal birth weight was mean blood pressure recorded just before the oral glucose test, significantly increased in the highest quartile of maternal birth weight. (91±6 mmHg in the highest vs 87±7 mmHg in the lowest; p=.03). In conclusion these results demonstrate that a higher maternal birth weight predicts the delivery of fatter babies, being, moreover, associated with a higher mean blood pressure duiring the pregnancy, while no relationship is shown between maternal birth weight and glucose tolerance or insulin levels after an oral glucose load in non diabetic pregnant women.

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IMPACT OF GESTATIONAL DIABETES ON INSULIN SECRETION, INSULIN SENSITIVITY AND GLUCOSE EFFECTIVENESS IN AFRICAN AMERICANS WITH FAMILY HISTORY OF NIDDM : K.Osei, and T. Gaillard, D.Schuster, The Ohio State University, Columbus, Ohio, USA.

Prior history of gestational diabetes mellitus(GDM) is an important risk factor for NIDDM. Nevertheless, the metabolic characteristics of African Americans (AA) with former GDM are unknown. In addition, whether a positive family history of NIDDM impact adversely on the putative metabolic defects in AA with former GDM is uncertain. We have measured, beta cell function, insulin sensitivity index(Si) and glucose - dependent glucose disposal(Sg) in 15 healthy, normal glucose tolerant former GDM females (mean±sem,38.5 ±2.0yrs) who were first degree relatives of AA patients with NIDDM and had average of 7 years after the index pregnancy. Their data were compared with those of 35 age -, weight -, BMI - and waist - hip ratio- matched healthy first degree AA females (mean age 43.0 ±1.8 yrs) but without prior history of GDM(CONT). Standard oral glucose tolerance test(OGTT) and insulin - modified, FSIGT were performed in each subject. Insulin sensitivity index(Si) and glucose effectiveness (Sg) were determined by the minimal model method. The mean fasting and postprandial (PP) serum glucose(G) levels were slightly greater but not significantly different in the GDM vs. CONT. While mean fasting serum insulin(INS) was no different, INS levels at 30-90 mins were significantly lower in former GDM vs. CONT. However, the mean fasting and PP serum c-peptide(C-P) were not different in the GDM vs. CONT. Following intravenous glucose load, serum G levels were not significantly different in the former GDM vs. CONT. Mean acute first phase insulin, but not C-P release, was blunted in the former GDM vs. CONT. Mean Si (1.87± 0.47 vs. 2.87± 0.35x10^{-4}. min^{-1}/uU/ ml^{-1}, p<0.05) and Sg (2.11 ± 0.15 vs 3.25 0.54 x 10^{-2}.$mins^{-1}$ p<0.05) were significantly lower in the post GDM than CONT. **Conclusion:** Our study demonstrates that defects in beta cell secretion, insulin clearance, Si and Sg persist 7 years after the index pregnancy in glucose - tolerant, first degree relatives of AA diabetic patients with former GDM. Thus,GDM confers greater metabolic derangement beyond that attributed to family history of diabetes alone and could partly explain the higher propensity for NIDDM in AA with former GDM.

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GESTATIONAL DIABETES MELLITUS: DIFFERENT OUTCOME IN WOMEN WITH ISLET CELL ANTIBODIES

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Gestational diabetes (GD) is an heterogenous entity, some women displaying positivity for islet cell antibodies (ICA). We aim to assess if glucose tolerance at follow-up differs in GD women with ICA. We have followed a cohort of 183 GD women for a mean of 7.34 ± 2.8 years. ICA were determined during pregnancy by indirect immunofluorescence with prolonged incubation: 22 women were ICA+ and 161 were ICA-. According to WHO criteria 17 have developed diabetes mellitus (DM) and 18 impaired glucose tolerance (IGT). Mean survival time for the whole cohort to develop IGT or DM is 14.75 years (95% CI 11.63-17.87). Despite a mean survival time of 14.46 years (95% CI 11.03-17.88) for ICA- and of 15.48 years (95% CI 11.51-19.45) for ICA+ GD women, the Breslow and Tarone-Ware statistics are significant (p<0.05) for a worse outcome in ICA+ women due to a faster deterioration in this group. We conclude that ICA+ GD women have an earlier deterioration of glucose tolerance than their ICA- counterparts.

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IS THERE A THRESHOLD OF HYPERGLYCEMIA FOR INCREASED RISK OF CONGENITAL MALFORMATIONS IN DIABETIC PREGNANCIES?

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Several studies in humans have shown that first trimester hyperglycemia is associated with an increased rate of malformations (MF): the higher the hemoglobin A1c (HbA1c), the higher the risk of MF. However, a threshold of HbA1c, under which the MF rate is not increased, is unknown. We studied 540 consecutive pregnancies of 405 IDDM patients during an 8-year period (from 1988 to 1995). The mean (range) maternal age was 29.6 years (17-44). HbA1c was measured at least once before the end of the 15th week of pregnancy in 94 % of the patients by a HPLC method with a mean (SD) of 5.0 % (0.32) in healthy non-diabetic subjects. There were 26 cases (4.7 %) with major MF and 36 cases (6.5 %) with minor MF among the 552 offspring of the 540 pregnancies. There were 10 twin pregnancies and 1 triplet pregnancy. A malformation was considered major when it was lethal or needed major surgical correction. Four cases with severe MF had pregnancy interruption between 16 and 20 weeks of gestation after ultrasound diagnosis. These 4 cases were included in the study group. The Table shows the frequencies of major and minor MF at different levels of HbA1c in early pregnancy. Only 6.5 % of the diabetics had a HbA1c value within the non-diabetic range. They had the lowest MF rate, which is roughly the same as in the general population. The diabetics with the highest HbA1c levels had the highest MF rate (Table). Our results suggest that only diabetics with HbA1c levels within normal limits in early pregnancy do not have an increased risk of MF.

HbA1c		N	Major MF		Minor MF		Total MF	
%	SD-units		N	%	N	%	N	%
>9.5	>14.0	50	4	8.0	4	8.0	8	16.0
7.6-9.5	8.1-14.0	197	8	4.1	14	7.1	22	11.2
5.6-7.5	2.1-8.0	234	12	5.1	15	6.4	27	11.5
<5.6	<2.1	36	1	2.8	1	2.8	2	5.6
Unknown		35	1	2.9	2	5.7	3	8.6

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POLYCYSTIC OVARY SYNDROME (PCOS) IN URBAN INDIAN WOMEN: ASSOCIATION WITH INSULIN RESISTANCE AND PARENTAL HYPERGLYCEMIA.

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We studied 55 women with PCOS and their parents for glucose tolerance (WHO 1985), plasma immunoreactive insulin (IRI) & lipids, blood pressure and anthropometry. The PCOS women were 22 y old (mean), 11 were obese (BMI>25 kg/m^2), 13 (24%) had abnormal glucose tolerance [4 diabetic (3 obese), 9 IGT (4 obese)]. Fasting IRI was significantly higher in the PCOS women compared to controls [149 vs 90 pmol/l, median, p<0.001]. Fasting IRI correlated with BMI (p<0.001), WHR (p<0.001), blood pressure (p=0.029), triglycerides (p<0.001) and luteinising hormone (p=0.036). Thirty six of sixty four (56%) parents studied were hyperglycaemic[27 diabetic (13 fathers,14 mothers); 9 IGT (2 fathers, 7 mothers)]. In 27 PCOS girls (50%) either or both parents were hyperglycaemic. Thus, Indian PCOS women suffer metabolic syndrome even though a large proportion are non-obese. High prevalence of parental hyperglycaemia suggests that the insulin resistance in PCOS may be familial.

OP 23

New Approaches in the Management of NIDDM

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Comparative effects of A-4166, sulfonylureas and repaglinide on meal induced glucose excursions in normal rats.

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The ability of A-4166, a rapid onset/short acting insulin secretagogue, to augment the early insulin response and thereby curb prandial glucose excursions during a meal was tested and compared to glibenclamide (GB) (slow onset/long acting), glipizide (GP) (rapid onset/short acting) and repaglinide (RP) (rapid onset/short acting). Chronically catheterized male SD rats acclimated to a meal feeding regime were administered the compound orally 10 min. before a 30 min. meal. Blood samples were obtained via the jugular cannula at -10, 0, 1, 3, 5, 10, 20, 30, 45, 60, 90 and 120 min. after commencement of feeding. Food consumed during the meal was unaffected by the compounds. In the controls, the 30 min. meal resulted in an increase in plasma glucose and insulin with peak levels occurring at 15 and 20 min. respectively. GB did not augment the early insulin response ($\Delta AUC_{-15 \text{ to } 3 \text{ min}}$) and glucose excursions paralleled those in the control. Total insulin release during the 120 min. test period ($\Delta AUC_{-15 \text{ to } 120 \text{ min.}}$) was not significantly higher (5±0.7 *v/s* 7±1.4 mU•min/ml, control and GB) but postprandial glucose levels were 30 mg lower than in controls. Conversely, A-4166 (60 and 120 mg/kg), GP (1.0 mg/kg) and RP (0.2 mg/kg) all augmented the acute insulin secretory response while glucose excursions during the meal ($\Delta AUC_{-15 \text{ to } 30 \text{ min}}$) were eliminated (814±91, 11±177, -415±106, 13±202 and -122±203 mg•min/dl for controls, A-4166 at 60 and 120 mg, GP and RP respectively). Total insulin release and postprandial glucose levels in the A-4166 treated groups were comparable to the controls. Conversely, GP and RP resulted in greater total insulin release and postprandial glucose levels were 33-34 mg lower that in the controls. A lower dose of RP (0.02 mg/kg) resulted in total insulin release comparable to the higher dose of A-4166 (10±2.3 and 10.1±1.6 mU•min/ml respectively). However, this lower dose of RP failed to augment the early insulin secretory response and eliminate the glucose excursion during the meal but postprandial glucose levels tended to be lower than in the controls albeit not significantly (-5±6.1 and 11±2.8 mg/dl respectively). In conclusion, the rapid onset of A-4166 allows for an augmentation of the early insulin response and an elimination of prandial glucose excursions while its extremely short duration of action prevents excessive insulin secretion and postprandial hypoglycemia in normal rats.

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GLP-1 tablet in NIDDM

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For establishing GLP-1 as a therapeutic regimen in diabetes, another route of administration would be advantageous. We showed that in healthy volunteers, a buccal tablet containing 400 µg of GLP-1 delivered potential therapeutic levels of the peptide. Furthermore, it reduced fasting glycaemia and increased plasma insulin. Now we have used that delivery system for studying the effect of GLP-1 in patients with NIDDM and poor metabolic control. In addition, we have evaluated the effectiveness of the insulinotropic effect versus the gastro-intestinal effect in the lowering of glycaemia. Ten NIDDM patients received a single tablet under fasting conditions and before a standard meal in this randomized, placebo controlled study. The mean peak GLP-1 concentration was 139.4±17 pmol/L and occurred 30 min following application. The mean placebo adjusted AUC was 5940±764 min•pmol/L consistent with a relative bio-availability of 6% vs i.v. injection and 46% vs s.c. injection. Half-life of total peptide activity after buccal administration was 17 min. Placebo adjusted glucose concentrations decreased by 1.4±0.4 mmol/L in fasting experiments and by 4.7±0.7 mmol/L after a standard mixed meal. In fasting state, plasma insulin increased by 185% and glucagon decreased by 20% consistently with the increase in plasma GLP-1 concentrations. The peptide exerted a significant insulinotropic effect during meal (calculated as an insulinogenic index) 93.1±27 vs 45.7±19 in placebo experiments. The calculated gastro-intestinal effect of the peptide (gastric emptying etc.) was 70% compared to 30% effect on pancreatic islets (insulin, glucagon, etc.). **We conclude** that potential therapeutic plasma levels of GLP-1 were achieved after administration of a single buccal tablet in NIDDM patients. The peptide had marked glucose lowering effect mostly by inhibiting gastric emptying. This new alternative treatment form may therefore be feasible in the future for NIDDM, although a more prolonged pharmaco-kinetic profile is required.

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INFLUENCE OF GLUCAGON-LIKE PEPTIDE 1 ON FASTING GLYCAEMIA IN NIDDM PATIENTS TREATED BY INSULIN (SULFONYLUREA FAILURE)

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Glucagon-like peptide 1 (GLP-1) has glucose-dependent insulinotropic and glucagonostatic actions in NIDDM patients on diet and on oral agents. It is not known, whether after secondary sulfonylurea failure GLP-1 is still effective. Therefore, 10 NIDDM patients (6 female, 4 male; 65 ± 10 years, BMI 30.4 ± 5.1 kg/m^2; HbA_{1c} 8.2 ± 1.5 %; 6 ± 3 years, range 2-13 years, after starting insulin treatment) were examined in the fasting state after discontinuing NPH insulin on the evening before the two study days. GLP-1 [7-36 amide] (1.2 pmol·kg^{-1}·min^{-1}) or placebo (NaCl with 1 % HSA) were infused over 6 h. Plasma glucoe (glucose oxidase) insulin (IMx), and C-peptide (ELISA) were measured. Statistics: repeated measures analysis of variance. Fasting plasma glucose was 9.4 ± 0.7 mmol/l, and was reduced by GLP-1 to 5.4 ± 0.4 (range 3.9 - 7.3) mmol/l (placebo: 8.2 ± 0.7 mmol/l; $p < 0.0001$). GLP-1 transiently increased insulin (from 114 ± 31 to 222 ± 65 pmol/l at 150 min; $p < 0.0001$) and C-peptide (from 1.00 ± 0.12 to 1.90 ± 0.23 nmol/l at 120 min; $p < 0.0001$) with no effect of placebo. After normalization of plasma glucose, insulin and C-peptide concentrations became lower again during the ongoing administration of exogenous GLP-1. It is concluded that exogenous GLP-1 effectively lowers plasma glucose concentrations also in advanced NIDDM (long after sulfonylurea secondary failure). These findings may broaden the applicability of GLP-1-derived drugs as a new treatment to nearly all NIDDM patients.

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THE AMYLIN ANALOGUE PRAMLINTIDE DECREASES POSTPRANDIAL PLASMA GLUCOSE AND GLUCAGON IN IDDM PATIENTS.

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Previous reports have suggested that administration of the amylin analogue pramlintide to IDDM subjects improves glycemic control. To further explore the effects of treatment with pramlintide (Pr) vs placebo (Pl) on glucose control and postprandial responses of glucose, glucagon and various metabolites following standardised meals in IDDM patients, we examined 13 male IDDM patients in a double-blind, placebo controlled, cross-over study. Patients were treated with Pr (120 μg/day, 30 μg qid) or Pl for 4 weeks. On the last day of this period, a diurnal profile (08.00-16.30 h) was performed, focusing on the postprandial response to a carbohydrate rich breakfast. Mean serum insulin (49.5±6.5 vs 46.6±5.0 pmol/l) on the diurnal day were comparable between treatment periods, however, mean plasma glucose was lower after Pr (8.4±0.7 vs 10.2±0.8 mmol/l, $0.05 < p < 0.10$). Furthermore, after Pr treatment, blood glycerol was lower (0.030± 0.003 vs 0.039±0.005 mmol/l, $p < 0.05$) and blood alanine higher (0.28±0.01 vs 0.25±0.01 mmol/l, $p < 0.05$), whereas no significant differences were observed in serum NEFA (0.26±0.02 vs 0.31±0.03 mmol/l) or blood lactate (0.73±0.03 vs 0.71±0.05 mmol/l)(Pr vs Pl). Following the carbohydrate rich breakfast, mean plasma glucose (9.3±1.0 vs 12.5±1.0 mmol/l) and glucagon (49.4±6.6 vs 65.4 ±7.5 ng/l) were lower after Pr vs Pl ($p < 0.05$), and area under the curve for both plasma glucose and glucagon were reduced by 26 % ($p < 0.05$). In conclusion, treatment of 13 male IDDM subjects with pramlintide (30 μg qid) for 4 weeks resulted in reductions in plasma glucose and glucagon as well as blood glycerol following a carbohydrate rich breakfast, while serum insulin remained constant. These observations suggest, that Pr mediated suppression of glucagon secretion may contribute to the reduction in postprandial hyperglycaemia observed during Pr treatment. Longer term studies are required to ascertain whether these findings are sustained over time.

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VANADYL SULFATE IMPROVES METABOLIC CONTROL AND INSULIN SENSITIVITY IN PATIENTS WITH NIDDM.

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Vanadium salts have significant insulin-like actions *in vitro*, reduce hyperglycemia and insulin resistance in animal models of diabetes and may be useful to treat subjects with NIDDM. STUDY AIM: to examine the effects of 6 weeks of treatment with vanadyl sulfate ($VOSO_4$) in 10 patients with NIDDM (age 59±2y, BMI 28.6±0.9 kg/m^2, fasting glucose (FPG) 187±15 mg/dl, HbA_{1c} 8.4±0.4%). We evaluated glycemic control, glucose tolerance (OGTT), insulin sensitivity (euglycemic insulin clamp, 40 mU/m^2•min, with 3-^{3}H glucose), lipids and 24h ambulatory blood presure (ABP) before (following a 4 week run-in period) and after follow-up for 6 weeks with $VOSO_4$ (150mg/day). Caloric intake and physical activity were constant. RESULTS: $VOSO_4$ reduced FPG(191±15 to 155±11mg/dl), HbA_{1c} (8.2±0.4 to 7.7±0.4%) and fructosamine (359±22 to 291±15 umol/L, all $p<0.01$). During OGTT, the mean plasma glucose conc was reduced by 30 mg/dl; this was entirely accounted for by a decrease in FPG and hepatic glucose production. Insulin clamp: glucose uptake (M) increased by 22% (2.7±0.3 to 3.3±0.4 mg/kg•min, $p<0.02$), but remained less than in non-diabetic controls (5.8±0.4 mg/kg•min, $p<0.001$). Total and LDL-cholesterol decreased (223±14 vs. 202±16, $p<0.01$; 141±14 vs. 129±14 mg/dl, $p<0.05$). Mean 24hr ABP was unchanged, but $VOSO_4$ enhanced the nocturnal MAP drop ($p<0.05$) and restored a normal circadian rhythm in 4/6 pts. Weight remained stable (76.1±4.2 vs. 75.9±4.1kg,NS). CONCLUSIONS: $VOSO_4$ treatment for 6 weeks in NIDDM is well tolerated, improves metabolic control and attenuates cardiovascular risk factors. Improvements in whole body insulin-mediated glucose disposal (muscle) and hepatic sensitivity to insulin contribute to enhanced glucose tolerance. The results suggest that $VOSO_4$ may be helpful in the treatment of NIDDM.

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THE PPARγ AGONIST, BRL 49653, PREVENTS PROGRESSION TO DIABETES IN ZUCKER DIABETIC FATTY RATS.

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The Zucker Diabetic Fatty (ZDF) rat, an animal model of NIDDM, progresses from an obese, hyperinsulinemic and glucose intolerant state to one of overt diabetes, with attendant impaired weight gain and secondary complications such as nephropathy and cataract formation. In an attempt to influence the course of the disease process, the PPARγ agonist, BRL 49653 (~10 μmol/kg body weight daily), was given via the diet to ZDF rats, aged 6 weeks (Prevention group; n=8) or 21 weeks (Intervention group; n=7) until termination at aged 28 weeks. Untreated ZDF and lean (ZL; n=10) rats were used as controls. ZDF control rats (n=16) were hyperglycemic at aged 11 weeks (15.2±6.1 vs ZL 5.7±0.4 mmol/L; mean±SD; $p<0.001$) and thereafter blood glucose fluctuated between 29.0±2.0 (22 weeks; n=8) and 17.9±6.8 mmol/L (25 weeks; n=8) until termination. The Prevention group, however, maintained a normoglycemia (eg 3.7±0.8 mmol/L vs ZL 3.0±0.9 mmol/L at 27 weeks; p=NS). Late Intervention with BRL 49653 at aged 21 weeks failed to influence the hyperglycemia. ZDF controls also developed marked glycosuria and proteinuria, neither of which were seen in the Prevention group. At aged 27 weeks, ZDF controls excreted in 24h 9.4±1.0g glucose and 1.0±0.1g protein. By contrast, glycosuria was barely detectable in Prevention group rats (~0.01g/24h) and proteinuria was similar to that in ZL controls (0.081±0.013 vs 0.051±0.008 g/24h; p=NS). Late Intervention treatment, however, failed to influence the established glycosuria and proteinuria. These data show that early treatment with BRL 49653 prevents the progression to diabetes in ZDF rats and simultaneously affords protection against the development of proteinuria.

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TROGLITAZONE AND METFORMIN ENHANCE GLUCOSE DISPOSAL THROUGH DISTINCT MOLECULAR MECHANISMS.

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Troglitazone and metformin are antidiabetic agents that belong to the thiazolidinedione and biguanide classes of drugs, respectively. These agents enhance glucose disposal and insulin sensitivity in both animal models and patients with NIDDM. To determine if these agents act through similar molecular mechanisms we compared their effects in several *in vitro* assays. Both drugs stimulated glucose transport and utilization in C3H10T1/2 cells, a pluripotent mesenchymal stem-cell line capable of differentiating into adipocytes. Troglitazone treatment stimulated glucose incorporation into lipids (*i.e.*, lipogenesis) and β_3-adrenergic receptor-mediated lipolysis but inhibited basal lipolysis and aerobic respiration. In contrast, metformin had no effect on lipogenesis or adrenergic-mediated lipolysis but increased basal lipolysis, β-oxidation of palmitoyl-CoA and aerobic respiration. Metformin was also more efficacious than troglitazone at decreasing the extracellular pH (*i.e.*, increasing acidosis) and increasing lactate accumulation in the medium (*i.e.*, anaerobic respiration). Insulin enhanced the effects of troglitazone, but not metformin, on glucose and lipid metabolism. Moreover, we show that troglitazone, but not metformin, binds and activates the nuclear receptor peroxisome proliferator activated receptor gamma (PPARγ). Taken together, these observations provide evidence that troglitazone and metformin affect distinct molecular pathways involved in carbohydrate and lipid metabolism. In sum, troglitazone had an anabolic effect while metformin had a catabolic effect on C3H10T1/2 cells. It is proposed that the antidiabetic action of troglitazone involves PPARγ-mediated transcription of genes involved in lipid and carbohydrate metabolism.

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THE METABOLIC EFFECTS OF TROGLITAZONE IN NON-INSULIN DEPENDENT DIABETES (NIDDM). Troglitazone Study Group (USA), Yale New Haven, CT, USA.

In a multicenter, double-blind trial, we studied the metabolic effects of 6 months troglitazone (TGZ) in 93 NIDDM patients (age 52±1yr, fasting plasma glucose 11.3±0.3mM, body mass index 33±1kg/m^2) randomized to take either 100, 200, 400 or 600mg of TGZ, or placebo; and assessed with a meal tolerance test and a 5h hyperinsulinemic (120 mU/m^2.min) euglycemic clamp with systemic di-deuterated glucose, before and after treatment. TGZ lowered fasting ($p<0.0001$) and post prandial ($p<0.02$) plasma glucose by ~25% and ~20% respectively at the high treatment doses (400 & 600mg) and lowered fasting ($p<0.02$) and post prandial ($p<0.01$) triglycerides, and fasting free fatty acids but only at the highest treatment dose (600mg, $p<0.02$). There was a lowering of plasma insulin in the higher treatment groups (200-600mg), whereas, c-peptide levels fell across all groups including placebo ($p<0.0001$). Of interest, basal hepatic glucose production was unaffected by TGZ in all treatment groups ($p<0.001$) except with the highest treatment dose (600mg) where basal hepatic glucose production fell. More importantly, TGZ had a most striking effect on insulin-mediated glucose disposal rates; increasing glucose disposal ($p<0.005$) by ~45% above pre-treatment levels in the 400 & 600mg treatment groups. Determining factors that predicted a favorable glucose lowering effect, regression analysis confirmed that the stongest predictor of a glucose lowering effect either fasting ($p<0.0001$) or post prandially ($p<0.02$) was treatment with TGZ itself. Controlling for TGZ effect, fasting c-peptide level was the next strongest predictor. To conclude, TGZ has powerful glucose lowering effects in fasting and post prandial states in NIDDM, especially in patients with good insulin secretory reserve; this effect is mediated by enhancing insulin-mediated glucose disposal in peripheral tissues.

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Genetics of IDDM

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ARE ATTRIBUTABLE RISK ESTIMATES IMPORTANT FOR IDDM PREVENTION?

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The WHO DiaMond Molecular Epidemiology Sub-Project, which is based on standardized IDDM incidence registries, is determining the contribution of host susceptibility to the occurrence of disease within and between populations from across the world. Using a case-control design, data from Japan, China, Mexico, USA (PA and AL), New Zealand and Finland have been analyzed to estimate absolute and attributable IDDM risks. This information is important not only for understanding the epidemiologic patterns of IDDM, but also for developing strategies for the prediction and prevention of the disease. Such approaches have typically been targeted towards first degree relatives, known to be at high genetic risk. However, most individuals who develop IDDM have no other affected family members. Thus, the definition of 'susceptible' individuals in the general population remains a complex and controversial issue that may be addressed, in part, by the DiaMond Molecular Epidemiology Sub-Project. For each racial group in these seven population, IDDM susceptibility was uniquely defined by the specific HLA-DQ genotypes that were significantly more prevalent among the cases than controls. Although it was consistently observed that individuals with two high risk genotypes were most likely to develop IDDM in all populations (1-33% through age 35 years), only 26-55% of the incidence of the disease in any of the areas could be accounted for by these high risk genotypes. One can, therefore, conclude that natural history studies and clinical trials, which are based upon the enrollment of only high risk individuals, identified by similar genetic screens, represent less than ½ of future incident cases. Therefore, even if other markers, such as autoantibodies, were subsequently utilized to improve predictive value, more than 50% of the individuals who will develop IDDM would be missed with strategies targeted towards those who screen at high genetic risk. Thus, attributable risk estimates provide important information that should be utilized, in conjunction with relative and absolute IDDM risk estimates, for the development of clinical and public health practices targeted towards the prediction or prevention of IDDM in populations from across the world.

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CONCORDANCE FOR IDDM IN IDENTICAL TWINS IS AFFECTED BY THE 'LOAD' OF BOTH MHC AND NON-MHC SUSCEPTIBILITY GENES.

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The environmental component of susceptibility to IDDM is reflected in the discordance for disease found in monozygotic twins. Twin studies have also provided valuable evidence for the genetic contribution to disease and might also help elucidate the potential importance of markers for IDDM in individuals who are 'genetically susceptible' to disease. We have examined the hypothesis that discordance in identical twins might also reflect a decreased load of diabetes predisposing genes in discordant compared to concordant twins. We studied 40 discordant and 40 concordant identical twins at 2 polymorphisms of the insulin gene region on chromosome 11p (INS) : the 3' untranslated region 1,127 Pst 1 and the 5' -23 Hph 1 restriction sites. Of the concordant twins 87.5% possessed the disease associated INS genotype at both polymorphisms; of the discordant twins it was only 60% at the 5' and 68% at the 3' polymorphisms (for Hph 1: $p=0.005$, RR=4.67; for Pst 1: $p=0.036$, RR=3.37). The stronger association with the 5' Hph 1 locus is consistent with reports implicating a functional effect of the VNTR in disease susceptibility. INS thus appears to have an effect on disease concordance. We also studied these twins at HLA-DQB1 and found a higher frequency of the disease associated 0302 allele in the concordant than in the discordant twins (67.5 v 42.5%; $p=0.027$, RR=2.8). The higher frequency of DQB1 0201/0302 heterozygosity in the concordant twins (50 v 30%) did not, however, reach statistical significance. Our results suggest that the 'load' of both MHC and non-MHC susceptibilty genes has an impact on the disease penetrance of IDDM.

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ROLE OF DQA1*0102/DQB1*0602 IN RISK CHARACTERIZATION: THE DIABETES PREVENTION TRIAL-TYPE 1 DIABETES (DPT-1).

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The aim of the study was to determine the role of DQA1*0102, DQB1*0602 (0602+) in risk characterization in relatives of patients with type 1 diabetes. This haplotype was previously found "protective" for type 1 diabetes, and is an exclusion criteria for DPT-1. Of >40,000 relatives screened, 3.44% are ICA positive (≥ 10 JDF U). Of 909 ICA+ relatives DQ typed, 62 (6.8%) were 0602+. Of 250 subjects with low first phase insulin response (FPIR) to IVGTT, 8 (3.2%) were 0602+, in comparison to 54 of 659 (8.2%) with FPIR that was not low ($p<0.001$). This suggests that the protective 0602+ haplotype may also be associated with lack of progression to low FPIR. We also analyzed expression of insulin (IAA), GAD65 (GAA), and ICA512bdc (ICA512AA) "biochemical" autoantibodies (AAs) for the first 52 ICA+ relatives with 0602+, and compared the results to analysis of a randomly chosen 52 similar relatives lacking 0602. Of the subjects studied in depth, 34% percent of the 0602+ relatives were ICA- (<10 JDF U) upon repeat ICA testing, as compared to 17% of 0602- relatives. The second DQ "haplotype" was either 0501/0201 or 0301/0302 in 81% of the confirmed ICA+ relatives with 0602+ in comparison to only 35% of relatives with 0602 but with negative repeat ICA. The remainder of analyses were done only on relatives with confirmed ICA positivity. Expression of biochemical AAs was greater amongst 0602- than 0602+ relatives (e.g. 60% of 0602+ relatives versus 21% of 0602- relatives expressed none of the "biochemical" AAs, $p<.001$).

	GAA	IAA	ICA512AA	0Ab	≥2Ab	3Ab
0602+	23%	23%	13%	60%	20%	6%
0602-	61%	59%	50%	21%	57%	30%

Amongst ICA+ first degree relatives of patients with type 1 diabetes, DQB1*0602 appears to dramatically "delay" rather than permanently suppress the expression of IAA, GAA, and ICA512AA.

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THIS ABSTRACT HAS BEEN WITHDRAWN BY THE AUTHOR.

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NON-Asp 57 RESIDUES IN THE HLA-DQB1 GENE CONTROL SUSCEPTIBILITY TO IDDM IN INDIA.

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Strong association of HLA-DR3/DR4 has been reported in IDDM patients around the world. Using PCR-SSOP, we have studied the polymorphism of HLA-DRB1, DQA1 and DQB1 genes in 38 patients with IDDM and 154 unrelated healthy controls from the homogeneous population of North Indian Hindus. A strong association of DRB1*03012 encoding HLA-DR3 was observed in IDDM as compared to controls (60.5% vs 8.4%, Pc<0.000000001, RR=16.6). HLA-DR4 did not reveal any deviation from controls in these patients. On the other hand, DR2 molecules showed a significant negative association (13.2% vs 40.3%, Pc<0.01, RR=0.2) in patients which is consistent with earlier studies. All HLA-DR2 positive IDDM patients were found to be DRB1*1502-DQA1*0103-DQB1*0601 which is the most common DR2 haplotype in Asian Indians (30%). Among DQA1 alleles DQA1*0501 occurred more frequently in diabetics as compared to controls (73.3% vs 40.3%, Pc<0.001, RR=4.1). Of the 4 DQA1 alleles that carry Arg 52 (0301, 0501, 0401, 0601), two i.e., 0301, 0501 were found to occur in 80% of IDDM patients as compared to controls (55.2%, Pc<0.05, RR=3.2). Similarly, non-Asp 57 carrying DQB1 alleles particularly, DQB1*0201 were found to be significantly increased in patients as compared to controls (78% vs 36%, Pc<0.000001, RR=6.5). Upto 95% of IDDM patients in this study had non Asp 57 residues in their DQ beta molecules (DQB1*0201, 0302, 0501, 0604, 0102) as compared to controls (55.2%, X^2=19.8, Pc<0.00001) thus giving a very high relative risk of 14.2. Further, a strong linkage disequilibrium was observed between DRB1*03012-DQA1*0501-DQB1*0201 in all patients and controls. The study indicates that i) IDDM in Asian Indians is primarily associated with HLA-DR3 and not DR4, ii) that the primary susceptibility genes lies in the DQ region represented by DQB1*0201 in strong linkage disequilibrium with DRB1*03012 and other non-Asp 57 containing alleles.

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EVIDENCE FOR ASSOCIATION OF SHORTER CLASS 1 ALLELES OF INSULIN GENE 5′ MINISATELLITE WITH IDDM IN JAPAN

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The role of the insulin gene-linked IDDM susceptibility (*IDDM2*) still remains unclear in Japanese: majority (~95%) of Japanese are homozygous for class 1 alleles of the variable number of tandem repeat (VNTR) in the 5′ region of the insulin gene. Therefore, we determined their exact length in number of repeat units (RUs) of class 1 alleles by PCR amplification of the VNTR of 152 IDDM patients and 167 control subjects in Japan. We found that the distribution of class 1 alleles in Japanese was bimodal with one peak located at 32/33 RUs and the other peak located at 41 RUs, and that 1S (25~38 RUs) alleles were significantly increased in the IDDM group compared with the control group (0.55 vs 0.46, p=0.032). The 1S/1S genotype was significantly increased in the patients (0.34 vs 0.20, p=0.005, RR2.1). Transmission disequilibrium test (TDT) of 21 Japanese families confirmed the association of 1S alleles with IDDM: 18 1S alleles and 7 1L (39~44 RUs) alleles were transmitted to affected offspring (p=0.028) while 1S and 1L allele transmission to non-affected offspring were 6 and 6. The present study suggests that shorter class 1 alleles may have a role in IDDM susceptibility in Japan. Because difference between 1S and 1L alleles is characterized by element sequence variations as well as repeat numbers, we prefer a possibility that sequence variations of class 1 alleles influence the disease susceptibility.

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HLA-DQB1^{57} BUT NOT *HLA-DQA1*^{52} ALTERATION IS ASSOCIATED WITH THE DISEASE IN POLISH IDDM FAMILIES

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Allele-specific amplification PCR method (*ASA-PCR*) allows to follow codon polymorphism at *HLA-DQA1*^{52} and *HLA-DQB1*^{57} thus giving a possibility of expressed amino acid identification. Frequency analysis of codon variants was performed in IDDM children (n=97) and control subjects (n=78) of Polish origin. 45 IDDM children, their parents (n=65) and healthy siblings (n=17) were also analyzed. Obtained results revealed aliphatic and hydrophobic amino acid residues (phenotypes: Val/Ala and Ala/Ala) to be present at significantly higher frequency at DQβ^{57} in IDDM children (63%) compared to control subjects (17%; RR=8,472) Values for parents and siblings were established at the level of 38% and 29%, respectively. Heterozygous Asp was present in 28% IDDM patients v. 47% control subjects (RR=0.784); in parents and siblings percentage did not exceed 58% and 65%, respectively. Homozygous Asp *was not found* in IDDM children while present in 28% of controls (RR=0.014). Only one parent and one sibling (1.5% and 6%, respectively) exhibited it. This suggests its enhanced protective effect. Simultaneous typing of both studied codons revealed that DQα^{Arg52+}/DQβ^{Asp57-} accompany IDDM phenotype at much higher frequency (64%) than the physiological one (20,5%), RR=6.86; respective values for parents and siblings are 38% and 29%, respectively. The correlation between IDDM and number of susceptible dimers DQα^{Arg52+}/DQβ^{Asp57-} one individual can assemble has been observed in dose respective manner. The highest relative risk (RR=4.87) was obtained for individuals who can express IV (100%) those dimers. *Amino acid residue at DQα*^{52} *concerned alone was of no importance in respect to IDDM.* The crucial role of DQβ^{57Asp} in respect to the disease was confirmed in second approach. 16 families with at least one affected and one non-affected child were selected from the set of subjects and subsequently analyzed. DQβ^{57Asp} was present in 12/17 (71%) healthy siblings, 16/31 (52%) parents, and only in 5/17 (29%) IDDM children.

Supported by KBN grant 4 P05E053 08

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INSULIN GENE 5' VNTR POLYMORPHISM IN BASQUE TYPE I DIABETICS

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Genetic analysis have shown association between several polymorphic genomic regions and susceptibility or protection to type I diabetes (IDDM). In addition to *IDDM1* (HLA class II region on chromosome 6p), a polymorphic region spanning 4.1 kb around the insulin gene on chromosome 11p (*IDDM2*) has been strongly related to the disease. Basques are a population living in the W-Pyrenees (SW France and N Spain), that has been isolated in the past. Anthropological studies have pointed out allelic frequency differences in polymorphic genes in Basques. Our aim was to characterize *IDDM2* among Basque families with IDDM. We studied 62 families of Basque ethnic origin, including 71 diabetic patients and 203 first-degree relatives, as well as 42 healthy and unrelated controls. For genetic analysis we studied the Variable Number of Tandem Repeats (VNTR) region 5' to the insulin gene using Southern blot hybridization of *Pvu* II digested genomic DNA with radiolabelled Phins 310 probe. Restriction fragments were classified according to size in class 1 (<600bp), class 2 (600-1600bp), and class 3 alleles (>1600 bp). Linkage analysis were performed with TDT (Transmission Disequilibrium Test). Comparison of the allelic frequencies between diabetic and non-diabetic alleles, using affected family based control alleles, and of the genotypic frequencies between diabetics and healthy controls are:

ALLELES	class 1	class 3
IDDM (n= 126)	84.1%	15.9%
Non-IDDM (n=110)	59.1%	40.9%

p= 0.000017

GENOTYPES	1/1	1/3	3/3
IDDM (n= 62)	71.0%	27.4%	1.6%
Controls (n= 45)	31.1%	51.1%	17.8%

p= 0.000054

Relative risks (RR) for each genotype were 5.41 for 1/1, 0.36 for 1/3 and 0.08 for 3/3. TDT analysis performed on heterozygous parents showed linkage of the VNTR region to diabetes susceptibility: 35 transmissions of class 1 *vs.* 9 of class 3 (p= 0.000089). There was no difference of allelic transmission between heterozygous mothers and fathers (p= n.s.). In conclusion, the VNTR region is linked to type I diabetes in Basques, and class 1 allele is associated with the disease. No evidence of imprinting has been observed.

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THE EFFECT OF HLA-B ALLELE ON THE IDDM RISK DEFINED BY DRB1*04 SUBTYPES AND DQB1*0302

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In this study we analyzed at the population level the effect of DR4 subtypes and class I, HLA-B alleles, on the IDDM risk when the influence of DQ locus was equalized. The genes encoding HLA-DQ heterodimer molecules, DQB1 and DQA1, have been found to have the strongest association with IDDM risk, although there is cumulative evidence for the effect of other gene loci within MHC gene region. HLA-DR locus besides HLA-DQ has most often been suggested to contribute to the disease susceptibility. In our research DRB1*0401 and DRB1*0404 were the common subtypes associated with DQB1*0302 in all three studied populations (Estonian, Latvian, Russian). DRB1*0401 was increased and *0404 decreased among IDDM patients. DRB1*0402 was also relatively common among Russian haplotypes, but was not associated with IDDM risk. When HLA-B alleles were analyzed, strong association between the presence of specific B alleles and DRB1*04 subtypes were detected. HLA-B*39 allele was found significantly more often in DRB1*0404-DQB1*0302 positive patients than healthy control subjects positive for this haplotype: 27/54 (50%) vs. 4/49 (8.2%), P<0.0001 in the combined series. The result demonstrates that DQ and DR genes cannot explain all of the HLA-linked susceptibility to IDDM and the existence of a susceptibility locus telomeric to DR is probable.

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IDDM2 ALLELE EFFECTS ON *IGF2* mRNA LEVELS IN THE MAJOR TISSUES IMPLICATED IN TYPE I DIABETES AUTOIMMUNITY.

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It has been shown that a polymorphism consisting of a variable number of tandem repeats, situated 5' to the insulin gene (*INS*-VNTR), is associated with type I diabetes (*IDDM2* locus). The shorter alleles (class I) predispose to diabetes, while the longer ones (class III) have a dominant protective effect. Since the VNTR is not transcribed, its contribution to *IDDM* susceptibility must be mediated by transcriptional effects on adjacent genes. We have presented evidence of strong VNTR allele effects on thymic (but not pancreatic) insulin expression, that may explain the effect on diabetes risk. In addition to --or even instead of-- effects on insulin, diabetes risk could be modulated by transcriptional effects on *IGF2*, the closely linked gene encoding insulin-like growth factor II. This gene is parentally imprinted, with exclusive expression from the paternal allele in most tissues, including pancreas and thymus. Parental effects have been described in the transmission of *IDDM2* alleles to diabetic offspring. We therefore, analyzed the effect of VNTR alleles on mRNA level in samples of human pancreas, thymus and leukocytes by quantitative-competitive-RT-PCR. *IGF2* mRNA level was 4.7 ±0.9 (mean ± SEM, arbitrary units) in thymi with a paternal class III *INS-VNTR* compared with 4.7 ± 1.3 in samples with a paternal class I. The same absence of a significant difference was found in pancreas: 28.4 ±4.2 vs. 29.5 ±5.2. We also examined control of *IGF2* expression in leukocytes where, as we have previously reported, *IGF2* imprinting is variably relaxed. A transcribed *ApaI* polymorphism was used to distinguish paternal from maternal alleles in heterozygous individuals. The (+) allele of this polymorphism was found to be associated with higher *IGF2* mRNA levels, when parental origin was taken into account. However, VNTR class had no effect on *IGF2* mRNA levels, as the paternal/maternal ratios were not significantly different between subjects with a maternal class I and a paternal class III vs. maternal class III and paternal class I (4.2±1.5 vs. 3.1 ±1.3). Thus, we found no VNTR effects on *IGF2* mRNA levels in three tissues relevant to diabetes autoimmunity. This corroborates our previous report suggesting that the gene involved in *IDDM2* is insulin.

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HLA ASSOCIATED GENETIC SUSCEPTIBILITY TO IDDM IN ORIENTAL AND CAUCASOID ETHNIC GROUPS IN RUSSIA

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A comparative study, using SSP method, on the association between HLA alleles and genotypes of the HLA DQ locus and IDDM susceptibility was conducted on two ethnic groups - Russians (Caucasoids) and Buriats (Orients). This choice is due to the fact that IDDM morbidity in Buriats is tenfold low as compared to Russians at the same region. Eight of HLA DQA1 and fourteen DQB1 specificities were discriminated. The genotyping had undergone quality control in term of the XII IHWC "HLA and IDDM" component. Among Russians the most frequent associations to IDDM are DQA1-0301 (RR=4,21), -0501 (RR=2,90),-0301 (RR=0,01) alleles and the genotype DQA1 0501/0301 (RR=9,13). In HLA DQB1 gene IDDM associated are HLA DQB1-0302 (RR=6,38), - 0602 (RR=0,42) and DQB1-0302/0201 (RR=9,60). The following genotypes were found to be IDDM associated: DQA1-0301/DQB1-0302 (RR=4,2), DQA1-0501/DQB1-0302 (RR=8,4) and DQA1-0103/DQB1-0201 (RR=0,03). In IDDM Buriat patients the associations with some "classical" HLA DQA1 and DQB1 "alleles-markers" we revealed which are typical for both Oriental and Caucasian populations. So alleles DQA1-0301 and DQB1-0302 were determined IDDM "markers" in spite of their extremely high frequency (cca. 25% for DQA1-0301) in healthy controls. On the other hand in IDDM Buriat patients the allele markers are typical for the subjects of Oriental origin and include HLA DQB1-0303 as IDDM "marker" and DQB1-0601 as the desease resistance "marker" were found. The most important conclusion of present observations is the total absence of the genotype HLA DQA1-0103/DQB1-0201 in healthy Buriat controls whereas that one is most frequently detected in Buriat IDDM patients (13,75% at RR=35,75%).

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INTERLEUKIN-1 RECEPTOR TYPE 1 GENE POLYMORPHISM IN SPANISH SUBJECTS AFFECTED WITH IDDM

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A diallelic polymorphism of the interleukin-1 receptor type 1 (IL1R1) gene has been differentially associated to IDDM according to the racial origin of the populations studied. A positive association has been found in British diabetics while not in Finnish and South Indians. We have studied the polymorphism of the 5'untranslated promoter region of the IL1R1 gene by using PCR-RFLP assay in the intermediate risk Spanish population. Analysis of samples from 100 type 1 diabetic subjects and 130 controls showed the following allelic distribution using sthe nomenclature from Southern analysis: 1.2kb/1.2kb 44% in diabetics and 40% in controls, 1.2kb/3.2kb 45% and 49% in diabetics and controls respectively, and 11% in both for 3.2kb/3.2kb (X^2=ns). Neither association was found when diabetic subjects were selected according to the presence or abscense of a high risk haplotype (DQA1*0301-B1*0302 and/or DQA1*0501-B1*0201). In conclusion, these results suggest that the polymorphism of the IL1R1 gene is not a valuable genetic risk factor for IDDM in Spanish subjects. **FISS 95/1065**

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ANALYSIS OF HLA-DRB1,-DQA1 AND -DQB1 GENOTYPE OF KOREAN IDDM PATIENTS

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To evaluate the association of IDDM and HLA class II genes in Korea, we compared allelic constitutions at HLA-DRB1, -DQA1, -DQB1 loci of IDDM patients with that of ethnically matched nondiabetic individuals by PCR-based reverse dot hybridization method. In IDDM patients, the frequency of DRB1 *03, *04 (RR=5.4;2.3) and DQA1 *03, *05 (RR=3.5;2.3) was significantly high and that of DQA1 *01 (RR=0.3) and DQB1 *06 (RR=0.4) was significantly low. Three haplotypes HLA-DRB1 *03 - DQA1 *05 - (DQB1 *02), HLA-DRB1 *04 - DQA1 *03 - (DQB1 *04) and (HLA-DRB1 *13) - DQA *01 - DQB1 *06 were in the state of linkage disequilibrium in IDDM. Of these three haplotypes, two (DRB1 *03-DQA1*05-DQB1*02 and DRB1*04-DQA1*03-DQB1*04) appear to be susceptible haplotypes, whereas the third (DQB1*13-DQA1*01-DQB1*06) appears to be protective haplotype. These findings suggest that DRB1*03-DQA1*05-DQB1*02 and DRB1*04-DQA1*03-DQB1*04 can be markers of IDDM in Korea.

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THIS ABSTRACT HAS BEEN WITHDRAWN BY THE AUTHOR.

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HLA DQA1 AND DQB1 ALLELES IN IDDM PATIENTS OF AN ARGENTINIAN CAUCASIAN POPULATION.

MULTICENTER TRIAL: PREDI-C-DIAB:
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The frequency of HLA class II alleles was analzyed in a population of 118 IDDM Argentinian patients and compared to that of 198 controls. Patients and controls belonged to an homogeneous Caucasian individuals with Spanish and/or Italian ancestry. Class II alleles were determined by DNA typing using primers and probes of the 12th International Histocompatibility Workshop. Results: 86.3% of the patients and 19.4% of the controls were homozygous for non-Asp_{57} - DQB1 alleles (odds ratio, OR: 26.2, X^2: 64.3, $p<10^{-5}$). To investigate the contribution of HLA-DQA1 to the susceptibility in this population, DQA1 alleles were determined in 64 patients and 180 controls: 43% of the patients and 1.7% of the controls were homozygous for non-Asp_{57} - DQB1/Arg_{52} -DQA1 susceptible alleles (OR: 38.9, X^2: 38.1, $p<10^{-6}$). On the other hand, DQB1*0602 was significantly decreased among patients (0,85% vs. 18,7% in controls, OR: 0.04, X^2:20.6, $p<10^{-5}$). These results confirm for this population the primary association of IDDM with DQA1 and DQB1 alleles. In addition DQB1* 0602 seems to be a protective allele also in our population.

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A *CTLA4* POLYMORPHISM AFFECTS LYMPHOCYTE mRNA LEVELS BUT IS NOT ASSOCIATED WITH TYPE 1 DIABETES IN A CANADIAN DATASET.

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The susceptibility locus *IDDM12*, mapped to 2q33, is linked to and associated with type 1 diabetes (IDDM) in Italian families. Association was confirmed in a Spanish dataset and, although it did not reach statistical significance in US and UK datasets, it was highly significant in combined data from all sources. The marker is an A/G polymorphism at nucleotide 49 of the coding sequence (threonine/alanine in the leader peptide) of *CTLA4*, a gene encoding a membrane receptor that mediates apoptosis in activated T lymphocytes. We sought to confirm the association in a group of diabetic children from Montréal. A PCR-based *Fnu4HI* RFLP was used to compare the frequency of the G allele in 128 IDDM patients to the control frequency in the two parental chromosomes not transmitted to the child. There were 161 A alleles transmitted (tr), vs 153 not tr and 95 G alleles tr vs. 103 not tr. This lack of association in our population may be due to different linkage disequilibrium of A/G with the susceptibility locus. Alternatively, if A/G is the true susceptibility locus, the population differences may be due to interactions with other loci. As a first step in examining possible differential biologic function of the A vs. G alleles, we measured relative, allele-specific *CTLA4* mRNA levels in human peripheral mononuclear cells (PBLC) from five A/G heterozygous diabetic subjects. The G/A allele intensity ratio in the internally radiolabeled RT-PCR product, measured in triplicate, was compared to the same ratio in genomic DNA, where both alleles are present in equal concentrations. The genomic DNA ratio was 0.419±0.024 (mean±SEM.) as expected by the smaller size of the digested G allele. The ratio was higher in RNA from both unstimulated (0.709±0.064, p=.002) and stimulated (0.568±0.022, p=.003) lymphocytes (paired *t*-test). This indicates a higher level of the G allele in *CTLA4* mRNA, which can be due an increase in either transcription level or mRNA stability. It provides a plausible mechanism of susceptibility to diabetes and other autoimmune disease, such as Grave's. The lack of association in our population may be due to population differences at other loci.

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IS THERE A METABOLIC, GENETIC OR IMMUNOLOGICAL CONNECTION BETWEEN IDDM AND NIDDM IN SOUTH INDIAN FAMILIES

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We studied 170 south indian families consisting of parents and a proband with IDDM (Type 1) to investigate whether there would be an increase in prevalence of NIDDM in the parents and the relationship of glucose tolerance with either HLA-DQ or GAD65 antibodies (GADA). Among the parents 11.2% had NIDDM and 13.6% had IGT on oral GTT and this was similar to that in age matched population of South India. DNA samples were typed for allelic variation at HLA-DQB1 in the families and studied using the transmission disequilibrium test. Both HLA-DQB1 *0201 (p=0.0003) and DQB1 *0302 (p=0.002) were associated with IDDM. There was no correlation of *0201 or *0302 with glucose tolerance in the parents excluding a genetic link between NIDDM and IDDM in these families. GADA were measured by a radiobinding assay. GADA was positive in 56.5% of the probands against 7% of control (p<0.0001) and in 33% of the parents. There was no correlation of GADA with glucose tolerance in parents. In conclusion,in South Indian families with an IDDM proband, there is no overlap between IDDM and NIDDM. The high rates of GADA in the parents are yet to be explained.

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NO LINKAGE OF THE *CTLA-4* GENE REGION (*IDDM12*) TO TYPE 1 DIABETES IN THE DANISH POPULATION

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Insulin-dependent diabetes mellitus (IDDM) is a multifactorial disease with polygenic susceptibility. Results from genome-wide screenings and partial ones have presented some evidence of linkage with several non-HLA linked loci. Recently, *IDDM12* was mapped to chromosome 2q making this chromosomal region particularly abundant of IDDM-linkage markers. *CTLA-4* is the candidate gene for *IDDM12*. We have analysed this locus in Danish IDDM family materials. Initially, we typed 152 families (688 individuals) with at least 2 IDDM affected siblings for the $(AT)_n$ microsatellite in the 3'untranslated region of the *CTLA-4* gene. An extended transmission disequilibrium test (TDT) for multi-allele marker loci was used to assess transmission from heterozygous parents to offspring. No evidence for neither allele-wise or genotype-wise transmission disequilibrium was found (p=0.6 and p=0.2, respectively). However, a distorted transmission of the $(AT)_{17}$ -allele was observed; p=0.007 (uncorrected). A point mutation in exon 1 of the *CTLA-4* gene (A→G transition) was analysed as well. Previously this polymorphism has revealed transmission disequilibrium in Italian and Spanish populations, but not in UK and US populations. In this analysis, the 152 sib-pair families and additionally 105 families (429 individuals) with only one IDDM affected offspring were included. Although a tendency towards a skewed transmission: 145 'A' passed and 166 'G' passed to IDDM offspring and 96 'A' and 88 'G' passed to unaffected offspring, this did not reach statistical significance (p=0.2 for IDDM offspring). In conclusion, we were not able to replicate the observation of linkage of the *IDDM12* locus to disease in a large Danish IDDM family data set. If the *CTLA-4* gene is important for conferring susceptibility to IDDM, the present data combined with previous data from UK and US, indicate that the A→G transition is unlikely to be the aetiological mutation.

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GENETIC AND ENVIRONMENTAL FACTORS IN TYPE I (INSULIN-DEPENDENT) DIABETES MELLITUS IN CHILEAN CHILDREN.

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HLA class II alleles play an important role in the genetic susceptibility to develop insulin-dependent diabetes mellitus (IDDM). Some environmental factors such as viruses prevalence and breast-feeding duration have been associated with the disease. Our objective was to clarify what heterodimers account for the higher risk of IDDM, and assess their interaction with the breast feeding duration (BFD). 63 IDDM children and 74 non diabetic children were recruited for this study. Alleles determinations were performed by means of PCR with oligospecific probes, while BFD and prevalence of infectious diseases were ascertained by retrospective survey. In diabetic patients, we found a significant different frequency of risk heterodimers (H4) regard to non diabetic children (0.59 vs 0.19, p<0.0001) with an OR=13.7 (IC 95%: 4.4-42.7) respect to the H0 group that serves as the reference category. H0 was more frequent between controls children (0.35 vs 0.08, p<0.01). In relation of the environmental factors, both groups had a similar prevalence for rubella, measles and mumps. For the BFD, the IDDM group showed a reduced average of effective breast feeding compared with the control group (21.5±15 vs 33.8± 20 weeks, p<0.01) and a early exposure to cow milk (15.9± 12 vs 21± 13, weeks, p<0.05). When stratifying by BFD, we observed a high OR for H4 respect to H0 in the group of children with breast-feeding < 3 months (OR 20.25, p<0.0001). For BFD > 3 months, the OR comparing H4 with H0 was 14 (IC 95%: 1.5 - 128). In conclusion, the H4 combinations is strongly associated with high risk for IDDM, and the long period of BFD could be very important as protector factor in the low incidence of IDDM in Chilean children.

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ASSOCIATION OF CTLA-4 GENE POLYMORPHISM WITH AUTOIMMUNE THYROID DISEASE BUT NOT WITH IDDM IN JAPAN

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CTLA-4 (cytotoxic T lymphocyte associated-4) gene on chromosome 2q33 (*IDDM12*) is a good candidate for autoimmune disease. Recently, associations of CTLA-4 gene polymorphisms with Graves' disease and IDDM were reported in Caucasian populations. Therefore, in this study, we determined an A/G polymorphism in exon 1 of CTLA-4 gene in Japanese patients with IDDM (n=97), autoimmune thyroid disease (AITD; Graves' or Hashimoto's disease)(n=142) or both(n=23). Allele frequencies of the G allele, which was increased in Caucasian patients with Graves' disease and IDDM, were 0.67 in the IDDM group, 0.71 in the AITD group (0.70 in Graves, 0.73 in Hashimoto) and 0.65 in the IDDM+AITD group. Compared with the control group, in which the frequency was 0.63, significant increase of the G allele was only observed in the AITD group (p=0.031). The G/G genotype was significantly increased in the AITD group (p=0.017, RR 1.7). Although further confirmation is required, the CTLA-4 gene region may have a susceptibility role in AITD but not in IDDM in Japan.

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DNA ANALYSIS OF HLA CLASS II GENES IN INDONESIAN IDDM PATIENTS

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The incidence of Diabetes Mellitus in Indonesia is obviously increasing every year, this is due in part to the following factor effect of different gene system, race, sex and patient age at the time of onset. The aim of this study was to determine the distribution of HLA Class II polymorpnism based on molecular investigation in Indonesia IDDM patients. Although, serological studies have investigated the association of HLA DR-3 and DR-9 antigen with the predisposition to IDDM, furthermore HLA DR-5 has been associated with a protection from IDDM.

In this study HLA class II alleles (DRB, DQA and DQB) were determined by PCR-SSO method in 64 unrelated IDDM patients, and 82 unrelated healthy controls. All samples originated from Java and Bali island. The results were observed in particular, a predisposing effect of the HLA DRB1 * 0301 (RR 14.8 p = 0.0014), DQA * 0501 (RR7.5, p = 0.00256), DQB1 * 0201 (RR 11.7, p = 0.000002) DRB3 * 0202 (RR 3.5, p = 0.04). Based on these study, it is shown in Indonesian IDDM patients , that DRB1 * 04 was not associated with a susceptibility to IDDM and HLA DRB1 * 1202, DRB3 * 0301 DQA * 0601, DQB1 * 0301 with a protection from IDDM. Conclusion : the data in Indonesian diabetics together with data on European IDDM patients, suggest agradient of various HLA class II predisposing and spesific protective markers link these populations.

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CTLA-4 GENE (*IDDM12*) POLYMORPHISM IN JAPANESE PATIENTS WITH IDDM

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Susceptibility to IDDM is determined by environmental and genetic factors. The main gene associated with a predisposition to IDDM is HLA and at least 12 other genes-unlinked to HLA contribute to the development of IDDM. Recent studies described linkage and association of IDDM to the CTLA-4 gene (*IDDM12*) in Caucasians. CTLA-4 is a candidate gene for T cell mediated autoimmune diseases because it is a negative regulator of T cell proliferation. As CTLA-4 association with IDDM may be influenced by the racial composition of the population, it is important to study it in other ethnic groups. We investigated the distribution of CTLA-4 gene polymorphism in 59 Japanese patients with IDDM and 59 controls. An A/G transition at position 49 of exon 1 was analyzed by the PCR-RFLP method. There was no significant difference in the distribution of CTLA-4 alleles between patients and controls (χ^2=1.69, P=0.43, 2 d.f.). The present study did not support the association of CTLA-4 gene with IDDM in Japanese, however this negative finding should be confirmed with increased number of subjects.

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THE FREQUENCY OF HLA-DR4 IS NOT INCREASED IN FAMILIES WITH IDDM MOTHERS AND MALFORMED CHILDREN

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The association between IDDM of the mother and the risk of congenital malformations of her children has been acknowledged for decades. The metabolic control of the IDDM mother is known to have a major effect on the risk. Despite the improvement in metabolic control of pregnant IDDM women achieved this far, the risk of malformations still continues to be 2-3 times higher than the risk of non-diabetic women. This study was undertaken to find out whether HLA, the major genetic contributor to the IDDM susceptibility, also exerts an additional risk for congenital malformations. Twenty families with an IDDM mother having child or children with congenital malformations were PCR-typed for DR and DQ loci. The malformations included different types of both minor and major birth defects. We expected that the strongest diabetogenic alleles, DR4 and DR3 would be more frequent in the case children and/or in their mothers. However, compared with our population-based study of HLA haplotypes of diabetic families, there were no differences between the diabetic malformation case families and the diabetic families with non-malformed offspring. Of the diabetic mothers of the malformed offspring, 15/19 (79 %) were DR4-positive; of the diabetic mothers with the non-malformed offspring 56/74 (76 %) were DR4-positive. Interestingly, 6/16 cases with malformations were DR4-positive, whereas the expected number based on mendelian segregation of DR antigens in the families would be 10. The frequencies of DR4 in the families with malformed offspring were the following: diabetic mothers 0.42, non-diabetic fathers 0.22, malformed offspring 0.28. In conclusion, it seems as if the DR4-antigen is not increased in the children with malformations, nor in the diabetic mothers of the malformed cases.

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CHROMOSOME 2 MARKERS AND IDDM IN SOUTH INDIAN FAMILIES

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A number of IDDM chromosomal regions have been assigned to the long arm of chromosome 2 including around the interleukin 1 gene cluster, HOXD8, D2S152 and CTLA4. We have previously investigated a South Indian population and failed to find an association between IDDM and either the insulin gene or the interleukin-1 receptor type gene using a case control study design. The aim of our studies was to investigate 82 IDDM probands and their parents for excess transmission of microsatellites in the regions on chromosome 2 that might harbour IDDM susceptibility genes. DNA was amplified by the polymerase chain reaction and primers for D2S160, D2S410, D2S326, D2S1244, D2S152, D2S72 and D2S206. Microsatellites were run on a polyacrylamide gel using an ABI373 sequencer and alleles sized using GENESCANNER software. The data was analysed using the extended transmission disequilibrium test (TDT). To date we have full data sets for D2S326, D2S1244 and D2S152; the Chi squared for allele wise TDT was 0.58, 0.59 and 0.21 for each of the loci respectively. In conclusion, the current results suggest that an IDDM locus is not located around HOXD8 or D2S152 on chromosome 2 in the South Indians.

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DOES INSULIN-DEPENDENT DIABETES MELLITUS OCCUR TOGETHER WITH MULTIPLE SCLEROSIS IN FINLAND?

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Finland has the highest incidence of diabetes mellitus (IDDM) in the world (40/100 000/year in 1991). The prevalence of multiple sclerosis (MS) is also high in different parts of the country. The genetic susceptibility to both IDDM and MS is conferred by genes in the HLA region on chromosome 6 although by different - mutually exclusive - alleles. Certain DR4,DQ8 haplotypes are specific markers for IDDM whereas DR15(2),DQ6 haplotypes - supposedly "dominant protective" in IDDM - are markers for MS. Our aim was to find out wheather IDDM and MS occur together in Finland and wheather DR17(3),DQ2 haplotypes might play a role in both diseases as suggested by Olerup. IDDM and MS were found to co-segregate in 23 of the 801 families (3%) ascertained in the DiMe Study, a nationwide population-based IDDM study of children under the age of 15 years. Second degree relatives of the DiMe probands were most likely to have MS. In three families the fathers had MS and in one family an IDDM mother had a child with MS. None of these parents transmitted known IDDM susceptibility haplotypes. DR17(3),DQ2 was not observed more frequenty in these Finnish families whereas B18 (or B35),DR1,DQ5 haplotypes were.

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LINKAGE ANALYSIS OF *IDDM13* IN DANISH IDDM MULTIPLEX FAMILIES

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Genetic susceptibility to insulin-dependent diabetes mellitus (IDDM) is polygenic and caused by both HLA and non-HLA loci. Several studies of linkage between non-HLA loci and IDDM have focused on the long arm of chromosome 2. Recently a locus at 2q34 providing some evidence for linkage to IDDM in a mixed population from UK and Australia was described and denominated *IDDM13*. The **aim** of the present study was to investigate if linkage between *IDDM13* (D2S164) and IDDM could be demonstrated in a large Danish IDDM multiplex **material** comprising 149 IDDM sibpair multiplex families with at least 2 IDDM affected siblings per family and a total of 676 individuals. **Genotyping** of the D2S164 microsatellite was performed by PCR followed by PAGE, blotting and visualization utilizing a streptavidine-peroxidase catalysed ECL reaction. **Statistical analysis** was performed using the extended transmission disequilibrium test (ETDT Version 1.1) for multi-allele marker loci. Borderline evidence for allele-wise transmission disequilibrium to affected offspring was found (χ^2=24.7; 15 df: p=0.054). No evidence for genotype-wise transmission disequilibrium to affected offspring was found (p=0.13). Normal allele- and genotype-wise transmission to unaffected offspring were observed. Transmission analysis of individual alleles, revealed a distorted transmission of the 285bp and the 297bp alleles to affected offspring. The 285bp allele was transmitted 62 and non-transmitted 42 times: p_{td}=0.05 (uncorrected) and the 297bp allele was passed 5 and non-passed 15 times: p_{td}=0.016 (uncorrected). Both alleles displayed normal transmission to unaffected offspring. In **conclusion**, marginal evidence for linkage of *IDDM13* to IDDM in a Danish IDDM multiplex population was demonstrated. The data suggested that the 285bp and the 297bp alleles may be in linkage disequilibrium with aetiological mutations that confer susceptibility and (partial) protection to IDDM, respectively.

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NO INDEPENDENT ASSOCIATION OF HLA-DP POLYMORPHISMS WITH SUSCEPTIBILITY TO DEVELOP IDDM IN NORWAY

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It is well established that the HLA complex (in particular DR and DQ) is involved in genetic susceptibility to develop IDDM. Polymorphisms within the HLA class II gene DPB1 have in recent studies been suggested to be associated with disease susceptibility. These studies have found the DPB1*0301 allele to be increased among patients, apparently independently of linkage disequilibrium to high risk HLA-DR or DQ alleles. To overcome the problem of linkage disequilibrium we have genotyped 237 IDDM patients and 285 HLA-DRB1-DQA1-DQB1 carefully matched controls, carrying high risk DR-DQ genotypes, from an ethnically homogenous population. We observed no significant differences between patients and controls comparing DPA1 or DPB1 allele frequencies. The frequency of DPB1*0301 is 12% in patients and 10% in controls (p_{nc}=0.2) in this selected population. Among DRB1*0301-DQA1*0501-DQB1*0201/DRB1*0404-DQA1*03-DQB1*0302 individuals there is a tendency of increased frequency of DPB1*0301 (19% in patients vs. 11% in controls; p_{nc}=0.07) among patients. Taken together, our data suggests that there is no independent effect of DPB1*0301 on IDDM susceptibility, suggesting that the DPB1 associations observed in previous studies may not reflect an independent effect of DPB1, but rather be secondary to linkage disequilibrium to high risk HLA-DQ and/or DR alleles. In conclusion, our data do not support previous reports on a primary association between HLA-DP polymorphisms and IDDM developmentand suggests that if such an association exists, it has to be a very minor one.

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THE VITAMIN D RECEPTOR; A CANDIDATE GENE FOR IDDM

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Vitamin D has important immunomodulatory properties and prevents the development of diabetes in the NOD mouse. We have investigated 96 probands with IDDM and their parents for an association with the Vitamin D receptor (VDR) gene (12q14). DNA was amplified using two separate pairs of primers to the VDR gene and at D12S85 (within 2 Cm from VDR). The VDR gene fragments were digested with BsMI, ApaI and TaqI and RFLPs, subjected to agarose gel electrophoresis and detected by ethidium bromide staining. D12S85 was analysed by polyacrylamide gel electrophoresis on a ABI 373 DNA sequencer using GENESCANNER software. Excess transmission of polymorphic markers was tested using the extended transmission disequilibrium test (TDT). There was significant excess transmission of VDR alleles containing the BsMI restriction site to affected offspring in these families ($p = 0.016$) as well as BsMI/TaqI haplotypes ($p = 0.01$). In contrast, in the first 60 families no excess transmission of D12S85 alleles were found. In conclusion, the vitamin D receptor gene or a nearby locus on 12q14 determines susceptibility to IDDM in South Indian subjects.

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Analysis of the HLA-DMB gene frequency in Japanese IDDM patients by PCR-RFLP method.

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The role of HLA class II antigens (DR, DQ, DP) is important to the onset of IDDM. We newly analized of HLA-DMB gene by PCR-RFLP methods (Tissue antigens 1996: 47: 530).

Materilas and methos: The genomic DNAs were isolated by phenol extraction of SDS-lysed and proteinase K-treated cells. After PCR-amplification of the third exon of DMB gene using a pair of primers, aliquots from reaction mixtures were digested with restriction endonucleases, ApaLI, BsrI, and HinpII (PCR-RFLP methods).

Results: The Japanese 89 IDDM patients typed for HLA-DMB alles by PCR-RFLP methods (TABLE). We compared the frequency of the normal Japanese populations (by Naruse etc.) to Japanese IDDM and listed. The DMB*0101 frequence of IDDM is higher than normal populations. The DMB*0102 of IDDM is significantly ($P<0.035$) lower than normal.

Discussions: HLA-DM is required for peptide loading into other HLA class II molecules, so by our data, it is possible to be related to the onset of IDDM.

TABLE. The HLA-DMB allels (%) in normal and IDDM Japanese.

	IDDM	normal	X2	P value
DMB*0101	56.2 %	49.3 %	2.6	p<0.105
DMB*0102	17.4 %	23.2 %	4.4	p<0.035
DMB*0103	26.4 %	23.2 %	0.004	NS
DMB*0104	0.0 %	0.4 %	---	---

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POLYMORPHISM OF ALDOSE REDUCTASE GENE IN RUSSIAN JUVENILE IDDM AND NIDDM PATIENTS: COMMON RISK MARKER?

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Aldose reductase (ALR2) is a key enzyme of polyol (sorbitol) pathway of glucose metabolism which activation results from hyperglycaemia in diabetes condition. Polymorphism of (CA)n dinucleotide microsatellite at the 5' region of ALR2 gene in DNA from 112 healthy Russian controls, 64 childhood IDDM and 46 NIDDM patients was studied using PCR technique. Seven alleles (from Z-6 to Z+6) were observed. The Z+4 allele was shown to be the most frequent in the Russian healthy subjects. A Russian population was characterized by strongly increased frequency of the Z+4 allele in comparison with one in British Caucasians (24.6% vs. 2.3%). Frequency differences between groups were estimated using two-tailed Fisher's exact test. The significant decrease of Z+4 allelic frequency was observed in both diabetic groups compared to healthy controls (10.2% vs. 24.6%, p=0.00056, and 15.2% vs. 24.6%, p=0.04443, for IDDM and NIDDM patients, respectively). Patients with juvenile IDDM had significantly increased frequency of both Z/Z-4 (10.9% vs. 2.7%, p=0.02845) and Z/Z-2 (21.9% vs. 10.7%, p=0.0387) genotypes compared to those in the non-diabetic group. The Z/Z-4 genotype was also increased in NIDDM subjects compared to normal controls (10.9% vs. 2.7%, p=0.04685). These preliminary data allow us to suggest that in a Russian population the particular ALR2 gene alleles might be associated with susceptibility or resistance to diabetes mellitus of both types, or to vascular complications characterized for IDDM and NIDDM in different extent.

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INVESTIGATION OF THE NON-HLA-DR-DQ CONTRIBUTION TO *IDDM1*

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The major histocompatibility complex (MHC) on chromosome 6 encodes the major determinant of genetic susceptibility to IDDM, (*IDDM1*). The HLA-DR and DQ genes are likely to contribute to this susceptibility but there is evidence that one or more additional MHC-encoded genes are also involved. Our previous studies have identified a DR/DQ haplotype (DR4/DQ4) which is predisposing to IDDM in the Japanese but is neutral in the Chinese. The aim of this study was to compare DR4/DQ4 haplotypes from each race at other MHC loci. Any allelic differences would implicate that locus as a candidate susceptibility determinant, this could then be tested in a case/control study. The Japanese subject was homozygous at all loci studied: DR4-DQ4-TAP2*0201-DMB*0101-DMA*0102-DPA1*02022-DPB1*0501. The Chinese individual was also homozygous at DR, DQ, TAP1, LMP7 and LMP2 with alleles identical to the Japanese individual, but heterozygous at the other loci: TAP2*0201/0302-DMB*0101/0103-DMA*0102/0101-DPA1*0103/N-DPB1*-N/N (N = new allele). One Chinese DR4-DQ4 haplotype identified is thus identical to the Japanese DR4-DQ4 haplotype from DRB1 to DMA. The divergence at DP is consistent with the recombination hotspot between DMA and DPB1. This study excludes a role for the TAP1, LMP7 and LMP2 alleles in determining the difference in susceptibility noted between Japanese and Chinese DR4/DQ4 individuals. Our previous population study of TAP2 alleles excluded a role for this locus. The DM and DP loci cannot be excluded as susceptibility determinants without population studies. Further population studies and comparison of other MHC loci on these haplotypes should allow us to map non-DR/DQ loci contributing to *IDDM1*.

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GLN-ARG 192 POLYMORPHISM OF PARAOXONASE IN IDDM

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Paraoxonase (PON) is an enzyme protecting organism from organophosphate poisoning and capable of lipid peroxides hydrolyzing. According to relative enzyme activities, two isotypes, A and B, have been defined. The actual molecular basis for the PON polymorphism is a Gln→Arg substitution. Serum paraoxonase activity is decreased in IDDM and NIDDM. We investigated 206 unrelated IDDM patients from St.Petersburg with mean age 18±9 years and 148 age-related healthy controls. In order to determine the amino acid 192 substitution the fragment 99 bp of genomic DNA was PCR amplified, then digested by MboI restrictase and subsequently analyzed on a 12% polyacrilamide gel. In PCR amplified fragment we have one original MboI restriction site and the second one forms in case of Gln-Arg (A-G) substitution. We have found the following PON genotype distribution : AA-50.5%, AB-41.25%, BB-8.25% in IDDM and AA-56.1%, AB-37.1%, BB-6.8% in control group. We used χ^2 test to compare frequencies in analyzed groups. No any statistically significant differences were obtained in IDDM patients compared to healthy controls, though there was a tendency of Arg allele increase in IDDM. Thus, the observing decrease of PON activity may be due the present of some epigenetic factors.

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EXAMINATION OF HUMAN β_2-MICROGLOBULIN (β_2-m) GENE AS A CONTRIBUTOR TO DEFECTIVE IDDM ANTIGEN PRESENTATION·

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We have shown that IDDM patients have a defect in antigen-presentation, in part, characterized by an altered conformation of surface class I molecules. Besides MHC class I genes themselves and genes coding for proteins involved in the processing of endogenous peptides (LMP and TAP), the β_2-m gene which encodes for the L chain of class I molecules, is a candidate for a polymorphism contributing to this altered conformation. β_2-m is required for normal class I surface expression and, in the mouse, can regulate the cell surface conformation and expression of class I H chains. The mouse β_2-m locus is polymorphic with 8 alleles. Some studies have suggested that one of these alleles can affect class I molecules/antigen conformation, and presentation and recognition by T cells. Furthermore, mouse β_2-m is linked to the autoimmune diabetes non-MHC locus Idd 13. In humans, 6 nucleotide discrepancies, including 2 with amino acid (AA) substitution, have been reported. It is not known whether they are true polymorphisms. Here, we report the β_2-m sequences from 4 IDDM patients shown to have the antigen presentation defect characterized by altered class I conformation. mRNA was isolated from B cell lines, RT-PCR was performed and a β_2-m cDNA probe was used for Northern blots. Specific PCR products of the β_2-m cDNA were transfected into E. Coli vectors and 5 inserts sequenced. The level of IDDM β_2-m mRNA was identical to non-diabetic control, making the presence of a functional polymorphism in the promoter region unlikely for constitutive β_2-m mRNA expression. The sequences of 4 β_2-m cDNA coding regions uncovered no difference and the deduced AA sequence is in agreement with the commonly submitted one. We have not found any of the previously reported nucleotide discrepancies. In conclusion, the HLA class I presentation defect observed in our patients is unlikely to be explained by a β_2-m gene polymorphism. We cannot rule out a mutation affecting the induced β_2-m mRNA expression or yet unreported alternative splicing with induction with interferon-α.

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ANALYSIS OF THE FAMILIES OF IDDM-MOTHERS WHOSE CHILDREN SUFFER FROM DIABETES

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The aim of our study was to analyse the occurrence of diabetes and its types in the families of IDDM-mothers with diabetic children (15 families A) and without them (115 families B). In our long-term follow-up of 177 adult children of IDDM mothers (CDM) we found 15 diabetic CDM: 9 IDDM and 6 NIDDM from 11 mothers, another 4 CDM of 4 mothers had transient gestational diabetes. In group A the diabetic girls were more frequent (73.7%) than in group B (46.7%), p <0.01. In A mothers IDDM manifested in 21.1±2.1 years, in B mothers in 17.1±0.6 years. IDDM was diagnosed in pregnancy in 46.7% of A mothers and in 13.9% of B mothers (p<0.001). In families A the occurrence of diabetes, mostly NIDDM, was higher than in families B. A mothers had also diabetic mothers (60%) and their parents (26.6%), B mothers had lower frequency of diabetic mothers (18.3%) and their parents (5.2%), p<0.001 and <0.01 resp. A mothers had diabetic fathers in 26.7%, B mothers in 16.5%. Diabetes in fathers of CDM (husbands of diabetic mothers) in 20% of group A and in 10.4 % of group B was present. Conclusion: We found high risk of diabetes of both types in offspring of those mothers, in whose families the high occurrence of diabetes is present and whose IDDM began in pregnancy. The special attention to this families should be paid.

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LACK OF MHC CLASS I POLYMORPHISMS IN NON-OBESE DIABETIC MICE

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Genetic susceptibility to autoimmune diabetes is predominantly linked to the major histocompatibility complex (MHC) genes. While the role of polymorphic MHC class II antigens in human and murine experimental diabetes has been extensively studied, the contribution of class I MHC is less defined. We here cloned and analysed MHC class I antigens of the non-obese diabetic (NOD/Lt) mouse, an animal model immunogenetically related to human autoimmune diabetes. From a (dT) primed NOD brain cDNA phage library 34 independent clones containing H-2 K^d, H-2 D^b and class I associated genes were isolated. When compared to wild type sequence, all H2-K^d NOD clones showed a single base exchange (T→G) at position 430, leading to a non-conservative amino acid substitution at position 114 (His→Gln^{114}) located in the extracellular α2 domain involved in antigen presentation and TAP interaction. To confirm this finding, wild type K^d cDNA was RT-PCR generated from DBA/2 mice and cloned. Complete sequence identity to NOD derived K^d was found, thus correcting the previously published DBA/2 sequence. In addition, no base exchanges were detected in NOD H2 D^b and class I associated cDNAs. In conclusion, MHC Class I antigens are not polymorphic in NOD mice compared to non-autoimmune strains, further emphasizing the contribution of class II polymorphisms to MHC associated diabetes risk.

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NUMBERS OF SAMPLES DESIRABLE IN LINKAGE DISEQUILIBRIUM MAPPING FOR DIABETOGENIC GENES.

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To isolate disease-predisposing genes for insulin-dependent diabetes mellitus (IDDM), linkage disequilibrium mapping is essential. It is desirable to estimate the number of subjects which is enough to detect significant results before conducting linkage disequilibrium mapping, and it is important to use an analytical method with a higher statistical power, especially when subjects available for analysis are limited. To investigate the number of subjects necessary to obtain significant results, and to compare the power of case-control study and transmission disequilibrium test (TDT), we conducted simulation studies. Genetic parameters, such as allele frequencies and penetrances, are calculated using the data from our extensive analysis on *IDDM2* (*TH*) and *IDDM7* (*D2S152*), which showed significant association with IDDM in Japanese population. In *IDDM2*, when 0.001 is adapted as the significance level, the power of case-control study using 200 patients and 200 control subjects is 0.70, and that of TDT using 200 patients and their parents was 0.50. In case of *IDDM7*, the power of case-control study using 400 patients is 0.38, and that of TDT using 400 patients is 0.26. Case-control study had higher power than TDT in any case studied, suggesting that it is a powerful method provided that there is no population stratification.

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ASSOCIATION OF TUMOR NECROSIS FACTOR-B IN INSULIN-DEPENDENT DIABETES (IDDM) PATIENTS FROM LATVIA.

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TNF -A and -B genes have been shown to be associated with IDDM in Caucasians. TNF genes are located close to the HLA-B genes in the short arm of chromosome 6. The aim of our study was to determine the frequency of TNF-B gene polymorphism in IDDM patients (n=105) and healthy controls (n=114) from Latvia.. TNF-B was genotyped by PCR-RFLP which identifies two alleles, allele 1 and allele 2. Allele 1 is positively associated with IDDM in Latvian patients and is present in 87/105 (83%) patients and 80/114 controls (Odds ratio 2.05; $p<0.05$). Analysis of TNF-B genotypes showed positive association for genotype 1/2 present in 77/105 (73%) patients and 68/114 (60%) controls (OR = 1.86; $p<0.05$). The genotype 2/2 is negatively associated, seen in 18/105 (17%) patients and 34/114 (30%) controls (OR = 0.49; $p<0.05$).

TNF-B is a cytokine gene that plays an important role in the inflammation and immune regulation. The polymorphism of the TNF-B gene linvolves NcoI polymorphism in intron 2 and is associated with substitution at amino acid position 26 encoded in exon 3 of the TNF-B gene. It is not clear how this polymorphism influences the secretion of TNF-β. We conclude that in IDDM patients from Latvia, TNF-B allele 1 and TNF-B genotype 1/2 is positively associated and TNF-B genotype 2/2 is negatively associated with the disease. It is not clear whether this association is primary or secondary to linkage with HLA-class II or class I region.

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GENETIC ANALYSIS OF AUTOIMMUNE TYPE I DIABETES IN THE KDP RAT

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Komeda Diabetes-Prone (KDP) rat has been established as a diabetes-prone substrain of the Long-Evans Tokushima Lean (LETL) rat, a desirable animal model for human IDDM. An MHC-linked gene has been reported to be involved in the pathogenesis of insulitis of the LETL rat. To detect non-MHC IDDM susceptibility genes in the KDP rat, we generated the three crosses: (TM x KDP) x KDP, (LETO x KDP) x KDP and (BN x KDP) x KDP backcrosses. Animals were checked for glycosuria and non-fasting blood glucose levels until 18 weeks of age. We genotyped markers throughout the rat genome on animals generated from the TM cross (54 diabetics, 114 non-diabetics), and detected strong evidence of linkage to IDDM on chromosome (Chr) 11 (D11M16Mit14, $\chi^2=62.0$, $p<0.001$). To confirm these findings, we genotyped Chr 11 markers on animals generated from the LETO (64 diabetics, 127 non-diabetics) and BN (9 diabetics, 48 non-diabetics) crosses. In these crosses, strong evidence of linkage to IDDM was found in the same region on Chr 11 (D11Mgh5 in the LETO cross, $\chi^2=64.8$, $p<0.001$; Mox2 in the BN cross, $\chi^2=9.0$, $p<0.01$), indicating that a recessively-acting gene on rat Chr 11, termed *Iddm/kdp1*, is a major diabetogenic factor in the KDP rat irrespective of the cross. Comparative mapping suggests that homologs of *Iddm/kdp1* are located in the region between D16Mit14 and D16Mit46 on mouse Chr 16 and in the human Chr 3p11-q13 region. To our knowledge, there are no potential candidate genes around these regions. *Iddm/kdp1* on rat Chr 11, therefore, seems to be a novel IDDM susceptibility gene.

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THREE TNF MICROSATELLITE MARKERS IN FINNISH IDDM HAPLOTYPES

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The polymorphic gene region encoding and regulating tumor necrosis factor is located between class I and class II HLA genes. It is a candidatefor an additional susceptibility gene in IDDM. We studied within HLA gene region three TNF microsatellite markers (a-b-c) in DiMe (Childhood Diabetes in Finland Study) families. TNFabc markers were identified in 249 IDDM associated and 202 control haplotypes. We used PCR and ABI PRISM 377 DNA sequencer GENESCAN fragment analysis program to identify these markers. TNF2-3-1 was significantly increased among IDDM haplotypes 16.4 % vs 8.9 % (p=0.0260) and TNF5-5-2 decreased 11.4 % vs 5.6 % (p=0.0408). TNF2-3-1 was associated with DQB1*0201 allele and there was no significant difference in the frequency of this TNF combination between IDDM associated and control DQB1*0201 posite haplotypes. When TNF microsatellite markers were compared in other haplotypes with defined DQB1 alleles with IDDM risk, no significant differences were found. In conclusion, we didn't find any conclusive evidence for an independent role of TNFabc microsatellite polymorphism in IDDM susceptibility.

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THE INCIDENCE OF INSULIN DEPENDENT DIABETES MELLITUS (IDDM) IN THE UNDER 16'S IN DEVON AND CORNWALL FROM 1985 TO 1996

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We aimed to study the incidence rates of insulin dependent diabetes mellitus (IDDM) in children under 16 years of age living in Cornwall (C)and Plymouth Health District (PHD) from 1985 to 1996. 310 children with IDDM were identified from 2 independent sources; a)paediatric and adult diabetes registers from Treliske and Derriford Hospitals, b) admissions for IDDM recorded in hospital records. All were diagnosed between 01/01/1985 - 3/12/1996. Ascertainment was 99%. Overall incidences were calculated using the 1991 census. The average annual incidence rates per 100,000 in C was 17.1 (boys 17.8, girls 16.5); in PHD was 19.7(boys 17.4, girls 22.3). The incidences differed between districts , lowest in the far south-west (10.2 Restormal), and highest in Caradon (25.4) around the Tamar Valley . These differences in incidence mirrored the prevalence of the HLA haplotype conferring low risk for IDDM (B44 DR4 *0301) which are most prevalent in the Celtic population . These observations may help us tounderstand the aetiology of IDDM

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THE RISK OF INSULIN-DEPENDENT DIABETES MELLITUS IS INDEPENDENT OF BIRTH WEIGHT - A DANISH TWIN-CONTROL STUDY.

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A relationship between birth weight and risk of developing insulin-dependent diabetes mellitus (IDDM) has been suggested. We conducted a nested twin-control study in order to investigate this. Twin partners of discordant pairs are matched for gestational age, maternal age, weight and parity. Furthermore, monozygotic twins are genetically identical.

In the 1953 through 1982 birth cohorts of the population-based Danish Twin Register, 95 twin pairs (26 monzygotic and 69 dizygotic) where one or both had IDDM were identified. These twins were found by sending questionnaire to the 20888 pairs of these birth cohorts. Zygosity was determined by questionnaire and by using serological methods. Information about birth weight and -length, prematurity, maternal age and parity was obtained from mid-wives records for the birth cohorts 1953 - 72 (incl.) and from the Danish Medical Birth Registry for the cohorts 1973 - 82 (incl.). Data regarding birth were available for 82 diabetic and 50 healthy twin individuals (67 twin pairs: 21 monozygotic and 46 dizygotic).

A paired t-test of discordant twins showed no difference in mean birth weight between diabetic and non-diabetic twin partners (2538 g *vs* 2549 g, $p > 0.05$, $n = 51$ pairs). Twin pairs divided according to zygosity showed a similar pattern. Paired t-test of twins who were born full term did not show any significant difference in birth weight between diabetic and non-diabetic twin partners (2911 g *vs* 2904 g, $p > 0.05$, $n = 20$ pairs). Concordant and discordant twin pairs did not differ significantly in gestational age ($p > 0.05$). In conclusion, no significant differences were found in birth weight between the twin individuals who later developed IDDM compared to the non-diabetic individuals in this material.

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INCIDENCE OF IDDM IN MERIDA, SPAIN, DURING THE PERIOD 1983-92.

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We studied prospectively the IDDM incidence in Mérida Health Care region of Extremadura, Spain, during 10 years (1983-92). Inclusion criteria of cases were those of IDDM, age at onset less than 30 years and residence in Mérida at diagnosis. All cases were identified by four sources: endocrinologists, general physicians, diabetes societies and diabetic summer camps (criteria of WHO). Population at risk (0-29 y) was 71.233 inhabitants (total population 158.296). The degree of ascertainment was 95%. Male/female ratio was 1.08. Age specific incidence rate/100.000 in the age group 0-14 were 8.00 (C.I. 95%: 7.95-8.07) and in the age group 0-29 were 7.80 (C.I. 95%: 7.79-7.94), with great variations between the years of study. There was a seasonal onset pattern, with highest incidence in spring (Mar-May). The highest incidence occurred in the age group 10-14 years. We conclude that incidence of IDDM observed in Mérida during the period 1983-92 is lower than that previously reported in other Spanish regions (Madrid or Catalonia), but similar to those of some Mediterranean countries (France, Portugal or Algeria). The highest incidence was observed in the age group 10-14 years, as in other studies. The hypothesis of the called "north-south gradient" in diabetes risk remains to be confirmed, having important regional differences in the same country.

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IDDM IN CHILDREN IS ASSOCIATED WITH EARLY NEONATAL COMPLICATIONS, LOW APGAR SCORE AND HIGHER MATERNAL AGE.

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Environmental risk factors for childhood IDDM may operate early in life. This study aimed to investigate neonatal exposures of children (0-15) diagnosed with IDDM in Yorkshire, UK, during 1993-4, using data from obstetric records of 521 mothers participating in a population-based case control study. Using 196 case control sets, matched for age and sex, univariate analyses were conducted on variables including birthweight, Apgar score, vitamin K, neonatal complications, jaundice and admission to a Special Care Baby Unit. Significant odds ratios (OR) were restricted to illness in the neonatal period (cases n=61; controls n=73; OR 1.61, 95% CI 1.06-2.44) and an Apgar score between 4 and 7 at one minute (cases n=49; controls n=57; 1.62, 1.04-2.51). No specific condition accounted for the risk of neonatal illness. Adjusting for mothers with pre-existing IDDM (cases n=4; controls n=0) did not effect the OR or significance of neonatal illness but removed statistical significance for low Apgar score. In a multivariate model, including mother's age *a priori*, only higher maternal age retains a significantly positive association. This study suggests complications in the neonatal period may contribute to the risk of IDDM in childhood, already associated with older mothers.

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PRELIMINARY DATA ON THE INCIDENCE OF DIABETES MELLITUS TYPE 1 IN THE PROVINCE OF BADAJOZ (SPAIN); Morales Pérez, FM., Barquero Romero J., Aguila López MA., Diaz Pérez de Madrid J. and Group of study of IDDM in the province of Badajoz(GEDIDB). Hospital "Infanta Cristina", Badajoz, Spain.

Introduction: The incidence of IDDM in Spain (10.6-12-5/100.000) has been shown to be higher than expected for countries in the south Europe area. Badajoz is the largest of Spanish provinces with a highly rural or semi-rural population.
Objective: To detect the incidence, geographic distribution, and seasonal variation of insulin-dependent diabetes mellitus (IDDM) in the province of Badajoz (Spain). **Research design and methods:** The study population involve all persons aged 0-29,9 years and diagnosed in the province of Badajoz since the 1st of January 1992 to 15th December 1996. IDDM was defined on the basis of a clinical diagnosis of idiopathic diabetes by a physician. Cases meeting these criteria were included if insulin treatment was started before their 30th birthday and had been living in the study area during at least six months before the diagnosis was made. The primary source of ascertainment were reports made by adult and pediatric diabetologists, and second source was the notification of Diabetes Associations. We present results of data referred to one of the four sanitary areas of the province, which includes Badajoz city, and comprising 251.520 of the 671 774 inhabitants of the province. **Results:** Sixty-eight patients (39 males and 29 females) up to 30 years of age have been diagnosed of IDDM in this sanitary area during the study period. The age-adjusted incidence of IDDM was 10,8/100.000 (males: 11,8/100.000; females: 9,8/100.000). The sex-specific incidence ratio was 1.34. The incidence in the pubertal age-group of 10 to 14,9 years of age (16,58/100.000) was higher than for other age-groups. The incidence was higher in metropolitan (13,01/100.000) than in rural (6,2/100.000), semi-rural (9,2/100.000), or urban areas (11,3/100.000). A seasonal variation in the incidence of IDDM was observed with the lowest rates during the spring and summer. **Conclusion:** Awaiting results for the whole province, preliminary data on one complete sanitary area suggest that the incidence of IDDM in the province of Badajoz is similar to that of other regions of Spain. There was a significant pubertal peak and an impressive clustering of cases was observed in metropolitan area of the Badajoz city (117. 834 citizens) compared with the rural (< 2.000 citizens) and semirural areas (2.000-10.000 citizens).

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COMPLICATED PREGNANCY AND LABOUR MAY PREDISPOSE TO THE DEVELOPMENT OF IDDM IN CHILDHOOD.

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Environmental risk factors for childhood IDDM have been investigated in Yorkshire, UK, using data from hospital obstetric records of mothers participating in a population-based case control study of children (0-15), diagnosed with IDDM during 1993-4. Univariate analysis of 196 age and sex matched sets of cases and controls gave significantly raised odds ratios (OR) for mothers over 35 years (OR 2.13, 95%CI 1.04-4.36). Antenatal risk factors included amniocentesis (3.85, 1.34-11.04), 'oedema, proteinuria and hypertension' (1.62, 1.03-2.54), excessive weight gain (7.12, 1.50-33.79) and labour complications (1.49, 1.00-2.21). The risk associated with oedema, proteinuria and hypertension was greatest when present throughout pregnancy. For the first trimester none of the cases (n=8) or controls (n=2) had IDDM but 4/8 cases and 1/2 controls had pre-existing hypertension. Caesarean delivery increased risk (1.90, 1.12-3.23), particularly for elective procedures (2.23, 1.03-4.81). Adjustment for mothers with IDDM (4 cases, 0 controls) did not affect the pregnancy risk factors or caesarean delivery but for labour complications and elective caesarians statistical significance was lost. In a multivariate model of significant OR, only excessive weight gain retained significance. This study presents a risk profile of older mothers whose babies may be exposed to adverse intrauterine conditions and caesarean delivery.

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INCIDENCE OF CHILDHOOD TYPE 1 (INSULIN-DEPENDENT) DIABETES IN COIMBRA, PORTUGAL,1987-95

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In order to investigate the epidemiology of insulin-dependent diabetes in the age group 0-14 years, a prospective, population based registry was established in Coimbra in 1990 (WHO DIAMOND project). Prior to the institution of that registry we undertook a retrospective study for the period 1987-89. Included were cases diagnosed between 1987-95, with age at onset less than 15 years and residence in the district of Coimbra at diagnosis. The population at risk was 76,784 and the total population 427,839 at the 1991 census. As the primary source of cases we used hospital admissions. Two other independent sources were used for validation of case ascertainment by the primary source (general practitioners of the Public Health System and endocrinologists who have a private practice in the region). The degree of ascertainment by the hospital source alone was 100%. The overall incidence rate (/100,000/year) was 8.5 (Poisson 95% CI: 6.5-11.0). The age-adjusted (world standard) incidence rate was 7.5 per 100,000 per year. The age group 10-14 years showed the highest incidence rate, 10.8 (95% C.I.: 7.3-15.4). The incidence rate was slightly higher in females, 8.7 (95% C.I.: 5.9-12.5) than in males, 8.2 (95% C.I.: 5.5-11.8). This difference was not statistically significant. The incidence of IDDM in the district of Coimbra is in the middle range of the incidence observed throughout the world and is comparable to those found in several Southern European countries and Brazil.

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FAMILIAL, PERINATAL AND DIETARY RISK FACTORS FOR INSULIN-DEPENDENT-DIABETES-MELLITUS (IDDM) IN CHILDREN AND JUVENILES IN VIENNA, AUSTRIA

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Environmental factors seem to play a role in the pathogenesis of insulin-dependent-diabetes-mellitus. In our case-control study the parents of all IDDM-patients with manifestation of the disease between 1989 and 1994 in Vienna were asked to complete a questionnaire (n = 119, mean age = 11.9 ± 3.4 yr). Control children (n = 476, 11.8 ± 3.4 yr) were recruited by random from all schools in Vienna matched for age and sex. The response rate was 87.4% in diabetics and 79.8% in controls. Additionally the obstetric hospital records could be traced in 72.3 and 68.1% respectively.

Both, fathers (mean age = 31.7 ± 6.7 yr) and mothers (27.8 ± 5.5 yr) of diabetic children were significantly older ($p<0.05$) than parents of controls (fathers: 30.0 ± 6.1 yr, mothers 26.9 ± 5.1 yr). There was no significant difference concerning duration of gestation (39.5 ± 1.5 vs 39.4 ± 1.8 weeks), birth weight (3348 ± 457g vs 3289 ± 510g), percentage (80.8% vs 81.3%) and duration (5.4 ± 4.4 vs 4.6 ± 4.3 months) of breast feeding. Neonatal icterus (40% vs 26%, $p<0.05$) and neonatal infections (7.1% vs. 2.6%, $p<0.05$) were significantly more often observed in children who later on developed IDDM.

Our results do not support the hypothesis of early introduction of cow milk and IDDM-development. Larger multicenter studies (EURODIAB) may help to clarify whether there are regional differences in the importance of trigger factors for the manifestation of IDDM.

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THE INCIDENCE OF INSULIN-DEPENDENT DIABETES MELLITUS IN SOUTH BRAZIL;THE PASSO FUNDO REGISTRY REPORT.

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The frequency of Insulin-Dependent Diabetes Mellitus (IDDM) varies among countries. The WHO is trying to access these differences, through standardised recommendations of the Multinational Project for Childhood Diabetes (DIAMOND), in order to identify possible environmental causes. In this context, we have studied the incidence of IDDM in Passo Fundo, a city with 152, 110 inhabitants in highland region in Southern Brazil (latitude 28.10S, longitude 52.20W) in 1996. The eligibility criteria were that the person had to be born in the city or be a resident for at least one year, be under 15 years old, using insulin and his diagnosis of IDDM had to be made by a physician. The technique of capture and recapture was used. The primary source (capture) consisted of paediatricians (n=39) and endocrinologists (n=5) who were contacted by mail and telephone calls and, the secondary source (recapture) was information obtained at basic and nursery-schools. Also for recapture, a message was sent to the whole population in December through the newspapers, the radio and the television reaching respectively 20, 60 and 84% of the local population. The primary source identified 6 patients and all the 3 patients from secondary source were common to both sources. The variance and confidence intervals were not computed since all cases from the secondary were included in primary source. This situation indicates the necessity tp improve our secondary source in the future using hospital registries and insulin selling The incidence of IDDM was 12 cases/100,000/year. The degrees of ascertainment were 100, 50 and 100% for the primary, secondary and both sources. Therefore, our preliminary findings places our city in the middle range of incidence, together with Italy, Spain, New Zeland and Netherlands.

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PANCREATIC FIBROCALCULOUS DIABETES BUT NOT MALNUTRITION RELATED DIABETES HAS DISTINCT FEATURES WHEN COMPARED TO IDDM.

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Malnutrition related diabetes mellitus (MRDM) may be difficult to identify among our patients with insulin-dependent diabetes mellitus (IDDM) who have low income, deficient nutrition and difficulty to obtain adequate control with moderate insulin doses. We selected 35 patients who additionally had shown to be resistant to ketosis. 14 had pancreatic calculi on X Rays and their diabetes was classified as fibrocalculous (FPD). Their age range was 14 to 37 yrs at onset and 10 were males. 7 had been initially diagnosed as NIDDM but finally required insulin. 7 out of 10 could recall manutrition during childhood and all had a BMI < 20 at onset.12 had a history of abdominal pain.10 had neuropathy, 2 had retinopathy (after 6&12yrs) and 4 had nephropathy (after 3,5,7&12yrs). When compared with 48 IDDM patients matched for age at onset and duration, FPD patients were leaner (BMI 16.8±2.9 vs. 20 ±2.5 kg/mt2,$p<0.001$), required higher insulin dose (1.08±0.61 vs. 0.70±0.20 units/kg.day, $p<0.001$) and still had higher blood glucose (252±72 vs. 192±51 mg/dL,$p=0.001$). On the contrary, the 21 patients suspected to have MRDM but with negative X Rays, although also leaner (BMI 18.6±2.6,$p=0.01$) required similar insulin dose as IDDM patients (0.78±0.28 units/kg.day) for a not so high blood glucose (215±75 mg/dL). We conclude that FPD is a distinct entity which may only be recognized if searched systematically (including X Rays). On the contrary, we believe that MRDM may just be the reflection of poor environment and poorer treatment among IDDM patients.

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ONE OF THE LOWEST VALIDATED INCIDENCE RATES OF INSULIN DEPENDENT DIABETES MELLITUS IN THE AMERICAS: SANTIAGO, CHILE

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There is an extraordinary variation in the incidence rates of Insulin-Dependent Diabetes Mellitus (IDDM) in different countries and ethnic groups. Little is known, nevertheless, about the incidence of IDDM in Latin America, and Chile. These are the first reported data from Chile using the standardized methodology from the Multinational Project in Childhood Diabetes (DiaMond). The goal of this study was to estimate the average annual incidence rate of IDDM in Santiago from 1 January 1986 to 31 December 1992. The rates were calculated among subjects under 15 years of age, through a retrospective search and confirmation method from Hospitals and private offices of endocrinologists and specialists. A total of 252 registered cases were found, 118 boys and 134 girls, for an annual incidence of 2.36/100,000 hab.year (IC95%: 2.07 - 2.67). The maximum incidence was found in girls 10 - 14 years (3.91; IC95%: 3.0 - 5.02). The estimated incidence in Santiago de Chile represents one of the lowest validated rates in the Americas.

Supported by Grant Fondecyt 1960395

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FOOD/NUTRIENT CONSUMPTION AND THE INCIDENCE OF INSULIN-DEPENDENT DIABETES IN CHINA.

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The association of insulin-dependent diabetes mellitus (IDDM) incidence and 25 food items and 25 nutrients was examined in China. The China IDDM registry had 22 centers and monitored more than 20 million children under age 15. There was an enormous geographic variation in the IDDM incidence rates across China. People living in different areas of China have quite different eating habits; thus this study provided a good opportunity to investigate the relationship of IDDM and food consumption. The food and nutrient consumption data were obtained from the third Chinese National Nutrition Survey. A three-consecutive-day dietary survey was carried out by a weighing method and a 24-hour dietary intake recall. Among the food items studied, 9 were found to be significantly correlated with IDDM incidence (5 positively and 4 negatively correlated with IDDM). These food items were milk (r =0.72), vegetable oil (r =0.5), cake/dessert (r = 0.51), sugar (r =0.77), paste/seasoning (r =0.46), dark colored vegetable (r = -0.46), animal fat oil (r = -0.42), rice (r = -0.41) and dry legume (r =-0.4). There were also 4 nutrients identified to be significantly positively correlated with IDDM (energy intake (r =0.46), fat (r =56), alpha tocopherol (r =53), vitamin E (total) (r =0.46). However, no nutrients were found to be negatively correlated with IDDM. The current study suggested that the huge geographic differences of IDDM incidence rates across China could be in part contributed to certain food consumption which is consistent in part with known risk factors (e.g. milk) but in an extremely low risk country. The existence of possible susceptible and protective foods toward IDDM may provide us directions to the prevention and treatment of IDDM.

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STUDY ON THE INCIDENCE OF INSULIN-DEPENDENT DIABETES IN THE LAZIO REGION (CENTRAL ITALY) FROM 1989 TO 1995.

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As part of the EURODIAB Subarea A, the study was carried out over a period of seven years, from January 1, 1989 to December 31, 1995. Evaluation of new cases of IDDM under 15 years of age was conducted at the region's antidiabetic centers and validated at centralized health centers. In seven years, 467 new cases of IDDM were identified, 230 female patients and 237 male patients; the average **annual incidence** was 8.43/100000 (in males 8.34/100000 and in females 8.53/100000). The incidence rates for each year, standardized on the data of Italian Statistical Year Book (1991) were: 8.09/100000 in 1989; 7.96/100000 in 1990; 7.84/100000 in 1991; 9.74/100000 in 1992; 7.58/100000 in 1993; 8.47/100000 in the 1994 and 9.36/100000 in 1995; differences by years are not significant. The incidence by age group was 6.84/100000/year in the 0-4-year-old age group, 10.09 in the 5-9-year-old age group and 8.33 in the 10-14-year-old age group. The **age** at which the most cases were shown was 9 years (10.7%). No statistical differences were observed in **sex** distribution. Place of residence of patients varied within the region, but the majority (66%) of them live in main towns in the Lazio region, 24% in smaller towns and 10% in rural areas, thus reflecting the distribution of the population in this region. The **seasonal trend** of incidence confirms that cases occurred more frequently during the winter (35.8%) and in spring (25.3%), whereas they diminished during summer and autumn (20.1% and 18.8%, respectively). 72.1% patients had been **breast fed** while the remaining 27.9% had been bottle fed. **Family history** of patients revealed that 23% of them have relatives who suffer from type 1 diabetes, while 55% have relatives with type 2 diabetes. With regard to the **disease severity at diagnosis**, the majority of patients (86.9%) were in ketosis and only 18 (3.9%) patients were in a state of coma. In conclusion, incidence of IDDM was found to be remarkably constant over the period of observation, higher in the 5-9-year-old age group, and in the winter period.

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COW'S MILK CONSUMPTION ASSOCIATED WITH DISEASE SPECIFIC AUTOANTIBODIES IN SIBLINGS OF CHILDREN WITH IDDM

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The association of infant feeding patterns and milk consumption with IDDM specific autoantibodies was studied in initially non-diabetic siblings of children with IDDM. The siblings were followed at half-year intervals for 4 years since the diagnosis of IDDM in the index child. At the end of follow-up 17.2 % (127/737) of the siblings were positive for ICA (≥2.5 JDF-units), GADA (>6.6 relative units), or IAA (>54 nU/ml). Those children who were positive for at least one IDDM specific auto-antibody at the end of follow-up (n=99) were compared to antibody negative ones (n=376) so that only one child from each family was included. Overall or exclusive duration of breast feeding were not associated with antibody positivity, neither was the age at introduction of cow's milk supplementary feeding. Cow's milk consumption during childhood was positively associated with antibody positivity, also when adjusted for age and sex (highest quartile vs. lowest OR=2.6, 95 % CI 1.2-5.5). To conclude, high consumption of cow's milk during childhood may be a risk determinant of seroconversion to positivity for IDDM specific autoantibodies.

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LOW INCIDENCE OF IDDM DURING 1985-1995 IN THE REPUBLIC OF MACEDONIA

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Nation wide registry for IDDM in childhood (0-14 years of age) has been set up for the Republic of Macedonia since 1985. This country is a cold spot for IDDM incidence in Europe and among Caucasian populations as a whole, since the overall incidence for the period 1985-1991 was 2.45 (95%CI 2.03-2.96) per 100 000 per year. Majority of the population is of Slavic origin. Genetic analysis of HLA DQB1, DQA1 and DR genes has confirmed high percentage of resistant and neutral haplotypes in the general population. The objective of the present study was to evaluate the dynamics of IDDM incidence over an eleven year period. Overall yearly IDDM incidence in the period 1985-1995 was in the range 1.84-4.11 (3.11+0.79)/per 100 000 per year. The IDDM incidence was lowest in the age group 0-4 years, 1.50 (95% CI 0.55-2.45), and highest in the age group 10-14 years, 4.46 (95% CI 2.84-6.08). Although the incidence among females was slightly higher 3.43 (95% CI 2.24-4.62), sex difference was not statistically significant. There were two sharp increments of the number of newly diagnosed patients during September 1995 and February 1996 (3-4 times compared to the number of cases in those months during previous years). Enteroviruses were isolated from all blood cultures at diagnosis during these events. Concerning the seasonality, the lowest incidence in this population appears in the period March-May, and the highest in December -February. We conclude that overall incidence during the last 11 years shows stable patterns in this population with a very low IDDM incidence.

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IMMUNOSUPPRESSING EFFECT OF COW MILK BETA-CASOMORPHINE IN PREDIABETIC MICE AND HUMANS

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Type 1 (IDDM) diabetes results from a genetically determined immune destruction of islet beta-cells, following early exposure to a diabetogenic environmental agent. The strong correlation of national milk consumption with diabetes incidence suggests cow milk (CM) as such an environmental agent. This notion is supported by the promotion of diabetes in the NOD mouse by CM casein in a diabetes prone rodent model (NOD mouse). This diabetes promoting activity is found solely in A1 beta casein not the A2 variant. A1 beta casein alone yields beta-casomorphine-7 (BCM) after intestinal digestion. The diabetogenic effect of A1 casein is neutralised by the u-opioid antagonist naloxone. Synthetic BCM is shown to inhibit the PMA stimulated oxidative burst of macrophages derived from prediabetic NOD mice or (islet antibody positive) children, but not Swiss mice or control children. BCM depresses lymphoblastic responses to Con-A by normal human peripheral blood and intestinal cells.
The primary immune response to ovalbumin is totally extinguished by co-administered BCM in the NOD mouse, but not Swiss mice.
The marked opioid like immunosuppression found with this peptide product of cow milk A1 beta casein digestion, in IDDM predisposed mice and humans is likely to be causally related to the specific diabetogenic effect of the A1 cow milk variant.

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THE INCIDENCE OF CHILDHOOD-ONSET INSULIN-DEPENDENT DIABETES IN ROMANIA

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A Romanian collaborative study group (ONROCAD) has been established to provide descriptive epidemiological information on insulin-dependent diabetes mellitus (IDDM)in nationwide Romania. Based upon submission of data from all 41 Romanian districts, 706 new cases (302 boys and 404 girls) of IDDM with onset 0-14 years were registered during 1992-1995 (4 years). The overall completeness of ascertainment was estimated at 93.5% (91.6%- 95.3%) with no major differences between the 3 main regions of Romania (Transilvania, Moldova and Muntenia). For whole Romania, the incidence of childhood onset was estimated at 3.57 per 100 000 per year. We found statistical differences in the incidence of IDDM between these 3 regions, after stratification for sex ($p < 0.05$). Girls had higher incidence than boys for each of the age groups 0-4, 5-9 and 10-14 years ($p < 0.05$). Between these age groups, the incidence showed statistically significant heterogeneity ($p < 0.0001$), due to a low rate for the age group 0-4 years. The incidence at district level showed significant variation, evident in girls, but not particularly in boys. We conclude that the incidence of childhood- onset IDDM is among the lowest recorded in Europe, with so far unexplained variability between Romanian districts.

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INSULIN-DEPENDENT DIABETES MELLITUS AND MILK'S CONSUMPTION: A CASE-CONTROL STUDY IN SÃO PAULO, BRAZIL

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A case-control study was conducted to test the assumption that breast feeding protects against insulin-dependent diabetes mellitus, and that the early introduction of cow milk into the infant diet is a risk factor for the disease. Three hundred and forty-six diabetic children, less than 18 years old at the moment of the interview, were identified in two institutions in the city of São Paulo. The duration of exclusive breast feeding and the age of introduction of cow's milk products in the infant feeding of the cases were compared with 346 paired control cases for sex, age and place of residence. The ages of the parents when the child was born, the history of congenital rubella, the duration of gestation, the weight and the height at birth, the vaccination status, the child's viral disease history, the child's history of severe attacks of diarrhoea, the date of insulin therapy, previous familial history of insulin-dependent diabetes mellitus and non-insulin-dependent diabetes mellitus, the per-capita income of the family, and the order of birth of the child were considered as possible confouding variables. All comparisions between diabetic and control children were done using paired tests. The findings of this study indicate that the lack of exclusive breast feeding, particularly during the first week of life, is a risk factor to insulin-dependent diabetes mellitus (OR=2.13; 95% IC:1.28-3.55) and that the age at introduction of milk products, particularly when these products are introduced during the first seven days of life, is a risk factor for the disease (OR=2.29; 95% IC: 1.37-33.83).

Grant 94/0943-0 from Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP)

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EPIDEMIOLOGY OF DIABETES: INCIDENCE, PREVALENCE AND MORTALITY IN MOSCOW CHILDREN.

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Epidemiolodgy study incidence, prevalence and mortality in group children with IDDM aged 0-15 years in Moscow population. Two sources of information were used: Children Hospitals' records and annual reports from all city's pediatric endocrinologists, to estimated we used capture-recapture method. Incidence was 5.17 per 100000 in 1967 - 1977 and during 1978-1990 - 9.7., 1990-1995 - 12.6 The increase in epidemiological "peak" was noted in 1985; number of new cases of IDDM 149, (incidence 10.1 per 100000). In the end of 1992 the prevalence was 50.9 per 100000 in Moscow-city, (children population was 1.772.581), the 1995 61,6. Mortality survival rate (Jan.1980- Dec.1989). All cases were verified by autopsy reports. In whole 28 deaths (20 girls and 8 boys) were revealed 12 cases occurred at the clinical onset of diabetes, and this makes 1.07 % of all diabetic children diagnosed during this 10 years. Mortality in 1995 was 0,06 per 100000. The remaining 16 deaths were classified as following: diabetic ketoacidosis -9,cases, infections-3, accident-1, cancer-1, others-2. Diabetic ketoacidosis was the main reason of death in analysed group. The above study is the first step of the National Register IDDM in Russia.

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NO IDDM PROTECTIVE EFFECT OF BREAST FEEDING IN ISRAEL.

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This study's aim was to evaluate effects of breast feeding and cow's milk introduction and their interaction with ethnicity on incidence of IDDM in Israel. Environmental factors, in addition to major genetic effects, likely have a crucial role in triggering beta cell autoimmunity in IDDM. Early introduction of cow's milk is suggested to predispose for, and breast feeding (BF) to be protective against, IDDM development. In Israel IDDM incidence varies among the different ethnic groups; thus, we initiated a study to evaluate the role and interaction of genetic and non-genetic factors in this disease. We obtained data concerning BF and cow's milk introduction in 199 IDDM patients (mean age 15.7 yrs±10.6 s.d.) and in 151 healthy controls (15.5±6.6). We applied several logistic regression models which initially examined feeding factors and their interaction with ethnic factors. As ethnic factor interactions had no significant effects, subsequent regressions applied reduced factor models as appropriate. The final resulting model contained only independent variables describing whether or not subjects were breast fed, and delay (months) of introduction of cow's milk. The overall regression model was significant (-2 log likelihood chi-square=9.575, df=2, p=0.0083; Score chi-square=9.497, df=2, p=0.0087). BF was significant (Wald chi-square=4.66, p=0.0309), with an odds ratio of 2.018 (95% CI 1.066 to 3.818) indicating BF as *predisposing* to development of IDDM. Delay of cow's milk introduction was also significant (Wald chi-square=7.08, p=0.0078), with odds ratio of 0.924 (95% CI 0.873 to 0.980) indicating a protective or sparing effect of 0.924 per month's delay. In conclusion, our results show that both BF and early cow's milk introduction are associated with development of IDDM in all ethnic groups in Israel,suggesting that the effect of feeding on IDDM development is not associated with a specific factor and that it spans across ethnicity.

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EPIDEMIOLOGICAL ASPECTS IDDM IN MOSCOW REGION

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OBJECTIVE: This study was designed to estimate the prevalence and incidence of IDDM and its main complications in Moscow Region. METHODS: During 1994-1995 years was created Regional Diabetes Register (RDR). RDR include all patients with IDDM in Mytishinsky District of Moscow Region. RESULTS: the prevalence of IDDM was 85,39 per 100 000. The prevalence level among teenagers from 14 to 17 years (88,11 per 100 000) was little high than among adults (85,22 per 100000). Study of IDDM distribution on age has shown heaviest prevalence in the age groups 20-24 years old (221,45 per 100 000), 35-39 years old (123,26 per 100 000) and 45-49 years old (158,12 per 100 000). We have estimated the incidence of Type 1 diabetes. The IDDM incidence in 1994 was 3,28 per 100 000, that is comparable with data of the national registers of many countries. The high incidence level was in age groups 14-15 years old and 18-19 years old (22,66 and 39,22 per 100 000). The analysis of a structure of IDDM complications has shown, that 59.23 % of patients has retinopathy, 55.38 % - neuropathy and 23,84 % - nephropathy. The HbA1 level was 14.86±3.40% (n=50). The prevalence of microalbuminuria was 52.28%.

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BREAST FEEDING; DEVELOPMENT OF DIABETES MELLITUS.

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The aim of this study was to determine the prevalence of infant feeding patterns and to compare the humoral immune response to bovine serum albumin (BSA) in patients with IDDM and NIDDM in Azerbaijan population. By the randomize method performed epidemiological analysis of 324 patients card. This data were the basis of study of serum samples from 90 patients with IDDM and 50 patients with NIDDM for anti-BSA-antibodies level by enzyme-linked immunosorbent assay (ELISA). By us was establish that nonexclusive breastfeeding was prevalent amongst patients with IDDM and constituted 60,5% and was rare occurence among patients with NIDDM (only 4,8%), P<0,001. The patients with IDDM who have had nonexclusive breast feeding in infancy had higher level of antibodies to BSA (in 1,5 time more) than did patients with IDDM but were exclusively breast fed. Furthermore, the level of anti-BSA-antibodies did not significantly differ between patients with NIDDM and IDDM, who have had only breast feeding in infancy. Results of researches confirm the provoke role of nonexclusive feeding in manifestation of IDDM and immediately participation of BSA in the autoimmune pathophysiological processes of induction of this disease.

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THE PECULIARITY OF TYPE 1 DIABETES INCIDENCE AT NOVOSIBIRSK AREA ACCORDING TO REGISTRY DATA

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The main aim of the study was to compare the Type 1 diabetes incidence at big Novosibirsk City and other smaller places of Novosibirsk Area. The analysis of incidence among children from 0 to 14 years was conducted for 1980-1994 years according to "DIAMOND" protocol. We have revealed significant statistical difference in incidence of IDDM among children from industrial Novosibirsk-City(total population about 1,5 million) in comparison with children living in smaller places: 6.1±0,5 (95% CI:5,0 - 7,1) and 3.9±0,4 (95% CI:3,1-4,6) cases per 100 000 children respectively. Also the mean annual incidence of Type 1 diabetes was significantly higher in 1990-1994 study period-7,9 per 100 000(95% CI:6,0-9,8) than in 1980-1984 study period where it was 4,4 per 100 000(95% CI:3,2-5,6) only among Novosibirsk City children. We have not found the time trends of incidence of Type 1 diabetes among children of small towns and villages (with the same genetic background) around the Novosibirsk City. The most likely reason for these phenomena are presence and increasing power of still unknown casual agents at the big Cities in Siberia.

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ENHANCED T-CELL RESPONSE TO COXSACKIE VIRUS B4 IN PATIENTS WITH NEWLY DIAGNOSED IDDM

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Enterovirus infections are associated with insulin-dependent diabetes mellitus (IDDM). We studied T-cell proliferation response from patients with newly diagnosed IDDM and from healthy children to a purified Coxsackie virus B4 (CBV4) (1 µg/ml) and to two vaccine antigens, a purified polio virus 1 (PV1) (1 µg/ml) and tetanus toxoid (TT) (10 µg/ml). IgG-antibodies to CBV4 were measured by RIA. HLA DQB1 genotyping was done in a subgroup of patients (n=36) and controls (n=23). T-cell reactivity to CBV4 was found in 27% patients and in 10% control children (20/74 vs 5/48 p=0.04), when the cut off was three multiples of median SI (SI>7.1) in the controls. No difference in the T-cell reactivity to PV1 (4/28 vs 0/16; p=0.3), or to TT was found between the groups. In IDDM patients, T-cell responses to enteroviral antigens, CBV4 and PV1, correlated (r=0.70, p<0.0001); the correlation was weaker in controls (r=0.49, p=0.05). The levels of antibodies to CBV4 did not differ between the groups (16.5 EIU vs 21.0 EIU; p=0.8). Correlation between humoral and cellular responses to CBV4 was better in patients than in controls (r=0.23 and r=-0.04). T-cell reactivity to CBV4 was found in 9 of 30 patients with HLA DQB1*0201 and/or 0302, whereas in none of 6 patients without risk alleles. Enhanced T cell reactivity to CBV4 was found at the time of IDDM diagnosis, although no increased levels of IgG-antibodies to CBV4 could not be demonstrated. Our results suggest that HLA risk alleles may influence to T cell reactivity to CBV4. Alternatively, enhanced T-cell response may reflect preceding enteroviral infection(s) as suggested by prospective studies.

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DATA FROM IDDMREGISTRY- BANGALORE DISTRICT (URBAN)

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Aim to know incidence and mortality of IDDM in Bangalore district Urban and other data. The IDDM registry is maintained at M.S.Ramaiah Medical College, as per the specification of Diamond Project. The primary objective is to register all known cases of childhood diabetes in Bnagalore, to know incidence and mortality. Secondary objective to analyze other data. The area studied - Bangalore district Urban- 2,1990 sq km with population 4,807,019 (census 1991) covering Anekal, Bangalore North and South and Bagalore city. Registry maintained by capture recapture method from primary and secondary sources. 214 patients registered till Nov 1996. 116 boys and 98 girls. In the year 1995 -96 18 new cases registered. Incidence 1.68/100,000/year. Age group 0 - 4 yr. - 6%, 5 - 9 yr. - 68%, 10 - 14 yr. 36%. Maternal age 21 - 25 yr. 26 %, 26 - 30 yr. - 54% more than 30 yr. - 30%. Rural urban ratio 52:162. Socioeconomic status 48.15% - middle income group 23.56% lower income group, 28.29%, upper income group. Complications- Nephropathy 3, Hypertension 1, Diabetic foot 3.retinopathy-3. Mortality -DKA 2 Nephropathy 1, Lung abscess 1, GI Bleed 1. Intensive insulin treatment 53.1% other endocrine abnormalities 15% had positive thyroid antibodies, 82.1% showed positive height compared to parental height. 11.1% height less than 3 SD. PCOS -1, Turner 1. Conclusion incidence of IDDM in Bangalore distirct Urban a south Indian district of Karnataka is 1.68%/100,000/year. Mortality is 2.33%.The age group most coomonly presenting is 5-9 yr.The maternal age and income was not significant.There was a positive corellation for height in 82.1% of IDDM.DKA was the most common cause of death.

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HLA RESTRICTED T-CELL RESPONSE TO ENTEROVIRUS ANTIGENS IN NEWLY DIAGNOSED IDDM PATIENTS

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Enterovirus infections may trigger the beta cell damaging process leading to clinical IDDM. Cellular immune response is important both in the elimination of the virus and in the autoimmune destruction of beta cells. In the present study lymphocyte proliferation responses to enterovirus antigens were analysed in 46 newly diagnosed IDDM patients and in 25 control subjects using standard blast transformation test and highly purified coxsackievirus B4, poliovirus types 1 and 3 and adenovirus hexon protein as antigens. There was no significant difference in enterovirus or adenovirus responses between IDDM patients and control subjects. However, proliferation responses to coxsackievirus B4 and poliovirus types 1 and 3 were significantly modulated by the HLA genotype. HLA- DRB1*03 positive patients had significantly lower responses than DRB1*03 negative patients (p<0.05-p<0.001). Median stimulation indices for coxsackievirus B4, poliovirus types 1 and 3 were 7.0, 3.0 and 1.6 in DRB1*03 positive patients compared to 11.4, 11.0 and 13.1 in DRB1*03 negative patients. No HLA association was found in adenovirus responses. T cell responses were not correlated with antibody levels to corresponding virus antigens. The results suggest that the DRB1*03 genotype is associated with weak cellular immune responsiveness to enterovirus antigens.

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INSULIN DEPENDENT DIABETES IN CHINA AS PART OF THE WHO DIAMOND PROJECT.

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The current study investigated the incidence rate of insulin-dependent diabetes mellitus (IDDM) in China. The China IDDM registry was established in 1991 as part of the WHO DiaMond project. Twenty-two centers were setup to monitor the IDDM incidence cases for children under age 15. The population under investigation was more than 20 million, which represents about 7% of children in China. Seven major ethnic groups were involved in the study. At least two sources were employed to identify IDDM cases and capture-recapture method was used to adjust for the underascertainment. The primary source was the hospital medical records. The secondary source includes the records from school health program, Family Planning Committee, insurance company, Child-Woman Care Network and anti-epidemic station. The investigation period was from 1985 to 1994. A retrospective registration by reviewing the records from the primary and secondary sources was employed to identify the cases diagnosed before 1991. In total, 592 IDDM cases were identified from 22 centers with an annual crude incidence rate 0.48/100,000. The capture-recapture method estimated that there were 630 IDDM cases with an ascertainment corrected incidence rate of 0.51/100,000. The sex-specific rates were 0.47/100,000 for boys and 0.59/100,000 for girls. There was a 12-fold difference among 22 centers (0.13 - 1.61/100,000), the largest within country variation even seen. In general, the incidence rates were higher in the north and in the east. There was also a 7-fold difference among various ethnic groups (highest: Mongol 2.12, lowest: Zhuang 0.32/100,000). The current study showed that China had the lowest IDDM incidence rate ever reported, as well as the largest within country variation in the largest IDDM registration system.

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ENTEROVIRUS RNA AND IDDM-RELATED AUTOANTIBODIES IN PREDIABETIC CHILDREN

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We have previously shown that enterovirus infections may initiate and accelerate the beta cell damaging process several years before clinical IDDM. In the present study the presence of enterovirus RNA was studied in the sera of initially healthy children who were followed until they manifested clinical IDDM, as well as in newly diagnosed IDDM patients and age- and sex-matched control subjects.

Altogether 33 follow-up samples from 7 prediabetic children were analysed using an RT-PCR method able to detect 1 PFU of virus per serum sample. Enterovirus RNA was found in 3 of these children in altogether 6 samples, taken 0.75 -3.4 years before the diagnosis of IDDM. In two children increases in IDDM-related autoantibodies were observed coincidently with the presence of enterovirus RNA in their serum. These findings are in line with our previous findings of enteroviruses as initiating and accelerating factors in the pathogenesis of IDDM. However, our analyses did not show an excess of enterovirus RNA in children with newly diagnosed IDDM (4 positive out of 35) as compared with control subjects (5/35). This suggests that the diabetogenic effect of enteroviruses may be most important during the early phases of the pathogenesis.

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EPIDEMIOLOGY OF CHILDHOOD DIABETES IN HONG KONG: A PROSPECTIVE STUDY

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Epidemiological data on childhood diabetes in Chinese are limited. A registry of childhood IDDM was established in 1991 to collect new cases of childhood IDDM from four districts in the northeast region of Hong Kong. Data were collected according to the WHO DIAMOND protocol. The registry currently covers a population base of approximately 1.1 million. Of these, 250,000 are under 15 years of age. Data were collected retrospectively from 1986 to 1990 and prospectively from 1991 onwards. Primary ascertainment was based on review of medical records at a regional hospital serving the area. Independent school surveys validated the registry data. Capture-recapture estimates indicated the overall level of ascertainment was 94%. A total of 35 cases of childhood IDDM were diagnosed between Jan, 86 to Dec, 95. The mean yearly incidence rates of childhood IDDM were 1.7/100,000 (95% CI 0.5-4.3) during 1986 to 1990 and 1.3/100,000 (95% CI 0.3-3.8) during 1991 to 1995. The yearly incidence rate for females was 1.9/100,000 (95% CI 0.2-7.0). For males, it was 1.0/100,000 (0.0-5.7). In conclusion, the incidence for childhood IDDM in Hong Kong Chinese is low and females appear to have a slightly higher incidence rate than males.

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CHILDREN WITH DIABETES SHOW NO TIME-SPACE CLUSTERING AT BIRTH

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The aetiology of IDDM remains largely unclear, but it is known the disease process involves an environmental component. Recent research has suggested perinatal events may be important, and the present study aimed to detect evidence of space-time clustering of births in children subsequently developing IDDM. Such a pattern would be suggestive of an infectious agent acting around the time of birth. The population basedYorkshire Childrens Diabetes Register collected details of children diagnosed with IDDM between 1978-1994 and is estimated as 97% complete. The residential address of the mothers at the time of birth of each child on the register was ascertained from birth certificates via the National Health Service Central Register and assigned a National Grid Reference. The Knox-Mantel test was applied to detect space-time clustering in the births of 1711 cases. Forty-two combinations of a wide range of spatial and temporal thresholds were tested (90-900 days, 1-20km). No evidence for space-time clustering was found ($p<0.05$) in the pattern of births of those children going on to develop IDDM during childhood. This the first UK study examining this issue and the results do not support the hypothesis of perinatal infections having an aetiological role in childhood IDDM.

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PREVALENCE OF INSULIN-DEPENDENT DIABETES MELLITUS AMONG YOUNG FINNISH MALES IN 1975-1995

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Finland has the highest incidence of insulin-dependent diabetes mellitus (IDDM) in the world. In Finland, all the males are ordered by the law to enter call-ups for common military service at the age of 18 years. The aim of the study was to describe the prevalence of IDDM among Finnish males at call-ups by municipality and year (birth cohort), during the study period 1975 - 1995. We used data provided by Finnish Defence Forces, Division of Health Care (FDF). The data contained aggregated information by district, service class, diagnoses, and numbers of checked males at annual call-ups. IDDM persons are without exceptions classified into service categories C or D (relieved from service in peace or war time, respectively) since 1973, and they enter call-ups only once.A case of IDDM was defined as a person, who at the time of the check-up had a well verified diagnosis of diabetes (ICD-8 or ICD-9: 250), and was classified into service category C or D. We calculated prevalence (i.e. cumulative incidence) per 1,000 persons by municipality and in the whole country. During the study period of 1975-1995, there were 4,281 IDDM cases among 715,212 males producing a crude prevalence of 6.0 per 1,000. The crude prevalence corresponds to an approximate annual incidence of 33.3 per 100,000. The lowest annual prevalence of 5.1 was observed in 1977, while the highest (6.9) in 1987. There was no clear trend in prevalence. By administrative municipality, the highest prevalences of IDDM were found in Eastern and Central Finland, and the lowest in Northern Finland. The observed variation between municipalities by study year was small as was variation by year within municipalities. Thus, the cumulative risk of IDDM in boys by age 18 remained unchanged while in young children the incidence has clearly increased during this period.

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THIS ABSTRACT HAS BEEN WITHDRAWN BY THE AUTHOR.

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MORTALITY IN YOUNG DIABETIC SUBJECTS. A TWELVE - YEAR LITHUANIAN CHILDHOOD COHORT STUDY

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Patients with IDDM are known to have increased risk of dying mainly because of acute and late complications of diabetes. The aim of present study was to determine mortality and causes of death among young patients with IDDM in Lithuania. The patients (n=698) were identified through the childhood register from 1983 to 1995. All these patients were young subjects: 360 males, age range (1.21-25.83 yrs) and 338 females (3.49-25.15 yrs). M/F ratio - 1.065. Cohort distribution by age was normal (skewness-0.096). Standard DIAMOND criteria for the diagnosis of IDDM were used. During the period of 01.01.95 - 01.06.95 the status of the cohort members was checked for 'alive' status. Search for IDDM onset deaths was a separate activity of mortality study in Lithuania. All deaths were coded according ICD-9 criteria. Totally, 25 deaths (2 onset) were registered. Hyperglycaemic coma was registered as a cause of death for 10 cases (40%). The observed/expected numbers of deaths (O/E ratio) of all cause mortality was 7.71 (CI 95% 2-sided 4.83-11.67, $p<0.001$), and 4.91 (CI 95% 2-sided 2.68-8.23, $p<0.001$) not directly attributed to diabetes. O/E ratio of all cause-mortality depending on duration of diabetes - for 0-5 yr duration group - 6.45 (CI 95% 2-sided 3.33-11.27, $p<0.001$), 6-13 yr duration group - 10.07 (CI 95% 2-sided 4.83-18.51, $p<0.001$). O/E ratio for causes not directly attributed to diabetes - 3.23 (CI 95% 2-sided 1.18-7.02, $p=0.012$) and 8.05 (CI 95% 2-sided 3.48-15.86, $p<0.001$), respectively. Conclusions: 1. Mortality risk for childhood onset diabetes in Lithuania is very high; 2. Mortality risk increases with longer duration of diabetes.

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SEASONAL VARIATION OF INSULIN-DEPENDENT DIABETES MELLITUS IN SARDINIA AND FINLAND

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The aim of this study was to compare the seasonal variation at the clinical onset of IDDM based on 1405 cases in Finland and 425 cases in Sardinia diagnosed at 14 years of age or under during 1989 to 1992. The average annual incidence of IDDM in Finland was 36.4/100,000 and 34.4/100,000 in Sardinia. Seasonal patterns were estimated presenting the data as short Fourier series up to three harmonics together with a possible linear trend. Likelihood ratio tests and Akaike's information criterion were used to determine the number of harmonics necessary to model the seasonal pattern. Seasonal patterns in both countries were compared between sexes and between the three 5-year age groups each controlling for the other's effect. In both countries, during a calendar year a significant seasonal pattern was found for the sexes combined and for two age groups (0-9 and 10-14 years). In Sardinia, two distinct cycles were found among the younger age group with a decreased incidence during May-August and a higher incidence during the autumn months. Two cycles were apparent for the older group with a nadir occurring during June-September. In Finland, one cycle with a decreased incidence in the summer was found in the younger age group. In the older age group, there were two distinct cycles with a decreased incidence in June and in the September-December period. The seasonal variation in the incidence of IDDM was clear in both countries, however, the pattern of variation was different. Although Finland and Sardinia have the highest IDDM incidence in the world, they are geographically remote from each other and therefore the difference in seasonal variation of IDDM incidence can be explained by climatic factors.

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CLINICAL CHARACTERISTICS OF TYPE 1 DIABETES AT ONSET IN CHILDREN AND ADOLESCENTS

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Objective: Aim of this study was to describe the clinical presentation of Type 1 diabetes at diagnosis in a population-based cohort of children and adolescents and to analyse the association of clinical characteristics and age at onset. **Methods:** During 1993-95 291 new cases of Type 1 diabetes diagnosed under the age of 15 years were prospectively registered via an active surveillance system in a geographically well defined region around Düsseldorf. Detailed information on demographic data, duration of symptoms before onset, and clinical and biochemical presentation at diagnosis were obtained from hospital records. **Results:** Hospital records of 233 patients (80% of all eligible, 123 boys) could be evaluated. The distribution of age at onset was 0-4yrs: 22.7%, 5-9yrs: 34.3%, and 10-14yrs: 42.9%. The most frequently reported symptoms were polyuria (93,6%), fatigue (66.1%), weight loss (59.7%), and abdominal pain (15.9%). Polyuria was the first symptom in 85.8% of the patients with a mean duration of 3.3 ± 3.0 (± SD) weeks. Mean weight loss before diagnosis was 9.3 ± 5.7% of body weight. Weight loss was significantly higher in the youngest age group ($p < 0.05$). 35% of the children had a reduced skin turgor at clinical examination and 21.7% an impaired consciousness. Mean values of plasma glucose and glycosylated haemoglobin (relative to upper normal value) at diagnosis were 451 ± 197 mg/dl and 191 ± 50.6 %, respectively. Whereas glucose levels were significantly higher in the youngest age group ($p < 0.05$), glycosylated haemoglobin values were significantly higher in the oldest age group ($p < 0.05$). Ketonuria was present in 80.5%, plasma bicarbonate values < 20 mmol/l in 48.3% and acidosis (pH ≤ 7.2) in 16.8% of the children. Prevalence of acidosis was more common in children under 5 years than in the older age groups (0-4yrs: 20.8%, 5-9yrs: 15.1%, 10-14 yrs: 15.6%). **Conclusions:** This population-based analysis showed less severe clinical symptoms and metabolic dearrangement at disease onset than previously reported in other studies. For the most part the degree of metabolic decompensation was relatively minor. Ketonuria and acidosis were more frequent in the youngest age group.

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THE SEASONAL DISTRIBUTION OF BIRTH OF PATIENTS WITH IDDM IS IDENTICAL TO ALL BIRTHS IN FINLAND

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It has been suggested that environment, e.g. virus infections, very early in life or even in utero would be important for the causation of IDDM. The aim of this study was to compare the seasonal distribution of birth of children with IDDM with all births in order to examine the possible differences in these distributions. The seasonal distribution of birth of 3604 children with IDDM diagnosed <15 years of age during 1987-1995 and born during 1976 to 1995 was compared with the seasonal distribution of births of 1,283,306 healthy children (children without IDDM) born in the same period. The fitting of the monthly distribution of births of children with IDDM and healthy children was tested using the X^2-test. The test was carried out for both sexes together and for boys and girls. Results showed that these two distributions did not differ from each others. Thus, in Finland, the monthly distribution of births of children with IDDM followed the monthly distribution of the healthy children born in the same period. The clear seasonal variation of IDDM incidence in Finland can hardly be explained by climatical factors or early exposure to certain infectious agents which are seasonal in the environment.

Distribution of births of children with IDDM and healthy children per month during 1976 to 1995 in Finland

		J	F	M	A	M	J	J	A	S	O	N	D	Tot
IDDM	n	321	278	305	342	313	311	296	291	301	312	264	270	3604
	%	8.9	7.7	8.5	9.5	8.7	8.6	8.2	8 1	8 4	8.7	7.3	7.5	100
Healthy	%	8.1	7.8	9.2	8.9	8.8	8.5	8.6	8.4	8.3	8 0	7.6	7.8	100

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THE DIABETES PREVENTION TRIAL-TYPE 1 DIABETES (DPT-1): PROGRESS REPORT.

DPT-1 Study Group, Nationwide, USA

The DPT-1 is a nationwide study designed to determine whether insulin based therapies can delay or prevent the clinical onset of Type 1 diabetes in relatives found to be at high risk of development of the disease. Parenteral insulin is used in relatives with >50% projected 5-year risk. Oral insulin is used in relatives with 26-50% projected 5-year risk. Screening began in February 1994. Randomization began in December 1995 for the parenteral protocol and in September 1996 for the oral protocol. By November 30, 1996 there were 43,544 samples received for screening, of which 40,657 (93.4%) were eligible samples, a rate consistent with initial projections of screening 15,000-20,000 subjects per year. Of the 40,381 samples analyzed for islet cell antibodies (ICA) by 11/30/96, 1391 were positive. The rate of positive samples, 3.44%, is virtually identical to that projected in calculating sample size (3.6%). Of the 937 individuals who were staged for risk, 259 (27.6%) had low first phase insulin response (FPIR) to IVGTT, a slightly lower percentage than projected (35%), but within an acceptable range. Of the subjects who completed Staging for risk categorization, 141 were eligible for the parenteral study, and of these 131 (92.9% of those eligible) were randomized to the parenteral study. Of the 28 subjects eligible for the oral study, 26 (92.9% of those eligible) were randomized. Overall enrollment rates are 74.4% of that projected for the parenteral study and 89.7% of that projected for the oral study, by this point in time. These enrollment rates are comparable to those found in most multi-center randomized clinical trials. These data indicate that the statistical projections used in the design of DPT-1 are appropriate. DPT-1 is proceeding as planned.

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MOLECULAR MECHANISMS FOR THE PREVENTION OF AUTOIMMUNE IDDM BY TREATMENT WITH A SUPERANTIGEN IN NOD MICE.

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We have previously shown that exogenous superantigens prevent the development of autoimmune IDDM in NOD mice. This investigation was initiated to determine the mechanisms involved in the prevention of IDDM by the superantigen staphylococcal enterotoxin B (SEB). First, we examined whether clonal deletion or clonal anergy of T cells is involved in the prevention of IDDM. We observed a mild but selective expansion of Vβ8 cells at three days after SEB injection, followed by a deletion of T cells at 14 days after injection. However, the number of Vβ8 cells and the proliferative responses of splenic T cells return to normal four weeks after SEB injection. Thus, the mild and transient effect of SEB is not a major factor in the prevention of autoimmune IDDM. Next, we examined whether SEB induces suppressor T cells when injected into NOD mice. We found that SEB induces CD4+ suppressor T cells. Finally, we examined the cytokine gene expression of the immunocytes that had infiltrated the pancreatic islets at different times after SEB treatment. We found an immediate increase in the expression of many cytokines, including IL-2, IFN-γ, IL-4, IL-10, IL-12 and TNF-α, as well as iNOS. With the exception of IL-4 and IL-10, expression of these cytokines and iNOS returned to normal three days after injection. At three weeks after injection a persistent increase in transforming growth factor β (TGFβ), IL-4, and IL-10 was observed. On the basis of these observations, we conclude that SEB persistently activates CD4+ suppressor T cells, which secrete increased amounts of IL-10, IL-4 and TGF-β. The increased secretion of cytokines may result in the prevention of cell-mediated autoimmune IDDM.

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PREVENTION OF INSULIN-DEPENDENT DIABETES BY CD8 γδ T CELLS INDUCED BY AEROSOL INSULIN

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Cellular immune hyporesponsiveness can be induced by the presentation of soluble protein antigens to mucosal surfaces. When we administered insulin aerosol to NOD mice even after the onset of sub-clinical disease, pancreatic islet pathology and diabetes incidence were both significantly reduced. Insulin-treated mice had increased circulating antibodies to insulin, and reduced splenic T-cell proliferation to the major epitope, insulin B chain amino acids 9-23, associated with increased IL-4 and particularly IL-10 secretion, as well as reduced proliferation to glutamic acid decarboxylase. The ability of splenocytes from insulin-treated mice to suppress adoptive transfer of diabetes by diabetogenic T cells were shown to be due to small numbers of CD8 γδ T cells. These cells also inhibit cyclophosphamide-accelerated diabetes and may be responsible for regulating the natural history of islet autoimmunity in NOD mice. Induction of regulatory CD8 γδ T cells by aerosol insulin has implications for the prevention of human IDDM.

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THE EFFECT OF SUPERANTIGEN ADMINSITRATION ON THE NATURAL HISTORY OF IDD IN NOD MICE.

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A recent study examining islet infiltrating cells from patients who succumbed to diabetic ketoacidosis at the onset of IDD showed a selective expansion of T cells bearing the Vβ7 T cell receptor (TCR); an observation consistent with a superantigen driven expansion of lymphocytes. Such a superantigen (if any) responsible for initiating or exacerbating human IDD is unknown. In order to identify the potential effects of superantigen in the pathogenesis of IDD, split litters of female NOD mice (10 per group) were intraperitoneally injected with normal saline alone or with 50μg of staphylococcal enterotoxin B (SEB) in normal saline @ 4 (i.e., pre-insulitis), 10 (i.e., established insulitits), or 4 & 10 weeks of age. Functional studies of SEB administration demonstrated that Vβ8+/CD4+ T cells respond to this dose of SEB, although to a lesser degree than other mouse strains. Life-table analysis revealed that treatment of NOD mice with SEB at 4 (P=0.4) or 4 & 10 (P=0.5) weeks of age did not alter the natural history of progression to IDDM. Interestingly, SEB injection into 10 week old animals significantly delayed rather than exacerbated the onset of IDDM (P=0.04). In addition to contrasting the hypothesis suggested by the aforementioned human results, this observation was in marked contrast to our previous findings in experimental allergic encephalomyelitits (EAE) where SEB administration re-induced disease in mice which had resolved a previous clinical episode. Therefore while provocative, the role for a superantigen driven pathogenesis of IDD is not supported by these studies and remains controversial.

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FEASIBILITY OF A MULTINATIONAL DIABETES PREVENTION TRIAL IN FIRST DEGREE RELATIVES OF A CHILD WITH IDDM

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The European Nicotinamide Diabetes Intervention Trial (ENDIT) set out to screen 40,000 first degree relatives of individuals who developed IDDM before age 20 yrs, with the aim of recruiting 528 high risk relatives to a 5 year randomized placebo-controlled trial of nicotinamide. Recruits were aged 5-40 years, non-diabetic by OGTT, positive for ICA on two occasions with one value ≥ 20 JDF units (JDFu), and fulfilled other eligibility criteria. The end point is development of diabetes (WHO criteria). Primary ICA screening was carried out in 10 laboratories, and eligibility confirmed at a central reference laboratory. By December 1996, 449 individuals had been randomized in 16 countries. 62% were under the age of 20 and 48% female. 29% were parents, 59% siblings and 10% children of the proband. 29% had ICA ≥80 JDFu, 55% had GAD autoantibodies (Ab), 38% IA-2 Ab and 29% insulin Ab ≥99th centile of 3000 schoolchildren. 43% had at least 2 Ab ≥ 99th centile in addition to ICA. OGTT was normal in 88%, and showed IGT in 12%. Of 263 IVGTT analysed, 17% had FPIR (1'+3' insulin) < 50 mU/l (standardized to the Seattle assay). Individuals <20 yr had more risk markers than those ≥ 20 yr. ICA ≥ 80 JDFu were found in 31% vs 23% (p=NS); 3-4 Ab ≥ 99th centile in 41% vs 23% (p<0.001), and FPIR ≤ 50 mU/l in 26% vs 9% (p<0.001). 30 of those recruited have developed diabetes, and 35 withdrawn from the trial. Recruitment is expected to end in 1997, with completion of the trial in 2002. Conclusion: multinational collaborative intervention trials are feasible in pre-IDDM. Simple robust entry criteria are essential. ICA have proved their value, but future trial entry will probably be based on multiple antibody testing. The ENDIT group are currently planning their next intervention trial.

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DOWN-REGULATION OF ISLET ANTIGEN EXPRESSION IN INSULIN STRATEGIES OF PREVENTION IN THE LD-STZ DIABETES MODEL.

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Aim of this study was to investigate in the LD-STZ mouse model of diabetes a preventive insulin administration evaluating its effects on disease onset, on the severity of the insulitis and on the pattern of islet antigen expression (ICA-target antigen, A2B5 monoclonal antibody recognizing islet glycolipids, GM3 and GM2-1 gangliosides) in different time points of the disease. Diabetes has been induced in male mice C57Bl6/J with LD-STZ. Seventy-one male C57Bl6/J mice were grouped as follows: Group A (n=25) injected i.p. with STZ only, Group B (n=21) injected subcutaneously with insulin in addition to STZ, Group C (n=15) injected with insulin only, Group D (n=10) used as normal control. Glycemia, insulitis and pancreatic islet antigen expression were evaluated at 12 and 24 days. At day 12 and 24 differences in mean glycemia and insulitis score between Group A and B were statistically significant (p<0.001, p<0.05), (p<0.001). At day 12 pancreas sections collected from Group B and Group C and incubated with 5 ICA+ human and normal mouse sera showed a lack of binding of these sera, at day 24 only sections from Group B showed positive reaction with the sera. At day 12 we observed in Group B and Group C the absence of the expression of monoclonal A2B5, in contrast at day 24 pancreata from both groups of mice showed positive binding to A2B5. Analysis of the pattern of ganglioside expression revealed that GM3 was equally expressed in all groups of treatment; in contrast expression of GM2-1 at day 12 was greatly reduced in Group B, while was not changed in animals belonging to Group C. At day 24, GM2-1 expression was reduced in Group C while was not unchanged in Group B of animals. In conclusion, our study confirmed that prophylactic insulin treatment is able to reduce diabetes and insulitis in the LD-STZ mouse model. Interestingly we showed that this effect was paralleled by a down regulation of the expression of some islet molecules, some of which, such as the cytoplasmic ICA antigens and the GM2-1 ganglioside, have been shown to be major targets of the autoimmune response leading to type 1 diabetes.

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MULTI-CENTER RANDOMIZED TRIAL AT DIFFERENT DOSES OF NICOTINAMIDE IN PATIENTS WITH RECENT-ONSET IDDM. The IMDIAB Group, Rome, Italy.

Recent evidence has suggested that nicotinamide (NA) when given at IDDM diagnosis may protect against residual beta cell damage. Studies in animal models indicate that when high doses of NA are given, beta cells are better protected from destruction. The aim of this study was to compare the efficacy of standard doses of NA (25 mg/Kg b.w., GROUP A) vs. high doses of NA (50 mg/Kg b.w., GROUP B) on residual beta cell function and the parameters of metabolic control (insulin dose and HbA1c) in patients with recent-onset IDDM (less than 4 weeks after diagnosis). A multi-center trial was carried out in 42 IDDM patients (20 females and 22 males, mean age 13.6 ± 8.1 years), who were randomized to one or the other dose of NA which was administered daily in addition to intensive insulin therapy. Data on the integrated parameters of metabolic control were collected at entry and after 3- and 6-month intervals. Patients are expected to be followed-up for up to 1 year. No significant differences were observed in C-peptide secretion, insulin dose or HbA1c levels at each interval. C-peptide values were 0.77 ± 0.57 ng/ml (baseline), 0.86 ± 0.77 ng/ml (3 months) and 0.72 ± 0.4 ng/ml (6 months) in GROUP A vs. 0.55 ± 0.53 ng/ml (baseline), 0.90 ± 0.62 ng/ml (3 months) and 0.78 ± 0.59 ng/ml (6 months) in GROUP B (NS), with comparable good metabolic control (HbA1c = 6.2 % ± 0.8 in GROUP A and 6.3% ± 0.9 in GROUP B, after 6 months of therapy). Adverse effects were not reported in either group of patients. We conclude that NA is equally effective at doses of either 25 or 50 mg/Kg in maintaining residual beta cell secretion 6 months after diagnosis in patients with IDDM.

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TROGLITAZONE REDUCES DIABETES INCIDENCE IN NOD MICE.

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Troglitazone (TGL) is a thiazolidinedione compound which increases sensitivity to insulin and is used to treat insulin resistance in NIDDM. Insulin resistance is also a feature of IDDM and may have pathogenic relevance as increased antigen expression on beta cells may occur as a consequence of insulin release. TGL was given to NOD mice, a spontaneous model of IDDM, to determine if increasing insulin sensitivity using this compound could reduce diabetes incidence. TGL was given by gavage in a methyl cellulose carrier (MCC) to 32 female NOD mice 5 x per week from 3 to 30 weeks of age at a dose of 800 mg per kg body weight. A further group of 32 age, sex and litter matched NOD's served as controls and received MCC alone. By 16 weeks of age there was a significant reduction in diabetes incidence in the treated vs control group (0 vs 5)($p<0.03$ - comparison of proportions) this level of difference was maintained up to the conclusion of the study (treated vs controls = 5 vs 16). However there was no difference in insulitis levels, nor in the destructive pattern of insulitis between treated and control groups. In vitro data (presented elsewhere) suggests that lymphocyte activation is altered by TGL by changing cytokine secretion patterns. Therefore we conclude that TGL may be effective in preventing IDDM in NOD mice by modifying cytokine secretion of TH1/TH2 lymphocytes.

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NICOTINAMIDE PREVENTS THE DEVELOPMENT OF INSULIN DEFICIENCY IN STREPTOZOTOCIN-TREATED NEWBORN RATS

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It has been shown that nicotinamide (NA), an inhibitor of poly (ADP-ribose)synthase and free radical scavenger, offers protection to pancreatic beta cells against a variety of toxic or immune-mediated insults. The aim of the study was to evaluate the protective effect of NA on the development of absolute and relative insulin deficiency in streptozotocin (STZ)-treated newborn rats. NA (500 mg/kg/day) was given to offspring's mother in diet for 10 days before and 5 days after STZ-injection (100 mg/kg i.p.). Pre- and postnatal treatment with NA prevented basal hyperglycemia (5.3±0.6 vs 11.4±0.5 mmol/l, $p<0.01$) and hypoinsulinemia (127.0±10.4 vs 78.0±5.5 pmol/l, $p<0.02$) on the absolute insulin deficiency stage (at day 5 after STZ-injection). NA administration also protected basal hyperglycemia ($p<0.05$) and forming of glucose intolerance on the relative insulin deficiency stage (at day 56): integral glycemia over the i.p. GTT was 32.1±1.4 vs 55.7±1.5 mmol/l, $p<0.02$ in untreated diabetic controls compared to 30.1±2.0 mmol/l in intact controls. All animals were sacrificed at 2 months of age and histological examination of pancreas revealed normalisation of morphological structure of islets with clear stimulation of beta-cells regeneration in rats treated with NA. Moreover, there were no differences in ascorbate and NADPH-dependent lipid peroxidation in liver of NA-treated animals and intact controls. We conclude that pre- and postnatal treatment with NA protects the development both absolute and relative insulin deficiency in STZ-treated newborn rats.

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Effect of testosterone on development of diabetes

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Nonobese diabetic (NOD) mouse strain has been utilized as a model for type 1 diabetes. The autoimmune diabetes is caused by lymphocytic infiltration and β -cell destruction mediated by inflammatory T-cells. The incidences of diabetes in female and male NOD mice are 65% and 5% at 30 weeks of age, respectively. This sexual dimorphism suggests that sex-steroid hormones may play an important role in the development of the disease. We, therefore, investigated the effect of a male steroid hormone, 5α -dihydrotestosterone(5DHT), on disease development, T-cell phenotype, T-cell proliferation, cytokine profiles and gene expression in female NOD mice. All mice (n=8) that recieved 5DHT for 120 days did not develop insulitis, whereas all untreated mice (n=8) developed the disease. The percentage of CD4+ T cells in peripheral blood mononuclear cells was decreased in the 5DHT treated mice compared to controls (37.1± 4.8% vs. 51.3± 9.3 %, $p<0.02$). Results of a syngeneic mixed lymphocyte reaction (SMLR) demonstrated an increased expression of a cytokine gene (IL-4) representing Th2 cell populations in splenic T cells obtained from 5DHT-treated mice. These results suggest that 5DHT may have direct effects on the expansion of Th2 cell populations with subsequent restoration of normal immune responses.

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PORCINE BRAIN GAD AND CFA MAY PREVENT INSULITIS AND DIABETES IN NOD MICE

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This study aimed to investigate the preventive effect of glutamic acid decarboxylase (GAD) and complete Freund's adjuvant (CFA) on insulitis and diabetes in non-obese diabetic (NOD) mice. Six female NOD mice were injected once intraperitoneally with 10^{-3}g purified porcine brain GAD at 4 weeks of age, 4 female NOD mice as control were injected once intraperitonearily with 10^{-3}g bovine serum albumin (BSA) at the same age; 5 female NOD mice were injected in the hind footpad with 0.05 ml CFA at 4 weeks of age, 6 female NOD mice as control were injected in the hind footpad with 0.05 ml normal saline (NS) at the same age. The severity of insulitis of GAD-treated mice were less than that of BSA-treated mice by 19 weeks of age (insulitis scores: 1.28±0.51 vs 2.36±0.93, $P<0.05$). By 30 weeks of age, none of 5 CFA-treated mice, compared with 3 of 6 control mice, had developed diabetes. The severity of insulitis of CFA-treated mice were less than that of control mice (insulitis scores: 1.84±0.75 vs 3.00±0.80, $P<0.05$). These data showed that porcine brain GAD purified by our laboratory and CFA may be able to lessen insulitis severity of NOD mice, and CFA may decrease incidence of diabetes in NOD mice.

ACKNOWLEDGMENT: This study was funded by grant from the National Natural Science Foundation of China (No. 39370343).

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Serological Markers of IDDM

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Detection of autoantibodies to the diabetes-associated antigen IA-2 with non-radioactive assays compared to a validated radioligand assay

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Autoantibodies to the tyrosine phosphatase-like protein IA-2 (IA-2A) are important markers of IDDM. IA-2A are currently measured in radioligand assays (RIA) using *in vitro* synthesised ^{35}S-labelled antigen. We have investigated non-radioactive methods for the detection of these antibodies using E. coli derived IA-2 (Pinpoint, Promega). Intracytoplasmic IA-2 (IA-2ic) and a control protein (non-sense protein produced by a frameshift) were affinity purified, and used to coat ELISA plates. Additionally IA-2ic was dot-blotted directly onto nitrocellulose, or subjected to SDS-PAGE followed by Western blotting. We tested 45 sera of RIA positive or negative IDDM patients and 47 control sera. The results are summarized in the table; all sera were negative in assays using the control protein.

	RIA	ELISA	Dot blot	Westernblot
all IDDM patients	31/45(69%)	32/45 (71%)	32/45 (71%)	12/39 (31%)
RIA positive IDDM patients		29/31 (93%)	29/31 (93%)	11/31 (36%)
RIA negative IDDM patients		3/14 (21%)	3/14 (21%)	1/14 (7%)
healthy controls	0/47 (0%)	0/47 (0%)	1/17 (6%)	1/17 (6%)

The sensitivity of the ELISA and the dot blot was comparable to the radioligand assay, but the dot blot had a lower specificity. The values of the radioligand assay and the ELISA correlated ($r=0,75$; $p<0,0001$). Only one third of IDDM patients was positive in the Western blot, indicating that the majority of IDDM patients has autoantibodies to conformational epitopes, and autoantibody binding to IA-2 may be heterogeneous in some patients. These non-radioactive assays are potentially useful for the screening of antibodies to IA-2 where radioactive facilities are not available.

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TEN DIABETES ASSOCIATED HUMAN MONOCLONAL GAD65-AUTOANTIBODIES DEFINE SIX DISTINCT EPITOPES IN GAD65.

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Autoreactive islet cell antibodies (ICA) directed to distinct target antigens are well-established markers for prediction and diagnosis of insulin dependent diabetes mellitus (IDDM). We attempted to isolate human monoclonal ICA to unknown islet cell antigens from patients newly diagnosed with IDDM but no stable ICA-reactive B cell lines could be obtained from 3 GAD-antibody-negative individuals. In contrast, four new human monoclonal ICA-reactive B cell lines (MICA 7-10) were stabilized from 2 out of 3 GAD-antibody-positive individuals tested. MICA 7 was derived from an HLA DR4/11^{+} patient, MICA 8-10 were isolated from an IDDM patient (HLA DR3/3) who also suffered from Graves' disease. MICA 7-10 were specific for GAD65. Therefore, they completed a set of six GAD65-reactive human monoclonal ICA (MICA 1-6) which were derived previously from an HLA DR1/7^{+} individual. This suggests a high frequency of GAD65-specific B cells compared to other ICA-reactive B cells in peripheral blood at diagnosis of IDDM. Analysis of 5 N-terminal and 3 C-terminal deletion mutants of GAD65 and blocking experiments by direct immunohistochemistry demonstrated that MICA 7-10 define three novel conformation dependent autoantigenic epitopes in GAD65. Using chimeric molecules constructed of GAD65 and GAD67 fragments we further narrowed down the epitopes of MICA 1-10. We identified three regions of distinct immunogenicity in GAD65: a) an NH_2-terminal region spanning amino acids (aa) 1-244 which was not sufficient for any epitope but contributed to the binding of MICA 8 and 9 requiring aa 39-585; b) the middle region encompassing aa 245-449 and harbouring the epitopes of MICA 4, 6 and MICA 10 and c) the COOH-terminal 135 aa which contained the epitopes recognized by MICA 1, 2, 3 and MICA 7. In sum, three analytical approaches demonstrated that MICA 1-10 define at least 6 distinct autoreactive epitopes associated with IDDM and detect two domain boundaries in GAD65.

Supported by grants of Deutsche Forschungsgemeinschaft Ri 707/1-2 to WR and NIH grant #DK47043 to SB and an HFSP grant to SB and WR.

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IA-2 ANTIBODIES IN RELATION TO OTHER AUTOANTIBODIES AND GENETIC RISK MARKERS IN CHILDREN WITH RECENT-ONSET IDDM

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To study the role of antibodies to IA-2 (IA-2A) in IDDM we analyzed 756 newly diagnosed patients younger than 15 years (mean age 8.5) for IA-2A, GAD65 antibodies (GADA) and insulin antibodies (IAA) with radiobinding assays, for islet cell antibodies (ICA) with immunofluorescence and for HLA DR alleles by serology. 73.4% of patients tested positive for IA-2A, 73.1% GADA, 48.6% for IAA and 84.2% for ICA. Boys and girls had similar frequencies and levels of IA-2A. There were no significant differences in the IA-2A frequencies between three age groups (0-4.99, 5.0-9.99 and 10.0-14.99), but patients aged 5.0-9.99 years had higher IA-2A levels ($p<0.05$) than the youngest children. There was a relatively strong correlation between IA-2A and ICA levels ($r=0.45$; $p<0.001$), while no relation was observed between IA-2A and GADA levels. A modest correlation was seen between IA-2A and IAA ($r=0.20$; $p<0.001$). A higher proportion of the patients tested positive for IA-2A and/or GADA than for ICA alone (91.5% vs. 84.2%; $p<0.001$). This difference was most conspicuous in the oldest age group (92.9% vs. 77.4%; $p<0.001$). An overwhelming majority of the patients (87.5%) tested positive for at least two disease associated autoantibodies. Only one antibody was detected in 8.8% of the children, and very few patients (3.7%) tested negative for all four autoantibodies. The patients carrying the DR4/x phenotype (x is other than DR3 or DR2) had higher antibody levels ($p<0.001$) than the other cases. Only about one third (35.3%) of the patients with DR 3/y phenotype (y is other than DR4 or DR2) tested positive for IA-2A and they had decreased antibody levels ($p<0.001$). These results indicate that the combination of IA-2A and GADA has a higher sensitivity for IDDM than ICA alone, especially in subjects older than 10 years. IA-2A are closely associated with the DR4 allele in contrast to GADA, which have been shown to associate with the DR3 allele.

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EPITOPE SPECIFIC PRESENTATION OF GLUTAMIC ACID DECARBOXYLASE, GAD_{65}, IN HUMAN B-CELL LINES

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Antigen processing and presentation of two immunogenic epitopes of GAD65 was analyzed using DRB1*0401 restricted murine T-cell hybridomas specific for peptides corresponding to GAD 274-286 and 114-126. Using immortalized B-lymphoblastoid cell lines derived from diabetic patients, subjects with GAD autoantibodies or healthy individuals, presentation of exogenously added GAD_{65} protein was investigated. Two B-cell lines pulsed with GAD protein were efficient for processing and presentation of both epitopes, but interestingly, four other B-cell lines displayed an epitope specific presentation pattern: The GAD 274-286 epitope was efficiently presented whereas stimulation of the T cell hybridoma specific for GAD 114-126 was not induced. All B-cell lines efficiently presented peptides corresponding to the particular epitopes, indicating that the differences occurred at the level of antigen processing. The deficiency of presentation in terms of epitope 114-126 generated from GAD protein was not due to a global defect in the processing machinery since these cell lines were capable of presenting exogenously added human serum albumin (HSA) protein equally well. These differences suggest either quantitative or qualitative variation in the intrinsic ability of antigen-presenting cells from different individuals to process and present specific antigenic peptides derived from an important autoantigen. Epitope specific presentation of GAD_{65} in human B-cell lines may have implications for events involved in triggering of the autoimmune response against dominant GAD_{65} epitopes or in determinant spreading towards subdominant epitopes during the disease course.

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CAN BIOCHEMICALLY BASED ASSAYS FOR ISLET AUTOANTIBODY DETECTION REPLACE THE CLASSICAL ICA TEST?

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Islet cell antibodies (ICA) continue to serve as the principal serological test for definition of active autoimmunity of the beta-cells. In the last decade biochemically-defined beta-cell antigens were described, leading to the development of sensitive and specific autoantibody assays, to be used in prediction of insulin dependent diabetes mellitus (IDDM). The aim of our study was to examine the value of using combinatorial biochemically-based serological assays such as autoantibodies to insulin (IAA), glutamic acid decarboxylase (GAD) and ICA512 in replacement of the traditionally used ICA assay. Blood samples of 114 newly diagnosed IDDM patients aged 12±5yrs (range 2months - 29 years) taken at or soon after diagnosis (mean 5±4 days, range 0 -30 days) were tested for ICA (indirect immunofluorescence), IAA, GAD and ICA512 (radiobinding assay). The latter 2 assays were performed using recombinant human [^{35}S]-labelled antigen produced by in vitro transcription/translation. Using these tests we found that 90.3% (103/114) of our patients expressed 1 or more, 78.9% (90/114) 2 or more and 58.8% (67/114) 3 or more markers. Despite the improvement provided by an enlarged battery of assays, 9.6% of the tested sera did not react in any of the tests performed. We found that a lower number of sera scored positive for ICA and/or IAA (80.7%, 92/114) than for 1 or more of IAA, GAD, or ICA512 (88.6%, 101/114). Interestingly, only 2/114 (1.7%) were positive for ICA and negative for the other 3 markers. We conclude that combinatorial testing for IAA, GAD and ICA512 can advantageously replace the traditionally used ICA/IAA test for prediction of IDDM.

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Molecular mimicry in IDDM: The PEVKEK motif of coxsackie B virus protein 2C and glutamate decarboxylase binds to HLA-DR3.

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Molecular mimicry between coxsackievirus B4 protein 2C and the autoantigen glutamic acid decarboxylase (GAD_{65}) has been proposed to play a role in the pathogenesis of insulin dependent diabetes mellitus. To investigate whether the sequence homology between GAD_{65} and p2C (PEVKEK) is restricted to coxsackie B4 we studied conservation of the homologous sequence within the family of enteroviruses. Sequence analysis of the p2C encoding genome of a number of coxsackie B-like enterovirus isolates showed that the mimicry motif and flanking sequences are highly conserved in different members of the coxsackie B-like group. In the polio-like enteroviruses no homology to GAD_{65} was found. Molecular mimicry is therefore restricted to the coxsackie B-like enteroviruses, which represent the most prevalent enteroviruses. Furthermore, the relevance of the mimicry motif for induction of autoimmunity to GAD_{65} was studied in an HLA-peptide binding assay using the diabetes-associated HLA-DR3, DR4 and DR1 molecules. Peptides derived from GAD_{65} and p2C, containing the PEVKEK motif, were found to bind with equal affinity to DR3. Replacement of amino acids in the motif showed that PEVKEK binds within the peptide binding groove of the DR3 molecule and thereby can be presented to the immune system. No binding was found with DR4 or DR1. These results suggest that molecular mimicry indeed may play a role in the development of diabetes. Furthermore, although exposure to the motif is frequent, molecular mimicry might be restricted to HLA-DR3 positive patients.

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GAD65 AND ICA512 AUTOANTIBODIES AS DISEASE MARKERS AND THEIR NATURAL HISTORY IN A POPULATION OF YOUNG INSULIN-DEPENDENT DIABETIC PATIENTS.

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Circulating autoantibodies to islet autoantigens, glutamic acid decarboxylase (GAD65) and tyrosine phosphatase ICA512, measured in combination, have been proposed as predictive markers of IDDM. To analyse their potential for screening children with these markers, we examined their sensitivity in identifying IDDM in a population of 59 children at diagnosis of IDDM (mean (SD) age 7.5 (4) years). We also examined the natural history of the autoantibodies amongst childhood diabetics in 91 adolescents with IDDM (age 14.7 (1.6) years; mean duration of IDDM 7 (3.5) years). 42 normal adolescents (age 14.6 (1.8) years) without family history of IDDM were the control group. GAD65 and ICA512 autoantibodies were assessed by a quantitative radioimmunoprecipitation assay, using in vitro transcribed/translated proteins and expressed as indices derived using standard positive and negative sera. GAD65 and ICA512 autoantibodies were detected in 53% and 64% of newly diagnosed IDDM children. There was no correlation between levels of C peptide and levels of autoantibodies. 80% had at least one autoantibody, but the autoantibodies occurred together in only 37%. 24% of ICA negative patients had at least one of the two autoantibodies. 1/42 of controls (2%) had GAD65 autoantibodies and another control had ICA512 autoantibodies. GAD65 and ICA512 autoantibodies were present in 44% and 45% of diabetic adolescents, and occurred together in 21% of the adolescents. The mean ICA512 index was significantly higher amongst the newly diagnosed patients ($p<0.001$), and levels tended to decline with diabetes duration ($p=0.058$). At diagnosis, levels of GAD65 autoantibodies correlated with age ($p=0.05$), and in the adolescent patients levels did not decline with diabetes duration. In the adolescent patients, the presence of ICA512 was significantly associated with ICA ($p<0.05$) and mean ICA512 index was higher in ICA positive than in ICA negative patients ($p<0.005$). Our findings indicate that positivity for either GAD65 or ICA512 autoantibodies is a highly sensitive marker of IDDM in the paediatric age group, while positivity for both has a diagnostic sensitivity of 37%. The association between presence and levels of ICA512 autoantibodies and ICA suggests tyrosine phosphatase as one of the ICA target autoantigens, supported by the finding of higher levels of ICA512 at diagnosis, declining with diabetes duration.

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STRUCTURAL PREREQUISITES OF ANTIGENIC MIMICRY BETWEEN ICA69 (TEP69) AND BSA (ABBOS)

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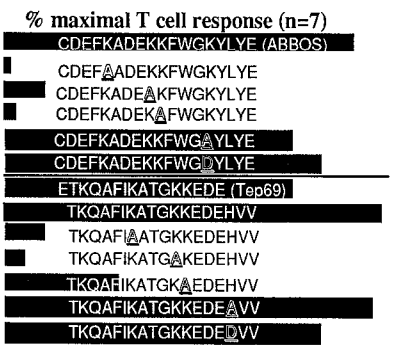
ICA69 is a neuroendocrine protein with maximal expression in β-cells. The function of the 4 ICA69 isoforms is unknown, but it is a frequent target of diabetic autoimmunity. There are 3 regions of homology between ICA69 and bovine serum albumin (BSA), one of several cow milk constituents that engender abnormal immune responses in diabetes. Studies of autoreactive T cells in newly diabetic children and NOD mice have delineated two dominant T cell epitopes in these two molecules that show fully reciprocal antigenic mimicry as well as cross-tolerance (in NOD mice): ABBOS (in BSA) and Tep69 (in ICA69). A model was proposed, where early exposure to dietary BSA would generate ABBOS-specific T cell repertoires that could target beta cell ICA69. As shown in the figure, the linear homology between ABBOS and Tep69 consists of 4 identically spaced amino acids (KA..KK). Here we have used ALA mapping with replacement peptides in patient T cell proliferation assays and find that these 4 residues are absolutely critical for the observed T cell mimicry. These and experiments in NOD mice define the structural constraints of mimicry between BSA and ICA69.



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ANTIBODIES TO ICA512 AND GAD65 IN INSULIN-DEPENDENT DIABETES (IDDM) PATIENTS FROM LATVIA.

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Antibodies to tyrosine pyrophosphatase (ICA512) and glutamate decarboxylase 65 (GAD65) are most prevalent in Caucasians with IDDM. The aim of our study was to determine the prevalence of ICA512 and GAD65 antibodies (Ab) in IDDM patients (n=87) and 90 healthy controls from Latvia.. ICA512Ab and GAD65Ab were evaluated by RIA using *in vitro* translated recombinant human 35S-ICA512 and 35S-GAD65 respectively. In controls, ICA512Ab were present in 5/90 (5%) and GAD65Ab in 3/90 (3%). In IDDM, ICA512Ab were present in 36/87 (41%)($p<0.001$ vs controls) and GAD65Ab in 58/87 (67%) ($p<0.001$ vs controls). The frequency of ICA512Ab in 0-5 years age was 8/16 (50%)which is higher than that observed in older (>5 years) patients (28/71,39%)(p=ns) whereas GAD65Ab was lower in patients with 0-5 years of age (6/16, 38%) than in older (>5 years) patients (51/71,72%)($p<0.01$). Both ICA512Ab and GAD65Ab were higherin frequency in female (20/42, 48% and 32/42, 76% respectively) than male patients (16/45, 35% and 26/45, 58% respectively). Both ICA512Ab and GAD65Ab were found simultaneously in 22/87 (25%); both Abs were absent in 15/87 (17%); either ICA512Ab or GAD65Ab was present in 72/87 (83%); A total of 12/87 (14%) IDDM patients was ICA512Ab+/GAD65Ab-, whereas 36/87 (41%) patients were ICA512Ab-/GAD65Ab+.

We conclude that IDDM patients from Latvia, have ICA512Ab and GAD65Ab at a significantly high frequency as compared to healthy controls. In conformity with previous observations in Swedish IDDM patients, GAD65 appears to be major autoantigen in Latvian IDDM.

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CONSTRUCTION OF AN ANTIBODY PHAGE DISPLAY LIBRARY FROM 7 PATIENTS WITH TYPE - I DIABETES MELLITUS

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The panel of human monoclonal IgG antibodies (hmabs) related to insulin dependent diabetes mellitus (IDDM) is still small. The prediction of IDDM and the standardization of islet cell autoantibody (ICA) assays will be improved when hmabs specific for diabetes-related autoantigens and their characteristic epitopes will be available. The phage display technology is a powerful tool for the generation of recombinant human antibody fragments (Fragment antigen binding, Fab). Aim of our approach was 1.) to generate a large combinatorial Fab phage display library characteristic for the humoral immune response at onset of IDDM and 2.) to isolate from this library new human islet cell autoantibody fragments directed to any relevant autoantigen in IDDM. To validate the applied techniques we established phage display of Fab and expression of soluble Fab molecules for MICA 2 and MICA 4, two human monoclonal GAD65-reactive IgG1 autoantibodies obtained by conventional methods. The genes of the light chains (L) and of the variable region plus first constant region (Fd) of the heavy chains (H) were cloned into phagemid pComb3HSS. The antibody fragments were expressed as fusion proteins with the coat protein cp III of bacteriophage M13 (Phab) and as soluble Fabs in E. coli. Phabs and soluble Fabs derived from MICA 2 and MICA 4 bound to human GAD65 in an indirect ELISA with immobilized antigen and in a dot blot analysis with immobilized phages. This demonstrates that the chosen phage display system is suitable for enrichment and production of IDDM-related Fab. The antibody phage display library was generated from 10^8 peripheral blood lymphocytes (PBL) pooled from seven individuals with high ICA titers at onset of IDDM. The library is now ready to be screened for high affinity Fabs to any autoantigen relevant for IDDM. The approach we described may be useful in identifying new IDDM-related antibody specifities to known and unknown islet cell antigens in type-I diabetes. Supported by grants of DFG Ri 707/1-2 and HFSP 361-95 to W.R.

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ANTIBODIES TO ICA512 AND GAD65 IN INSULIN-DEPENDENT DIABETES (IDDM) PATIENTS FROM EASTERN INDIA.

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Antibodies to tyrosine pyrophosphatase (ICA512) and glutamate decarboxylase 65 (GAD65) are frequently present in Caucasian patients with IDDM. The prevalence of ICA512 and GAD65 antibodies (Ab) in IDDM (n=74), and 123 healthy controls from Cuttack in Eastern India. was determined. ICA512Ab and GAD65Ab were evaluated by RIA using *in vitro* translated recombinant human 35S-ICA512 and 35S-GAD65 respectively. In controls, ICA512Ab were present in 3/123 (2%) and GAD65Ab in 8/123 (7%). In IDDM, ICA512Ab were present in 32/74(43%)($p<0.001$ vs controls) and GAD65Ab in 12/74 (16%)($p<0.001$ vs controls). In IDDM, the frequency of ICA512Ab was lower in patients with short term (0-4 years) (14/44,32%) than long term duration (>4 years)(18/30, 60%)($p<0,05$) whereas GAD65Ab frequency was higher in patients with short-term (0-4 years) (9/44, 20%) than with long-term duration (>4 years) (4/30, 13%)($p=0.42$). Both ICA512Ab and GAD65Ab were higher in frequency in male (20/42, 48% and 9/42, 21% respectively) than female patients (12/32, 38% and 3/32, 9% respectively). In IDDM patients, 30/74 (41%) were both ICA512Ab and GAD65Ab negative; 4/74 (5%) were both Abs positive; 36/74 (49%) were either ICA512Ab or GAD65Ab positive; 28/74 (38%) were ICA512Ab positive and GAD65Ab negative; and 8/74 (11%) were ICA512Ab negative and GAD65Ab positive.

We conclude that patients from Cuttack in Eastern India, have ICA512Ab and GAD65Ab in IDDM at a low but significant frequency compared to controls. ICA512 and not GAD65 is the major autoantigen in the IDDM patients from Eastern India. ICA512Ab and GAD65Ab account for only 49% of patients suggesting that an additional major autoantigen is involved in the etiopathogenesis of IDDM in this population.

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ACTIVITY DEPENDENT BIOSYNTHESIS AND EXPRESSION OF SULPHATIDE IN ISLET CELLS

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The aim of this study was to describe activity dependence of sulphatide, 3'-sulphogalactosylceramide, which has been identified in insulin granules of beta cells in the islets of Langerhans in the pancreas. Sulphatide is thought to play a role in the pathogenesis of IDDM, as circulating sulphatide antibodies are found in both BB rats and diabetic patients. It has been suggested, that beta cell activity is of importance during the development of IDDM. In fact, islet cell activity dependent cytotoxicity has been shown. This might be explained by certain antigens being co-released or expressed with insulin. By FACS analysis, glucose stimulated activity dependent islet cell expression of sulphatide was examined using a sulphatide specific monoclonal antibody, Sulph I. Activity dependent islet cell synthesis of sulphatide was also examined. This was done by adding radiolabelled precursors to islet culture medium followed by extraction of lipids and TLC-ELIZA of the purified sulphatide fraction. FACS analysis showed a significant influence of glucose stimulation upon sulphatide expression. After 12 h, the mean percentage ± SEM of Sulph I positive cells after glucose deprivation (4 mM) was 23 ± 8%, whereas the percentage of positive cells after glucose stimulation (18 mM) was 33 ± 7%, $P=0.01$ ($n=4$). After 18 h incubation the picture is reversed, as 42 ± 2% beta cells are positive after deprivation and only 37 ± 6% are positive after stimulation, $P=0.3$ ($n=4$). Chromatography and colometric scanning of the Sulph I positive bands showed, that after 24 h in culture, the sulphatide content in stimulated islets was half (1 nmol/600 islets) that observed in glucose deprived islets (2 nmol/600 islets). In conclusion, expression of the beta cell antigen sulphatide is activity dependent, but exhaustion after a longer period of glucose stimulation is acompanied by an insufficient capacity to synthesize and express sulphatide.

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HIGH CONCORDANCE OF GAD 65 ANTIBODIES AND ISLET CELL ANTIBODIES IN RECENT ONSET IDDM PATIENTS AND IN IDDM OF LONG DURATION

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In recent onset IDDM most of the islet cell antibody positive patients are also positive for GAD 65 antibodies. In this study we investigated the frequency and levels of co-existence of GAD 65 antibodies and ICA in 105 longterm IDDM patients (median duration: 21 years; range: 10-46 years) compared to 259 recent onset IDDM patients. GAD 65 antibodies were detected in a radioligand-binding-assay, using recombinant human GAD 65 as tracer and islet cell antibodies were determined by indirect immunofluorescence on human pancreas. Among the long-term IDDM patients GAD 65 antibodies were present in 32%, whereas, islet cell antibodies were observed in 20% ($p<0.05$). By contrast, in the group of recent onset IDDM patients 78% were positive for GAD 65 antibodies but there was no significant difference in the frequency of islet cell antibodies which were found in 82% of the patients. In both groups of patients autoantibodies to GAD 65 were significantly more frequent in ICA positive individuals ($p<0.01$) and the index levels of GAD 65 antibodies were positively associated with higher ICA titres ($p<0.01$). In summary humoral autoimmunity to GAD 65 is closely associated with the presence and higher titres of ICA and tend to disappear more slowly than islet cell antibodies. The high concordance of GAD 65 antibodies with islet cell antibodies is striking supporting the hypothesis that there is an overlap in the humoral immune response directed to islet specific antigens although the time course appears to be different.

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MONOCLONAL ANTIBODIES AGAINST THE SURFACE OF INS-1 INSULINOMA CELLS

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INS-1 is an established cel line from the rat (parent cell RIN tumour), β-mer- captoethanol dependent, characterised by functional and morphological properties similar to the rat β-cell. Confluence of cultured INS-1 cells is reached by 10-11 days and the doubling time is 100 hours. In this study we generated monoclonal antibodies to INS-1 in order to study the various functional and differentiation antigens of this line. Balb/c mice were immunised with whole cells and fusion of immune spleen cells with NSO myeloma cells generated several hybrids. Screening of hybridoma supernatants for reactivity in an ELISA assay of INS-1 extract or whole cells yielded a number of positive clones. After two rounds of cloning by limiting dilutions two clones, 2F8 and B2D6, that were positive in both assays were selected for further analysis. Both clones are of IgM class and are positive for cell surface immunofluorescence. Analysis by flow cytometry revealed that antibody 2F8 reacted with INS-1 cells in a time-of-culture-dependent manner (% positive cells from the 7th to the 11th day of culture 24 and 65 respectively). B2D6 was also reactive on this assay. Dilutions for flow cytometry for both assays were from 1:1000 to 1:4000, with a less than 10% decrease in staining at the latter dilution. By contrast, both antibodies were reactive with RIN-5AH cells throughout the culture period of 3 days (e.g. on day 2, 44.3% pos. with 2F8, and 75.0% with B2D6). Neither antibody reacted with rat splenic lymphocytes or monocytes. These antibodies could not inhibit the growth of RIN-5AH cells in culture, in three-fold dilutions of 1:2500 up to 1:202500, when added concurrently with or 24, or 48 hrs after the seeding of the cells. The antigenic specificity(ies) of these antibodies and their possible effect(s) on INS-1 and RIN-5AH function is currently under investigation.

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FREQUENCY OF AUTOANTIBODIES AGAINST SIX DIABETES-RELATED AUTOANTIGENS IN NEWLY DIAGNOSED TYPE 1 DIABETIC PATIENTS.

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Several autoantibodies have been found in sera of patients at or before the onset of type 1 diabetes; despite that various studies showed that a number of new onset type 1 diabetics at diagnosis (15-20%) seem to be autoantibody negative (whatever the test used); this finding could reflect the use of low sensitivity assays or more probably the study of an insufficient number of autoantigens in each patient serum. In this work we have combined the search for autoantibodies towards both protein and non protein antigens to better characterize this potential subgroup of autoantibody negative patients. Antibodies to islet pancreatic cells (ICA), glutamic acid decarboxylase (GAD65Abs), insulin (CIAA), amino acid residues 256-976 of the IA-2 molecule (ICA512bdc), monosialoganglioside GM2-1 (GM2-1Abs) and disialoganglioside GD3 (GD3Abs) were investigated in 78 new onset type 1 diabetic sera (45 males, mean age 12.9±8.0; 33 females, mean age 11.3±8.2). ICA were measured by indirect immunofluorescence, CIAA by a competitive fluid-phase radioassay, GAD65Abs and ICA512bdc by radioassay using in vitro transcribed and translated recombinant human GAD65 or ICA512 bdc respectively, GM2-1Abs by an immuno-TLC method and GD3Abs by ELISA. ICA were detected in 56.4% (42/78), CIAA in 57.7% (45/78), GAD65Abs in 70.5% (55/78), ICA512bdc in 53.8% (42/78), GM2-1Abs in 61.5% (48/78), GD3Abs in 35.9% (28/78) type 1 diabetic sera respectively. Patient sera positive for at least one autoantibody were 97.4% (76/78). 9.0% of type 1 diabetic sera were positive for one, 19.2% for two, 19.2% for three, 26.9% for four, 15.4% for five and 7.7% for all the antibodies tested; it is of note that of the 7 patients with only one autoantibody positivity, 3 were GM2-1Abs positive and none ICA512bdc or GD3Abs positive. In conclusion, the combined study of a large panel of diabetes-related antibodies against protein and non protein antigens at disease onset restricts to less than 3% the percentage of type 1 diabetics apparently without humoral autoimmune phenomena.

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CLASSICAL INSULIN DEPENDENT DIABETES VERSUS LATENT AUTOIMMUNE DIABETES IN ADULTS: TWO DIFFERENT ENTITIES?

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We evaluated conversion to diabetes in 53 individuals from a cohort of 5034 first degree relatives who have been followed for periods up to 17 years and who presented signs of B-cell autoimmunity, either islet cell antibody (ICA), antibodies to glutamic acid decarboxylase 65kd (GAD65) or to ICA512 (IA-2). We were struck by the fact that 14 appeared to be latent autoimmune diabetes in adults (LADA) and thus we prospectively investigated differences between the classical IDDM and the LADA group. The 53 converters were classified into two groups: group I (classical), who started insulin treatment immediately or within 1 year of diagnosis (n=39) and group II (LADA), who started insulin treatment 1 - 8 years after diabetes diagnosis (n=14). The mean age at start of insulin treatment was 23 years in group I and 43 years in group II. The frequency of antibodies in the first blood drawn prior to diabetes diagnosis was:

	Group I (n = 39)	Group II (n = 14)
ICA*	92.3% (36)	64.2% (9)
GAD*	87.2% (34)	50.0% (7)
IA-2	53.8% (21)	35.7% (5)
ICA + GAD*	84.6% (33)	42.9% (6)
ICA + IA-2	51.3% (20)	28.5% (4)
ICA + GAD + IA-2	46.1% (18)	28.5% (4)

* significant at 0.05 level, when comparing group I vs group II

We conclude that the characteristics of these two groups before the onset of diabetes are different in that the prevalence of ICA and GAD 65 autoantibodies is lower in LADA as compared to the classic converters. These results clearly suggest the existence of a slow progressing autoimmune form of diabetes which has been called LADA and which can occur in families of pediatric IDDM probands.

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CORRELATION BETWEEN THE PRESENCE OF IAA, GAD-AB AND ICA AND AB-BSA IN CHILDREN WITH NEWLY DIAGNOSED IDDM.

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It has been suggested that anti-bovine serum albumin (BSA) antibodies may participate in the autoimmune process leading to the destruction of pancreatic B-cells. The aim of the present study was to assess correlation between the frequency of anti-islet cell autoantibodies (ICA), anti-insulin autoantibodies (IAA), and anti-glutamic acid decarboxylase (GAD) autoantibodies with anti-BSA-antibodies in newly-diagnosed diabetic children.

We examined 67 children (age 4-16) during 1 month after diagnosis of IDDM. Anti-IAA and GAD autoantibodies were measured with RIA, and anti-ICA and BSA antibodies were measured with FIA.

Anti-IAA, GAD and BSA antibodies were found in 13%, 31% and 22%, respectively. Anti-BSA-positive subjects were anti-IAA and anti-GAD positive in 44% and 57%, respectively. In 5 cases all kinds of examined antibodies were found.

Our results confirm the possible role of BSA antibodies in the IDDM development.

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DEFINING DIFFERENCES IN ANTIGEN-SPECIFIC ANTIBODIES ASSOCIATED WITH TYPE 1 AND TYPE 2 DIABETES

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Islet antigens associated with Type 1 insulin dependent diabetes (IDDM) include a protein tyrosine phosphatase-like molecule, IA-2 of 979 aminoacids containing an intracellular fragment from 603 to 979 (IA-2ic) and glutamic acid decarboxylase (GAD 65). The extent of the immune response, reflected by a combination of antibodies to these two antigens is highly predictive of IDDM. GAD 65 antibodies have also been found in patients with non-insulin dependent diabetes (NIDDM) who can progress to insulin dependency. To determine for the first time whether antibody responses to different antigens or different specificities of the same antigen distinguish NIDDM and IDDM patients we studied antibodies to IA-2, IA-2ic and GAD 65 in: a) 260 NIDDM subjects, b) 60 newly-diagnosed IDDM patients, c) 156 Controls. Of the 260 NIDDM patients antibodies to both IA-2 and IA-2ic were tested in 115. In NIDDM and IDDM, antibodies to GAD 65 were detected in 10% (26/260) and 77% (46/60) respectively, to IA-2ic in 5% (12/260) and 67% (40/60) respectively, and to either in fewer NIDDM 10% (26/260) than IDDM 87% (52/60) patients ($p<0.0001$); 1/156 controls had IA-2 but no other antibodies. Combinations of GAD 65 and IA-2ic antibodies were less prevalent in NIDDM 27% (7/26) than IDDM 63% (32/51) ; $p<0.002$). Of the NIDDM patients with antibodies to IA-2 or IA-2ic only 4/17 had antibodies to both, compared with 37/40 IDDM patients ($p<0.01$). In summary, antibodies to IA-2ic and GAD 65 in NIDDM compared to IDDM patients are less prevalent, less likely to occur in combination and, for IA-2, show a more restricted specificity. Thus, antibodies in NIDDM patients show restricted specificity both between antigens and within an antigen.

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GLUTAMIC ACID DECARBOXYLASE AND ISLET CELL ANTIBODIES IN IDDM AND FIRST-DEGREE RELATIVES IN ANDALUSIAN

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The aim of this study was to evaluate the frequency of glutamic acid decarboxylase and islet cell antibodies (GADA and ICA) in IDDM and first-degree relatives (FDR). 136 subjects with IDDM, 280 FDR and 100 controls were analyzed as a part of the European nicotinamide diabetes intervention trial. GADA were measured by RIA, and ICA were determined by indirect immunofluorescence. Age, sex and IDDM duration were analyzed and related to both GADA and ICA. In IDDM group, GADA were positive in 38.5% (35/91; males: 16; age: 22 ± 10; IDDM duration: 3.3 ± 3.9), and ICA were positive in 20.5% (25/122; males: 12; age: 14.7 ± 6.1; IDDM duration: 1.7 ± 2.6), and both antibodies were positive in 13% (10/77). Age and sex were not related to the presence of these autoantibodies. However, IDDM duration was related inversely to ICA and GADA. In FDR group, GADA were positive in 5.6% (11/195; males: 6; age: 17.8 ± 9.5), and ICA were positive in 1.5% (4/271; males: 3; age: 19.2 ± 11.3), and both antibodies were positive in 1.1% (2/186). All controls were negative. The frequency of GADA and ICA in Andalusian affected IDDM and relatives is similar to other Caucasian population. Both autoantibodies could be useful for diagnosis of IDDM and screening of high risk subjects to prevention trials among first relatives.

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DIAGNOSTIC SENSITIVITY OF AUTOANTIBODIES TO TYROSINE PHOSPHATASE-LIKE PROTEINS IA-2 AND IA-2ß IN IDDM AND NIDDM

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To evaluate the diagnostic sensitivity of antibodies to tyrosine phosphatase-like proteins IA-2 (anti-IA-2) and IA-2ß (anti-IA-2ß) for IDDM and latent insulin-dependent diabetes in adults (LADA) we tested sera from IDDM patients (age 0-40, median 17 years) and patients classified as non insulin-dependent diabetes (NIDDM, age 40-66, median 53 years) with diet failure 0.5-10 years after diagnosis who had preserved residual beta cell function (C-peptide >0.6 nmol/l). Autoantibodies were measured by radioligand assay using the recombinant intracytoplasmic IA-2 or IA-2ß and were compared to antibodies to glutamic acid decarboxylase (GADA) and cytoplasmic islet cell antibodies (ICA). Out of 200 patients with IDDM, 112 (56.0%) had anti-IA-2 and 76 (38.0%) were positive for anti-IA-2ß. The frequencies of anti-IA-2 and anti-IA-2ß were 70% and 49% in young children below age 10, 70% and 48% in patients aged 11-20 years but only 34% and 21% in patients above 20 years of age. Among subjects with anti-IA-2ß 73 of 76 (96.1%) were found positive for anti-IA-2. In all age groups both anti-IA-2 and anti-IA-2ß were significantly associated with the presence of ICA but not with GADA ($p<0.001$). Combined screening for anti-IA-2 and GADA identified 89% of all patients with IDDM and 94% of ICA positive subjects. In patients classified as NIDDM 19% (36 of 200) were found positive for one autoantibody specificity suggesting the presence of LADA. Only a few sera of LADA patients had anti-IA-2 (n = 3) or anti-IA-2ß (n = 1) whereas GADA were present in 18 and ICA in 27 patients. All sera positive for anti-IA-2 or anti-IA-2ß had ICA, 18 patients had ICA alone and 11were positive for GADA alone.

Our data indicate that antibodies to IA-2 are a sensitive marker for IDDM in childhood and adolescence but are of only limited value for IDDM in adults. The low prevalence of anti-IA-2 in NIDDM emphasizes the importance of both ICA and GADA to identify patients with LADA. Anti-IA-2ß may indicate a heterogeneous autoimmune response in a subgroup of anti-IA-2 positive individuals whose significance is still unknown.

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MEASUREMENT OF GADA USING RADIOLIGAND BINDING ASSAY, RIA AND IRMA IN KOREAN PATIENTS WITH IDDM.

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IDDM is an autoimmune disease in which serum antibodies against islet antigens have been recognized. These include insulin autoantibodies, cytoplasmic islet cell antibodies (ICA), ICA 512 and GAD antibodies(GADA). Recently, there is increasing interest in the use of GADA for identification of subjects at increased risk of developing IDDM. However, because the classic assays of GADA are still rather time-consuming, a more simple and reproducible radioligand binding assay is widely used recently. Also, RIA and IRMA for GADA using ^{125}I - labelled human GAD have been developed. To evaluate the prevalence of GADA (measured by radioligand binding assay, radioimmunoassay and IRMA) in Korean patients with IDDM and to observe the associations of GADA with ICA, we tested 26 IDDM sera (M:F=10:16, mean age 14±3) and 20 control sera (M:F=10:10, mean age 15±1) for GADA (using radioligand binding assay, RIA and IRMA) and ICA (using indirect immunofluorescence method). The results are follows : The overall prevalences of ICA and GADA in Korean patients with IDDM were 46% (12/26) and 31%(8/26)-38% (10/26) respectively. In a subset of these patients with recent onset IDDM (<1year), the prevalences of ICA and GADA were 50%(2/4)-75% (3/4). The frequency of GADA (by RBA, RIA and IRMA) increased as the JDF unit of ICA increased. There is significant correlation between ICA titer and GAD index (by RBA) & GAD concentration (by RIA and IRMA). There is significant correlation between GAD index (by RBA) and GAD concentration (by RIA and IRMA). These results suggest that GADA (by RIA, IRMA or Radioligand binding assay) test is useful for screening and diagnosis of Korean patients with IDDM.

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LATENT AUTOIMMUNE DIABETES IN THAI NIDDM PATIENTS WITH SECONDARY ORAL HYPOGLYCEMIC AGENTS FAILURE.

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To study the clinical characteristics of and the prevalence of glutamic acid decarboxylase antibody (GAD-Ab) in Thai NIDDM patients who had secondary oral hypoglycemic agents (OHA) failure. Sera from 40 adult NIDDM patients who were followed at out-patient diabetic clinic of Songklanagarind University Hospital and had history of successful treatment, for at least a year, with and currently failed to respond to OHA were obtained for analysis of fasting c-peptide and GAD-Ab. Clinical characteristics of all patients were obtained by reviewing medical records and/or direct patient interview. Of 40 patients, 10(25.0%) were positive for GAD-Ab with a mean level of 59.9 U/ml (median 58.5, range 3.4-127). Patients with GAD-Ab(+) had a significantly lower fasting c-peptide levels than those with GAD-Ab(-) albeit shorter duration of diabetes (0.21± 0.19(SD) VS 0.52±0.33 nmol/L; p=0.003). Duration of treatment with OHA in patients with GAD-Ab(+) was also shorter (4.6±3.5 VS 10.4±5.5 years; p=0.001). Age of onset of diabetes did not differ between these two groups. Forty percent of patients had insulin deficiency (fasting c-peptide level <0.33 nmol/L); 50% of which had GAD-Ab(+). Patients with insulin deficiency had significantly lower BMI before insulin therapy regardless of their GAD-Ab status (18.6±2.8 VS 22.9±4.3 Kg/m^2;p=0.002). Among insulin-deficient patients, those with GAD-Ab(+) had significantly shorter duration of OHA treatment (3.3±2.3 VS 10.0±7.9 years;p=0.018). In conclusion, the prevalence of GAD-Ab in Thai NIDDM patients with secondary OHA failure was 25%. Almost all GAD-Ab(+) patients had insulin deficiency and most had been initially treated with OHA for a few years before depending on insulin. This group represents a slowly pregressive form of IDDM or latent autoimmune diabetes in adult diabetic population.

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PREVALENCE OF GAD-ANTIBODIES IN A SELECTION-FREE DIABETIC POPULATION (JEVIN)

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Up to the present only a few data have been available concerning the prevalence of diabetes specific autoantibodies (anti-GAD, ICA, IAA, IA-2) in closed populations. Hence, the aim of the present trial is to determine the prevalence of anti-GAD in a selection-free population of diabetic patients (IDDM n=127, NIDDM n=117) aged 16 to 60 years and living in the city of Jena (100,247 inhabitants). In order to test sera for anti-GAD, serum samples were taken in 75% of IDDM (n=95) and in 80% of NIDDM (n=94) patients. **Results:** In the group of IDDM patients, according to a cut-off of 8 U/ml, 55% of all the patients tested positive for anti-GAD (102.9±141.5, range, 8.3-585.0 U/ml). In the group of NIDDM a total of 21% were positive (153.0±212.8, range, 9.2-626.0 U/ml). In IDDM patients anti-GAD titers were strongly correlated to diabetes duration (r=-0.23, p=0.024) and the duration of insulin therapy (r=-0.23, p=0.025), but not to HbA1c, BMI and insulin dose/kg body wt. In NIDDM patients there were no correlations. Also, no correlations were found between diabetes long-term complications (retino-, neuro- and nephropathy) and anti-GAD titers, neither in IDDM, nor in NIDDM. However, looking at the high number of anti-GAD positive NIDDM patients, although these patients showed no differences ($p<0.05$) in clinical parameters (HbA1c, age at diabetes onset or time up to initiation of insulin therapy), it must be suggested, that a part of them are patients with latent autoimmune diabetes mellitus in adults (LADA).

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ANTIBODIES TO ICA512 AND GAD65 IN MALNUTRITION-RELATED DIABETES (MRDM) PATIENTS FROM EASTERN INDIA.

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Antibodies to tyrosine pyrophosphatase (ICA512) and glutamate decarboxylase 65 (GAD65) are major markers for IDDM in Caucasians. The prevalence of ICA512 and GAD65 antibodies(Ab) in MRDM (n=128) and 123 healthy controls from Cuttack in Eastern India. was determined. MRDM was subdivided into Protein deficient diabetes (PDDM)(n=71) and Fibrocalculous pancreatic diabetes (FCPD)(n=47). MRDM patients are typically young at onset with low body mass index, require insulin treatment for glycemic control, have insulin resistance and do not develop ketosis on withdrawal of insulin. FCPD, but not PDDM, patients have abdominal pain and calculi in the pancreas. ICA512Ab and GAD65Ab were evaluated by RIA using *in vitro* translated recombinant human 35S-ICA512 and 35S-GAD65 respectively. None of the FCPD patients were positive for GAD65Ab suggesting that they have secondary form of diabetes. In controls, ICA512Ab were present in 3/123 (2%) and GAD65 Ab in 8/123 (7%). In PDDM, ICA512Ab were present in 17/71(24%) ($p<0.001$ vs controls) and GAD65Ab in 11/71 (16%)($p<0.001$ vs controls). In PDDM, the frequency of ICA512Ab and GAD65Ab was higher in patients with short-term (0-4 years) (12/46, 26% and 7/46, 15% respectively) than with long-term duration (>4 years) (5/25, 20% and 3/25, 12% respectively)(p=ns). ICA512Ab was higher in frequency in male (12/44, 27%) than female patients (2/27, 19%) while GAD65Ab was higher in frequency in female (5/27, 19%) than male patients (6/44, 14%). In PDDM patients, 53/71 (75%) were both ICA512Ab and GAD65Ab negative; 5/71 (7%) were both Abs positive; 23/71 (32%) were either ICA512Ab or GAD65Ab positive; 12/71 (17%) were ICA512Ab+/GAD65Ab-; and 6/71 (8%) were ICA512Ab-/GAD65Ab+. We conclude that PDDM patients from Cuttack in Eastern India, have ICA512Ab and GAD65Ab at a low but significant frequency compared to controls. ICA512 and GAD65 are important autoantigens in PDDM patients. ICA512Ab and GAD65Ab positivity suggest an autoimmune pathogenesis suggesting that protein deficiency may induce β-cell autoreactivity in susceptible individuals.

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AN ELISA FOR ANTIBODIES TO GLUTAMIC ACID DECARBOXYLASE IN DIABETES MELLITUS PATIENTS

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Many data suggested that insulin-dependent diabetes mellitus (IDDM) is marked by circulating antibodies to a 64000-Mr islet cell antigen identified as glutamic acid decarboxylate(GAD),antibodies to GAD were measured by ELISA using recombinant GAD as antigen in 30 healthy subjects,30 autoimmune disease,37 IDDM and 30 NIDDM(non-insulin dependent diabetes mellites) patients.The prevalence of GAD antibodies was 83.8%(31/37) in IDDM patients and 16.7%(5/30) in NIDDM patients but the prevalence of GAD antibodies was in none of the other antoimmune disease patients and healthy subjects.islet-cell antibodies(ICA) were measured by immunofluorescence the results showed the prevalence of antibodies to GAD in IDDM patients was significantly higher than that of ICA($P<0.05$)the antibodies to GAD will be of clinical value in the diagnosis and indicate of diabetes mellitus patients.

Key words:Diabetes mellitus,Glutamic acid Decarboxylase. Islet-cell antibodies.

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ISLET CELL AND GLUTAMIC ACID DECARBOXYLASE ANTIBODIES IN FIBROCALCULOUS PANCREATIC DIABETES

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Fibrocalculous Pancreatic Diabetes (FCPD) is a type of diabetes secondary to tropical, chronic non-alcoholic pancreatitis. Little is known about etiopathogenesis of FCPD. We studied islet cell antibodies (ICA-b) and Glutamic Acid Decarboxylase antibodies (GAD-Ab) in healthy controls and patients with Fibrocalculous Pancreatic Diabetes, Insulin Dependent Diabetes Mellitus (IDDM) and Non Insulin Dependent Diabetes Mellitus (NIDDM) seen at our centre in southern India. Prevalence of ICA-b was 4.7% in controls, 0% in FCPD, 54% in IDDM and 9.9% in NIDDM. Prevalence of GAD-Ab was 0% in controls, 7% in FCPD, 47.5% in IDDM and 5.6% in NIDDM. Combined ICA-b and GAD-Ab positivity was seen in 0% of controls, 0% in FCPD, 40% of IDDM and 1.4% of NIDDM. The data suggests that in FCPD the frequency of auto-antibodies is low and its etiology is probably not linked to auto-immunity.

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CLINICAL VERSUS LABORATORY CLASSIFICATION OF DIABETES TYPE IN YOUNG ADULTS

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The classification of diabetes is based on descriptive clinical signs and the uncertainty in the classification increases with age of the patient. In this study a combination of clinical based criteria and laboratory tests for the classification of the type of diabetes are studied.

Methods: Blood samples from consequtively diagnosed patients with diabetes in the ages 15-34 years were collected during two years. A clinical classification of the diabetes was done at diagnosis by the doctor who treated the patient, without knowledge of the laboratory results. ICA (islet cell antibodies), GAD65-ab (glutamic acid decarboxylse antibodies) and C-peptide were analysed.

Results: Of 586 patients classified as Type 1 236 were negative for ICA and GAD65-ab (Type 1-), and these showed higher C-peptide (0.38±0.27 nmol/l) compared with autoantibody positive Type 1 patients (Type 1+) (0.30±0.21 nmol/l; $p<0.001$). Among Type 1- patients 20 (8%) were found to have C-peptide >0.50nmol/l and BMI>25. Of the 110 clinically classified Type 2 patients, 37 (34%) had ICA or GAD65-ab (Type 2+) and 51% were on insulin. C-peptide in Type 2+ were lower than in Type 2- (0.51±0.40 nmol/l vs 0.87±0.50; $p<0.001$), but higher than in Type 1+ patients (0.30±0.21; $p<0.001$).

Conclusion: The levels of C-peptide and BMI indicated that both Type 1 and Type 2 patients are misclassified. The estimated frequency of clinically classified Type 2 who are Type 1 is about 8%, and even higher, about 30%, are initially classified as Type 2 but should be in the Type 1 group. An identification of combined clinical and laboratory criteria are needed for an improved classification of diabetes in these ages.

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PERSISTENT GAD 65 ANTIBODIES IN LONGSTANDING IDDM■ ARE NOT ASSOCIATED WITH RESIDUAL BETA CELL FUNCTION, NEUROPATHY OR A CERTAIN HLA-DR STATUS

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Persistent humoral autoimmunity to the enzyme glutamic acid decarboxylase (GAD) has been described in a substantial proportion of patients with insulin-dependent diabetes mellitus (IDDM) of long duration. The source of the stimulus for this autoimmune reactivity is still unknown. Because the GAD 65 isoform is mainly expressed in pancreatic beta-cells and in the nervous system we investigated in 105 patients with longstanding IDDM (median duration: 21 years; range: 10-46 years) the presence of autoantibodies to GAD 65 (radioligand-binding-assay) and their relationship to a residual C-peptide response (Hendriksen's criteria) or peripheral and autonomic neuropathy. Additionally we studied the HLA-DR status relative to GAD 65 antibodies in 86 individuals. Autoantibodies to GAD 65 were present in 32% of the long-term diabetic patients, 82% of the recent onset IDDM patients and in 3% of the healthy control subjects. A preserved C-peptide response was observed in 23%, autonomic neuropathy was found in 67% and peripheral neuropathy in 79% of the patients. The HLA specificity DR 4/X was observed in 47% and HLA-DR 3/X in 22%. Patients who were heterozygous for DR3/DR4 were found in 23% of the cases. Diabetes duration showed a significant negative correlation with GAD 65 antibody index levels ($r=-0.22$; $p<0.01$). Interestingly, GAD 65 antibodies were not significantly correlated with residual beta cell function or neuropathy and no particular HLA-DR status was associated with persistent GAD 65 antibodies. In conclusion neither residual beta-cell function nor diabetic neuropathy or a certain HLA-DR specificity are exclusively associated with persistent autoimmunity directed to GAD 65 in longstanding IDDM. The stimulus for the persistent humoral immune response and its significance for the disease process and its complications remain to be established.

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C-PEPTIDE LEVELS IN AUTOIMMUNE DIABETES DURING THE FIRST TWO YEARS AFTER DIAGNOSIS

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The aim of this study was to follow the C-peptide levels, the influence of autoimmune markers and other factors that may predict the course of C-peptide during the first two years after diagnosis of Typ 1 diabetes.

Method:In1992-93 all new onset diabetic patients aged 15-34 years, donated a blood sample.Only patients positive for either ICA (islet cell antibodies) or GAD65-ab (glutamic acid decarboxylase-antibodies) or both were included. 890 patients were registered of whom 607 were positive, 8,4% were classified as Type 2 and 7,5% unclassifiable. ICA were analysed with IF, GAD-ab and C-peptide with RIA. The reference range for the C-peptide was 0,25-1,0 nmol/l.

Results:At onset 57% had C-peptide values within the reference range, after one year it was 54% and after two years 39%. The mean value of the C-peptide levels was 0,33±0,25 nmol/l at diagnosis and unchanged after one year, 0,34±0,26. However, after two years the levels decreased with a mean of 0,05±0,28 to 0,28±0,26 nmol/l (p=0,003). The decline of the C-peptide during the first two years positively correlates to the initial level (rs=0,60;p<0,001), and also to age (rs=0,15;p=0,007). The initial levels of ICA or GAD65-ab and the decline of the C-peptide levels do not correlate.

Conclusion: Young adults with Type 1 diabetes have the same C-peptide levels after one year as at onset, but during the second year the levels decline. After two years 39% have C-peptide levels within the reference range. The decline of the C-peptide is larger if the initial level is high. The levels of ICA and GAD65-ab do not correlate to the decrease.

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"The UK and Padua Polyendocrine Prospective Study". Association of antibodies to IA-2 and phogrin with progression to IDDM.

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Almost all ICA positive patients with polyendocrine (PE) autoimmunity have antibodies to GAD, but only a proportion (approximately 30%) develop IDDM. Previous studies have shown that antibodies to 40kD and 37kD fragments of islet antigens, now identified as tyrosine phosphatase (PTP)-like proteins IA-2 and phogrin respectively, can identify those PE patients with highest risk for IDDM. In this study, we have analysed antibodies to IA-2 and phogrin using assays with *in vitro* translated recombinant antigen in a larger population of PE patients to re-evaluate the value of these antibodies in prediction of IDDM. Of 154 ICA positive PE patients, 19 developed acute onset IDDM and a further 11 required insulin after a period of >1 year of non-IDDM. 37 of the PE patients, had antibodies to IA-2, of whom 10 developed acute onset and 7 slow onset IDDM, whereas 38 had antibodies to phogrin, 9 of whom developed acute onset and 5 slow onset IDDM. The sensitivity, specificity and positive predictive value for IDDM development within the PE population for IA-2 antibodies were 57%, 84% and 46% and for phogrin antibodies 47%, 80% and 36%. The majority (26/49) of PTP-antibody positive patients had antibodies to both IA-2 and phogrin, but a higher proportion of patients who developed acute onset IDDM (42%) were positive for antibodies to both IA-2 and phogrin than those who progressed to IDDM slowly (18%). However, 11 patients were positive for IA-2 antibodies in the absence of phogrin antibodies, and 12 patients were positive for phogrin antibodies alone; in spontaneous IDDM patients, individuals with phogrin antibodies alone are rare. Thus, antibodies to IA-2 and phogrin are markers for IDDM in PE patients and can develop independently, suggesting that autoimmunity to phogrin is not always the result of cross-reactivity between these two homologous proteins.

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PANCREATIC RESERVE AND GAD ANTIBODIES IN MALAYSIAN YOUNG DIABETICS.

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In Malaysia, it can be difficult to categorize and treat young diabetics. We investigated the pancreatic reserve and GAD antibodies in 320 diabetics, between the ages of 12 and 40 years and diagnosed within 5 years. Blood samples for GAD antibodies, C-peptides, insulin and IGF-I were taken at fasting and 6 minutes post-1mg glucagon, IV. Post-glucagon stimulated C-peptides of less than 600 pmol/L was used as the criteria of ß-cell deficiency. 93 (29%) were treated as IDDM and 227 as NIDDM. 60 patients (18.8%) were GAD positive, 29 in the IDDM (31%) and 31 in the 227 NIDDM (13.7%). 23/29 of the GAD positive IDDM had low C-peptides (79.3%), the other 6 (20.6%) had adequate reserve. Conversely, 38/61 (62.3%) IDDM with low C-peptides were GAD negatives. Of the 227 NIDDM, 59(26%) had low and 168 (74%) had high C-peptides. 16/59 (27.1%) of the NIDDM with low C-peptides and 15/168 (8.9%) with high C-peptides were GAD positive. The GAD-positive NIDDM were older and had significantly higher mean BMI, waist-hip ratio (WHR) and C-peptides than GAD positive IDDM. GAD antibodies had negative correlations with WHR and C-peptides only if the C-peptides were low. Using criterion of GAD positivity, 13.7% young NIDDM in Malaysia were latent autoimmune diabetes in adults. Conversely, 28% of young IDDM were GAD-negative and had adequate C-peptides, implying NIDDM.

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METABOLIC, AUTO IMMUNE AND GENETIC MARKERS IN IDDM PATIENTS AND THEIR FAMILY

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In a prospective family study (over 3 years) we screened 45 families of IDDM patients (diabetics : 28M, 17F,age : 21 ± 2 yrs ; duration of IDDM : 7 ± 6 yrs ; siblings : 45M, 23F, age : 19 ± 2 yrs, parents : 40M, 41F) for the presence of metabolic, autoimmune and genetic markers. Despite higher sHbA1c levels (HPLC) in the diabetics (7.2 ± 0.2%, versus 4,6 ± 0,04%), cholesterol and triglyceride levels were slightly higher in the nondiabetic subjects (182 ± 5 vs 203 ± 5 mg/dl and 94 ± 7 mg/dl vs 114 ± 5 mg/dl).
IAA are present in most diabetics (84%) and in 3,3% of the control group. GAD Ab and ICA are still present in 40% of the IDDM patients compared to 6.7% and 2% respectively in the controls.
A high prevalence of auto Ab against colon (13% vs 21%), thyroïd (15% vs 24%), and ANF (17,8% vs 13,4%) are found as well in patients as in family members. No antibodies towards other endocrine glands have been demonstrated. In all diabetic patients at least 2 genetic high risk alleles (DQβ and DQα) are present. In family members only one has not at least one high risk allele. During the study period, the sib with ICA and GAD Ab developed IDDM. In two parents NIDDM was diagnosed. One patient with ANF developed SLE.
In conclusion : we observed a high prevalence of autoimmune markers in IDDM patients and their family members. Whether this is related to a higher prevalence of other autoimmune diseases remains to be evaluated. All subjects with positive antibody titers need further follow up.

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EFFECTS OF ANTIBODIES TO GLUTAMIC ACID DECARBOXYLASE (ANTI-GAD) ON β-CELL FUNCTION IN DIABETIC PATIENTS.

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To determine the effect of antibodies to glutamic decarboxylase (Anti-GAD) on β-cell function, we measured Anti-GAD and pancreatic β-cell secretory function in diabetic patients. Materials and Methods : Anti-GAD antibody titers in serum were measured using ELISA kits. The increment of serum C-peptide at 6 min after 1mg glucagon injection ($\triangle$CPR), 24 h urinary C-peptide excretion (UCPR), HbA_1c, the duration of DM, and daily insulin dosage were evaluated in insulin-dependent diabetes mellitus (IDDM) (11 males and 14 females) and in non-insulin dependent diabetes mellitus (NIDDM) (16 males and 15 females on insulin, 13 males and 20 females on SU agents). Results : The prevalence of Anti-GAD was 44% in patients with IDDM (11 GAD positive, 14 GAD negative) and 1.5% in patients with NIDDM. The values of $\triangle$CPR (ng/ml) in GAD positive and negative groups (IDDM groups) were 0.4 ± 0.1 and 0.3 ± 0.1, respectively, being significantly lower than those in NIDDM patients on insulin and on SU agents (2.3 ± 0.2, 2.8 ± 0.2). UCPR (μ g/day) levels in GAD positive and negative groups were 9.9 ± 2.3 and 4.8 ± 1.3, being significantly lower than those in NIDDM patients on insulin and SU agents (46.0 ± 1.9, 61.2 ± 3.2). The values of $\triangle$CPR and UCPR in GAD negative patients were significantly ($P<0.05$) lower than those in GAD positive patients. The duration (years) of DM in GAD negative group was significantly ($P<0.05$) greater than that in GAD positive group (10.3 ± 0.8 vs 4.2 ± 1.2). Daily insulin dosage (U/day) in GAD positive, GAD negative and NIDDM patients on insulin were 29 ± 1.5, 32 ± 2.6 and 17.9 ± 1.3, in which the level of the last was significantly lower ($P<0.01$) than the others. No significant differences in HbA_1c value were found between any groups except that the value in GAD positive group was significantly ($P<0.035$) higher than that in NIDDM patients on SU agents. Conclusion : The prevalence of Anti-GAD in IDDM patients decreases gradually with a progressive functional exhaustion of pancreatic β-cell.

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GAD ANTIBODIES ARE ASSOCIATED WITH THYROID ANTIBODIES IN CHILDREN WITH IDDM AND IN THEIR SIBLINGS

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We have earlier reported no or only weak associations between ICA or IAA and other signs of humoral organ-specific or non-organ-specific autoimmunity. To explore the possible relationship between antibodies to glutamic acid decarboxylase (GADA) and other autoantibodies we analysed GADA and parietal cell (PCA), reticulin, adrenal, thyroid microsomal (TMsA) and thyroglobulin (TgA), smooth muscle, anti-nuclear and mitochondrial antibodies in 656 0-15-year-old children with IDDM and in their 588 3-19-year-old healthy siblings at the time of diagnosis of the index case. GADA were detected in 74.2% of the children with IDDM and in 6.5% of the siblings, TMsA in 11.1 and 7.9%, TgA in 5.3 and 3.3% and PCA in 6.7 and 4.6%, respectively. Diabetic children with TMsA tested more often positive for GADA than those without at the clinical manifestation (91.8 vs. 72.0%, p=0.0003) and they had higher GADA levels than those negative for TMsA (medians 46.2 vs. 22.0 RU, p<0.001). GADA positivity was also more often seen in TgA-positive index cases than in TgA-negative ones (88.6 vs. 73.4%, p=0.046). Siblings positive for TMsA were more often positive for GADA than those testing negative for TMsA (15.2 vs. 5.8%, p=0.012), but there was no significant difference in the GADA levels between TMsA-positive and negative siblings. A trend of association was also seen between GADA and PCA in siblings: 14.8% of PCA-positive tested positive for GADA, whereas only 6.1% of those negative for PCA (p=0.07). A similar trend was observed when comparing GADA levels in PCA-positive and negative siblings (medians 0.8 vs. 0.0 RU, p=0.075). No other associations were found between GADA and other autoantibodies. Our observations indicate that humoral autoimmunity against GAD may be determined by the same factors as those controlling susceptibility to thyroid autoimmunity, and GADA may predict susceptibility to thyroid disease in diabetic children and in their siblings who are at risk for IDDM.

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COMPLEMENT DEPENDENT ANTIBODY MEDIATED CYTOTOXICITY IN TYPE 1 DIABETES: WHAT'S HAPPENING UNDER THE ICEBERG?

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In order to investigate the activation system of the classical and alternative pathway of complement, circulating immune complexes (cIC), human IgG and its subgroups, and ICA this study was conducted in 78 patients with Type 1 diabetes (mean age 28.4±12 yrs, duration of diabetes 2.1±1.1 yrs) and in 20 non-diabetic controls (mean age 27.1±11yrs). CH50 and AH50 were determined using immune haemolytic assay by microcomplement technique, cIC by microcomplement fixation test. The precipitation of cIC is accomplished by caprilic acid-ammonium sulphate method, purification of IgG by agarose gel filtration electrophoresis, fractioning of IgG using protein A sepharose column in HPLC, ICA by indirect immunofluorescence technique. Activation of classical pathway and cIC was significantly elevated in Type 1 diabetics compared to the controls (CH50 (ml) 271.2±122.1 vs 208.6±34, p<0.05; cIC (%) 21.5±11.1 vs 10.8±4, p<0.001), but the alternative way was not activated. In 86.2% of elevated CH50 group, cIC was found to be high (p<0.001). In the group with ICA>20JDF CH50 and cIC were significantly higher than in the ICA(-) group (CH50 (ml) 332.8±119 vs 226.9±104.5, p<0,001; cIC (%) 28.7±11.8 vs 16.2±7.1, p<0.001). In 77.4% of patients with ICA(+) cIC was high. Human IgG which is obtained by precipitation and purification of cIC was significantly higher in diabetics than in non-diabetics (3.9±1 vs 1.72±0.8mg/ml). IgG1κ had the highest peak (52.7%) in fractions of IgG. IgG1k was significantly elevated in Type1 diabetics compared to the non-diabetics (2.03±0.9 vs 1.03±0.23mg/dl, p<0.001). In conclusion, complement dependent antibody mediated cytotoxicity (C'AMC) which is activated by beta cell antibodies cIC play an important role in the destruction of beta cell. IgG1κ is a reliable indicator for the activation of C'AMC.

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PREVALENCE OF SUBCLINICAL HYPOTHYROIDISM IN SIBLINGS OF PATIENTS WITH TYPE I DIABETES.

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A higher prevalence of clinical hypo and hyperthyroidism as well of subclinical hypothyroidism (SCH) has been reported in IDDM. In order to evaluate the prevalence of SCH in siblings of patients with IDDM not previously suspected of having thyroid disease, TSH levels before and following the administration of TRH and thyroid microsomal (MCS -Ab) and thyroglobulin (TGB - Ab) antibodies were determined in 70 controls (C) (AGE 27±9, 41 F and 29 M), 42 patients with IDDM (AGE 24 ± 7, 25 F and 17 M) and 64 non-diabetic siblings (AGE 29 ± 10, 34 F and 30 M). TSH was measured by RIA, thyroid Ab by hemoaglutination. The prevalence of increased TSH concentrations (Basal≥ 5 uU/ml and/or post TRH≥ 25 uU/ml) was 2.8, 19.0 and 10.9 %, MCS-Ab were positive (≥ 1/100) in 4.2, 26.1 and 14%, and TGB-Ab in 2.8, 14.2 and 6.2% in C, patients with IDDM and their healthy siblings, respectively ($p<0.01$). Goiter was detected in 2.8, 14.2 and 7.8 % in the 3 groups. These results support the existence of a higher prevalence of SCH both in patients with IDDM and in their non-diabetic siblings, emphasizing the need to look for it in both groups.

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THE DIABETES PREVENTION TRIAL-TYPE 1 DIABETES (DPT-1): DEMOGRAPHICS OF RELATIVES SCREENED AND ICA POSITIVE.

DPT-1 Study Group, Nationwide, USA

The DPT-1 is a nationwide study designed to determine whether insulin based therapies can delay or prevent the clinical onset of Type 1 diabetes in relatives of patients with established disease found to be at high risk of diabetes development. Parenteral insulin is used in relatives with >50% projected 5-year risk. Oral insulin is used in relatives with 26-50% projected 5-year risk. Screening for islet cell antibodies (ICA) began in February 1994. By November 30, 1996, there were 40,381 samples which had been received and analyzed for ICA. The age and gender distribution of subjects tested is as follows:

Age	Female	Male	Unknown	Total
0-9	5677	6268	3	11948
10-20	6455	6355		12810
21-45	10434	5189		15623
Total	22566	17812	3	40381

Rates of ICA positivity are:

Age	Female	Male	Total
0-9	3.3%	4.0%	3.7%
10-20	3.2%	4.5%	3.9%
21-45	2.6%	3.6%	2.9%
Total	3.0%	4.1%	3.4%

In terms of ethnicity, of subjects screened, 83.7% were non-Hispanic White, 8.2% were Hispanic, 2.5% were non-Hispanic Black, 0.8% were Asian or Pacific Islander, and 0.2% were native American Indian or Alaskan. Rates of ICA positivity were: 3.6% for non-Hispanic Whites, 2.3% for Hispanics, 3.4% for non-Hispanic Blacks, 2.2% for Asian or Pacific Islanders, and 1.0% for native American Indian/Alaskans. The rates of ICA positivity vary only slightly amongst different age, gender, and ethnic groups. Differences may be accounted for either by ascertainment bias or real biological variation.

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GAD ANTIBODIES IN LONG TERM DIABETICS WITHOUT OVERT ENDOCRINOPATHY: A MARKER OF THYROID IMMUNITY AND DISEASE.

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Persistent pancreatic antibodies have been described in diabetic patients with overt autoimmune thyroid disease. In diabetics without clinical thyroid disease, contradictory results have been reported about predictive value of persisting ICA in developpment of thyroid autommunity and disease . We have studied pancreatic markers (ICA and GAD antibodies), TPO antibodies and TSH values in 115 long standing (> 5 years) diabetic patients without known clinical and biological endocrinopathy Results: 16 of the 41 patients who were positive for ICA and/or GAD antibodies displayed also TPO antibodies (40%) versus only 5 of the 74 other subjects (7%, p<10-4).This result was also found when ICA positivity alone was analysed (p<0.05) but also for GAD antibodies with a greater significativity (p<10-4). ICA fluorescences in patients with ICA ≥20 units were restricted to ß cells and were totally or partially blocked after preincubation with brain GAD extracts. Eight patients, all with TPO antibodies, displayed abnormal TSH values, 6 of the 41 patients with persistent pancreatic autoimmunity (15%) versus only 2 of the 74 patients without ICA or GAD antibodies (3%, p=0.03). Among the 6 patients with persistent pancreatic immunity, 4 developped overt clinical thyroid disease (one hypothyroidism and 3 Graves'disease) in the next year. In conclusion, persistence of a humoral GAD reactivity is not only found in diabetic patients with overt clinical thyroid disease but is also associated with markers of thyroid autoimmunity and risk of further developpment of a thyroid dysfunction in diabetic patients without known clinical or biological autoimmune endocrine disease.

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ADHERENCE OF FINNISH FAMILIES TO FREQUENT ISLET CELL ANTIBODY FOLLOW-UP OF CHILDREN AT GENETIC DIABETES RISK.

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The Finnish Diabetes Prediction and Prevention (DIPP) project, which began in 1994, is a population-based, prospective trial aiming at 1) recognizing the newborn babies who are at genetic risk for IDDM, 2) determining the onset of autoimmune destruction of beta cells at an early stage, and 3) preventing/delaying the onset of clinical IDDM. Cord blood samples are examined for 2 susceptibility and 3 dominant protective HLA-DQB1 alleles associated with IDDM risk. Children with genotype associated with increased IDDM risk are invited for islet-cell antibody (ICA) follow-up. ICA titers are measured at 3-month intervals for 2 years, then every 6 months. The aim of this study was to evaluate 1) the parental acceptance of genetic screening of newborn babies for IDDM susceptibility, and 2) the adherence of Finnish families to immunological follow-up of the children at genetic IDDM risk. Altogether 6977 babies were born in the University Hospital of Turku between 11/94 and 11/96 (non-Caucasian babies excluded). Informed consent for the genetic screening was obtained from the parents of 6860 (98.3%) newborns. By 11/96, the genetic IDDM risk of 6438 babies has been determined. Of these, 811 (12.6%) carry an increased IDDM risk. In 11/96, 179/811 (22.1%) of the at-risk children have been lost from ICA follow-up. The majority (134/179 = 74.9%) of these families were unwilling to participate the follow-up either immediately after they had been informed about their child's increased IDDM risk by phone, or after the first counseling visit. When the children reach the age of 9 months, drop-outs become extremely rare; so far only two children have been lost from the follow-up after the 9-month visit, and none after the 15-month visit (to date, more than 500 children have visited at 15 months of age). In conclusion, the cord blood screening for genetic IDDM susceptibility is well accepted by Finnish families, and the adherence of families of the at-risk children to the rather laborous ICA follow-up is excellent, especially after the child has reached the age of 15 months.

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HIGH PREVALENCE OF GAD65 ANTIBODIES IN JAPANESE IDDM PATIENTS BY A NEW IMMUNOPRECIPITATION ASSAY.

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Marked differences have been reported in the prevalence of glutamic acid decarboxylase (GAD) antibodies between Caucasian (63-84%) and Japanese (30-50%) or Asian (5-50%) IDDM patients. Using a new immunoprecipitation assay based on ^{125}I-labeled recombinant human GAD65, we reassessed prevalence of GAD65 antibodies in Japanese patients. GAD65 antibodies were detected in 83.3% of sera taken within 1 year of onset, comparable to the prevalence reported in Caucasian patients. Positivity decreased to 66.7% after 2 to 3 years and to 54.3% after 3 years from onset, still higher than previously reported Asian prevalence. Except in one patient, high antibody levels persisted chronically, up to 12 years. GAD65 antibodies were detected in 2.6% (1/38) of new-onset NIDDM sera, in 2.6% (1/38) of untreated Graves' disease sera, and in 1.5% (1/65) of normal subjects sera. GAD65 antibodies were not found in 18 Hashimoto's thyroiditis and 36 SLE patients sera. Even it positive, levels of GAD65 antibodies were very low. There was no difference in GAD65 antibodies between Japanese IDDM patients with and without autoimmune thyroid disease (AITD). The difference in positivity in Asian IDDM patients between present and previous reports arose from the sensitivity of our assay for GAD65 antibodies. Additionally, the patients we studied had classic IDDM with a well-defined onset. We conclude that prevalence of GAD65 antibodies in Japanese patients with IDDM is comparable to that in Western studies. There was no relationship of GAD65 antibody positivity to coexistence of AITD. Our results suggest that autoimmunity is the most significant cause of Japanese IDDM.

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SEROCONVERSION TO AUTOANTIBODY POSITIVITY OF CHILDREN AT GENETIC RISK FOR INSULIN-DEPENDENT DIABETES.

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The Diabetes Prediction and Prevention (DIPP) project aims at finding the newborns at genetic risk for IDDM development, recognizing early the appearance of autoimmune markers preceding clinical IDDM, and delaying the onset of the disease. In the population-based DIPP trial, which began in 1994, cord blood samples of all newborns are examined for 2 susceptibility and 3 dominant protective IDDM-associated HLA-DQB1 alleles. As 85-90% of new cases of IDDM occur in subjects with no family history of the disease, effective prevention has to be based on screening of genetic susceptibility and/or analysis of preclinical IDDM markers in the entire population. HLA-DQB1 allele combinations *0302/*0201 and *0302/*x (*x ≠ *0602, *0603 or *0301), which associate with ~7% and ~3% IDDM risk (risk of background population 0.66%), respectively, before the age of 15 years, define a population which in Finland includes 60-80% of those who develop IDDM in childhood. Children with these genotypes are followed for development of islet-cell antibodies (ICA) at 3 to 6 month intervals. By November 1996, the genetic IDDM risk of 6438 newborns has been determined in Turku. Of these, 811 (12.6%) carry an increased risk. Eight at-risk children have been ICA positive at 3 months; 3 of them have lost ICA by 6 months, 2 children by 9 months, 2 by 12 months, and 1 child by 15 months of age. Now that the oldest children are 24 months of age, 7 have seroconverted to ICA positivity at the age of ≥6 months; these titers often fluctuate. First rapidly-rising ICA titers appeared in 2 children between 9 and 12 months of age; these children have also antibodies against GAD or insulin. In conclusion, ICA detectable at birth are lost by 15 months of age in all children with increased genetic IDDM risk. Positive "own" ICA titers were first recognized between 3 and 6 months of age, but these titers often fluctuate. First aggressive-looking ICA titer curves appeared between 9 and 12 months of age.

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ISLET CELL CYTOPLASMIC ANTIBODIES, GAD ANTIBODIES, AND ANTI-THYROID ANTIBODIES IN JAPANESE IDDM PATIENTS.

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Onset of insulin-dependent diabetes mellitus(IDDM) is in consequence of autoimmune B-cell destruction. Islet cell cytoplasmic antibodies(ICA) and anti-GAD antibodies as immunological markers of the B-cell damage detected in sera from patients with IDDM in early stage were reported during the past decades. In Japan, it is well known that Hashimoto's thyroidis, an organ-specific autoimmune disease, is often co-existent with IDDM, so we investigated frequency of anti-thyroid antibodies in Japanese IDDM patients, and relations of the antibodies to anti-islet antibodies. In 46 IDDM patients, ICA, anti-GAD, thyroglobulin antibody, and TPO antiboby were determined in same serum samples, and inter-relationships of these antibodies were analysed in respect of antibody titer and positivity. In 50 non-diabetic patients with thyroid disease, these antibodies were also measured as control study. Of 46 IDDM, ICA were found in 36(78.3%), anti-GAD were in 28(60.9%), thyroglobulin antibody was in 23(50.0%), and TPO antibody was in 22(47.8%). Thyroglobulin antibody or TPO antibody were frequently positive in patients having high titer ICA(160≦) than in those having low titer or negative ICA(80≧)($P < 0.001$). Furthermore, these thyroid antibodies were also frequently positive in patients having high titer anti-GAD(700U/ml <) than in those having low titer or negative anti-GAD(700U/ml >)($P < 0.01$). In 50 non-diabetic controls, ICA and anti-GAD were detected in only one (2.0%) and two (4.0%) patients, respectively. The results that frequency of anti-thyroid antibodies in IDDM is associated with high titer of anti-islet antibodies indicate close relation of the two organ-specific autoimmune diseases in Japanese subjects. It should be resolved what is common immunological abnormality lying between two autoimmune diseases, IDDM and Hashimoto's thyroiditis.

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DIRECT COSTS OF COMBINED GENETIC AND IMMUNOLOGICAL PREDICTION OF INSULIN-DEPENDENT DIABETES IN CHILDREN.

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In the Diabetes Prediction and Prevention (DIPP) project two HLA-DQB1 alleles associated with IDDM risk and three dominant protective alleles of all newborns in Turku and Oulu (8,500 annual births) are analysed in cord blood. The screening finds 60-80% of those who will develop IDDM before the age of 15 years. Islet-cell antibody (ICA) titers of the at-risk children (12.6% of the newborns) are measured in cord serum, then at 3-month intervals for 2 years, and thereafter at 6-month intervals. The aims of this study were to analyse the direct costs of genetic screening for IDDM risk, to analyse the direct costs of repeated ICA follow-up, and to compare the direct costs of the approaches based on A) genetic screening with subsequent ICA follow-up of the at-risk children and B) repeated ICA screening of the entire population. The major part of the costs was caused by collection and handling of samples and by counseling of the families. The mean fixed (i.e., overhead costs 65%) and variable direct cost of one risk allele determination in our project is US$83. The direct cost of finding one child at genetic IDDM risk is US$653. If the at-risk child remains ICA negative, the direct costs of ICA follow-up are $239, $212 and $117 in the 1st, 2nd and 3rd year, respectively. If the child is ICA positive in all measurements, the costs increase to $478, $404 and $212, respectively. The 10-year costs of genetic screening and subsequent ICA follow-up of the at-risk children are in the mean $261/child; without genetic screening the mean costs of ICA follow-up are $680/child. Using 5% discount rate the present direct costs are $232 and $557 per child, respectively. At Finland's annual birth rate (65,000) the 10-year direct costs of extending the trial to all newborns and using the two approaches would be $15.0 million and $37.7 million, respectively. In conclusion, population-based genetic screening for IDDM risk is clearly cost-saving compared to the approach based on repeated ICA screening of the entire population.

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IN NEW-ONSET IDDM PATIENTS THE PRESENCE OF IA2-ANTIBODIES IS NOT ASSOCIATED WITH INCREASED THYROID AUTOIMMUNITY

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In previous studies a strong association between the presence of anti-thyroid peroxidase antibodies (aTPO) and a positive level of islet-cell antibodies (ICA $\geq$ 12 JDFU) has been demonstrated in new-onset IDDM patients aged 10-39 yr but this observation could not be confirmed in patients with GAD 65 Ab positivity. Since recently a liquid phase radiobinding assay became available to measure autoantibodies against a transmembrane PTP-ase, IA2, a known antigen in IDDM, it seemed interesting to look after a possible relation with aTPO positivity. In 105 new-onset IDDM (71 men, 34 women, ages 10-39 yr) 49% showed an increased level of IA2-Ab ($\geq$0.51% of tracer binding). The median age of the IA2-Ab positive group was significantly lower (21 yr versus 26.5 yr, $p = 0.02$). The distribution between men and women was not different in the IA2-Ab+ and - groups. Positive thyroid autoimmunity (aTPO > 100 Uml^{-}) was equally distributed between both groups (10/51 IA2-Ab+ versus 11/54 IA2-Ab-). Although more women (35%) showed aTPO positivity as compared to men (12.8%, $p<0.01$) no sex differences could be disclosed for thyroid autoimmunity in the IA2-Ab+ group. When patients of this group were subdivided in a young (10-25 yr) and an older group at onset (26-39 yr) the equal distribution of aTPO positivity was maintained in contrast to previous observations where ICA and IAA positivity were significantly associated to aTPO+ in the older group. Finally in this limited number of patients Fisher Exact Test showed a significant relation ($p=0.02$) of the risk haplotype HLA-DQ DQA1*0301-DQB1*0302 with aTPO positivity but not with IA2-Ab positivity ($p=0.1246$). In conclusion it looks that IA2-Ab positivity at onset of IDDM does not permit a prognosis of future increased thyroid autoimmunity.

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THE SARDINIAN SCHOOL CHILDREN - IDDM (SSI) STUDY. PREDICTIVE VALUE OF IDDM-RELATED AUTOANTIBODIES IN A COHORT OF HEALTHY SARDINIAN SCHOOL CHILDREN.

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In order to define the predictive value of islet-related autoantibodies, as single or combined specificities, over 10,000 sera have been collected from school children living in the 4 provinces of Sardinia. To date, 3080 of them (from the 3 provinces of Cagliari, Oristano and Nuoro), have been followed-up for a period of 2 to 10 years. Their sera were tested for ICA, GADA and IA-2icA. 10 (M:F=1) developed IDDM between 2 and 60 months since they were recruited into the study. All but 1 were 'sporadic' cases of IDDM and none live in the province of Nuoro.

Test	Positive No (%)	Se %	Sp %	PPV %
ICA ≥5	255 (8.3)	100	92	4
ICA >20	64 (2.1)	80	98	13
GADA	37 (2.1)	40	98	11
IA-2icA	49 (2.8)	70	98	14
ICA >20 & GADA	19 (1 1)	30	99	16
ICA >20 & IA-2icA	22 (1.3)	70	99	32
GADA & IA-2icA	15 (0 9)	30	99	20
ICA >20 & GADA & IA-2icA	14 (0 8)	30	99	21

All were positive for ICA, 7 were positive for IA-2icA and 4 had also GADA. The overall results are summarized in the Table, which indicates that: 1) ICA alone, ≥5 JDFu (100%) or >20 JDFu (80%) showed the highest values of Se for pre-IDDM and 2) combination of ICA >20 JDFu and IA-2icA can still be regarded as a promising test for pre-IDDM (Se 70% & Sp 99%) also showing the highest PPV (32%). In summary, it is only by consistenly following these children, that prediction of IDDM among sc will be assessed in Sardinia.

Se sensitivity, Sp Specificity, PPV positive predictive value

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INSULIN AUTOANTIBODIES BEFORE AND AFTER CARBIMAZOLE THERAPY IN ASIAN INDIAN PATIENTS WITH GRAVES' DISEASE.

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Spontaneous occurrence of Insulin autoantibodies(IAA) has been reported in patients with Graves' disease (GD). Also in Japanese patients with GD, IAA has been reported to be induced with carbimazole (CBZ) therapy. However CBZ has not been shown to cause induction of IAA in Caucasians. The HLA haplotypes associated with GD in Asian Indians have been reported to resemble Caucasians rather than Japanese. We assessed IAA levels in 114 north Indian patients with GD before and after CBZ therapy (mean duration 6.2 $\pm$ 3.9 months). We also evaluated the functional significance of IAA in them by assessing insulin response to intravenous glucose (IVGTT) and oral glucose tolerance test (OGTT). Thyroid hormones and insulin were measured by in-house radioimmunoassays. IAA was measured using radioligand assay and expressed in standard deviation scores (SDS) above healthy controls. On participation in the 5th IAA proficiency program (University of Florida, U.S.A.) our assay had 100% specificity and 90% sensitivity. Thyroid microsomal antibody (TMA) was measured by haemagglutination method. Pretreatment, 22 of 114 (19%) GD patients had positive IAA (mean$\pm$SD 5.5 $\pm$ 4.4 SDS). After CBZ therapy additional 11 (9.6%) showed IAA positivity (mean$\pm$SD, 3.5 $\pm$ 1.0 SDS). Of the 22 patients with IAA +ve before treatment, 12 became negative after CBZ therapy. The fasting insulin levels and area under insulin curve in IVGTT (mean$\pm$SD) were comparable in IAA+ve and -ve GD (43.7 $\pm$ 64 vs 56 $\pm$ 33 pmol/L and 3142 $\pm$ 215 vs 3458 $\pm$ 2195 pmol/10min respectively). OGTT results and %TMA positivity were also comparable in the IAA+ve and -ve groups. Thus, north Indian GD patients, though resembling Caucasians in HLA haplotypes, behave like Japanese in co-prevalence of IAA and their tendency to become IAA +ve on CBZ therapy.

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THE SARDINIAN NEWBORN - IDDM (SNI) STUDY. EARLY ONSET OF IDDM CORRELATES WITH THE APPEARANCE OF ISLET-RELATED AUTOANTIBODIES IN A COHORT OF YOUNG CHILDREN FOLLOWED UP FROM BIRTH.

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In order to study the natural history of IDDM in reference to the appearance of islet-related autoantibodies, between Nov 1993 and Sep 96, 15,939 newborn were recruited and 12,858 cord blood sera were tested for ICA. Their prevalence was 2.4% (314) for titres ≥5 JDFu and 0.7% (86) for titres >20 JDFu, with a similar trend in the 3 provinces studied (Cagliari, Nuoro and Sassari). To date, of the 12,858 children initially recruited, 1897 blood samples have been collected at year 1 and 278 at year 2. The sera were tested for ICA (yr1=1853; yr2=238), GADA (yr1=1893; yr2=277) and IA-2icA (yr1=1894; yr2=275). The results are summarized in the Table.

AAbs	Year 1		Year 2	
	Pos	%	Pos	%
ICA	4	0.2	4	1.7*
GADA	8	0.4	4	1.4#
IA-2icA	3	0.2	2	0.7
ICA & GADA	2	0.1	3	1.5*
ICA & IA-2icA	1	0.1	2	1.0*
GADA & IA-2icA	0	0	2	0.7*
ICA & GADA & IA-2icA	0	0	2	1.0*

*p<0.001 vs Year 1, #p<0.05 vs Year 1

At year 1, 8 children were positive for GADA. At year 2, 4 of them did not attend the pre-IDDM Clinic, 4 were followed-up and confirmed GADA positivity, 3 showed ICA and 2 had also IA-2icA. To date 6 children (M:F=1) developed IDDM. They were between 11 and 29 months old and all but one were ICA negative at time of birth. At year 1, 2 out of 5 attended the pre-IDDM Clinic, and both were ICA positive, together with GADA or IA-2icA. At the time of onset, sera from 3 newly IDDM out of the 6 were available and all were positive for ICA and GADA, 1 was also positive for IA-2icA. In summary: 1) GADA developed before ICA and IA-2icA at year 1; 2) there is a significant increase in the appearance of islet-related autoantibodies at year 2, when compared with year 1; 3) children developing IDDM before the age of 3 years appeared to be ICA negative at birth, but islet-related autoantibody positive at the onset of the disease. In conclusion, a possible increase of incidence of IDDM under the age of 3 is presently monitored in Sardinia.

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CO-APPEARANCE OF ANTINUCLEAR, MITOCHONDRIAL AND ISLET CELL ANTIBODIES IN HUMAN PANCREAS SECTIONS

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In order to evaluate possible relationships between islet cell antibody (ICA) and antinuclear antibody (ANA) we screened totaly 1359 sera using I.F. technique in human pancreas sections. Sera were belonged to 378 non-diabetic (non-DM) first degree relatives of Type 1 DM cases and 491 Type 1 DM patients at different stages (177 early-clinical; < 3 months, 255 clinical; 3-12 months, and 59 late clinical period; ≥ 5 years). Specifically stained small clusters of islet cells (Askanazy cells; mitochondrial antibody, "MITO") were also investigated in the same sections. Results were compared with data of 305 non-DM first degree relatives of Type 2 DM cases, 146 Type 2 DM patients, and 38 healthy control subjects who have no diabetic relative. Antibody frequencies were shown as follows:

Type 1 DM	ICA≥10 JDF	ICA≥20 JDF	ANA	MITO
1°st non-DM relatives	15.1	4.0	2.6	0.003
Early clinical period	60.5	15.9	1.7	0.8
Clinical period	34.5	13.7	1.6	0.4
Late clinical period	20.3	1.7	-	-
Type 2 diabetes	6.8	3.4	-	-
1°st non-DM relatives	7.5	5.9	1.3	-
Control group	7.9	2.6	-	-

Both low and high titre ICA were most frequent in early period Type 1 DM patients, the frequency have shown a continuously decreased trend as expected from early to clinical, and to late periods ($p<0.005$ and $p<0.001$). ICA positivity among 1°st relatives of Type 1 DM were more prevalent than 1°st relatives of Type 2 DM, Type 2 DM patients and control subjects. Frequency of ANA was high both in 1°st relatives of and in early and clinical stages of Type 1 DM cases as compared to late period Type 1 DM, 1°st relatives, and clinical Type 2 DM and control subjects. MITO showed a similar trend to ANA. This antibody pattern might have suggest activaton of autoimmunity along with high titre ICA.

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THE ULM SCHOOLCHILDREN STUDY: RISK ASSESSMENT FOR IDDM BY COMBINED ANALYSIS OF GAD65 AND IA2 ANTIBODIES.

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Risk assessment and prediction of insulin-dependent diabetes mellitus (IDDM) in the Ulm schoolchildren study of 1988 was based on islet cell autoantibodies (ICA) detected by the classical immunofluorescence test on human pancreatic tissue. We here developed a new strategy to replace the laborious and difficult to standardize histochemical ICA test in large scale screening programs for risk assessment of IDDM in the general population. We established radioimmunoassays for separate detection of autoantibodies to GAD65, the cytoplasmic part of IA2 ($IA2_c$) (one parameter tests) and for combined detection of GAD65-A and $IA2_c$-A in a single-step assay (two parameter test). Sera from 93 patients newly diagnosed with IDDM and 695 healthy controls were used to optimize the tests and to determine the optimal decision threshold levels for each assay by receiver operating characteristics (ROC) plot analysis. The cut-off value of the combined assay was selected in a way that no sera positive in the one parameter tests escaped detection in the two parameter test. This allowed to discriminate antibody-positive sera from antibody negative sera in one step. Only positive sera had then to be re-analysed in the GAD65- and $IA2_c$-A to determine their exact antibody specificity. By retrospective analysis, 4021 sera from the Ulm schoolchildren study sampled in 1988 were evaluated by our new tests. 31 sera (0.77%) were positive for GAD65-A and 7 sera (0.17%) were positive for IA2-A compared to 44 of 4287 sera (1,05%), which had been found ICA-positive. 5 sera were positive for ICA, GAD65-A and $IA2_c$-A; 4 sera showed 2 antibody specificities. 5 individuals developed IDDM during the follow up according to a questionaire sent out to all participants of the 1988 study. The data demonstrate that our strategy based on quantitative, reproducible and combined detection of GAD65-A and $IA2_c$-A allows cost-saving and most effective large scale screening of sera for prediciton of IDDM.

Supported by grants of Deutsche Forschungsgemeinschaft Ri 707/1-2 to W.R. and Bo 849/6-2 to B.O.B and the Deutsche Diabetes Gesellschaft to W.R..

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AUTOANTIBODIES TO THYROID PEROXIDASE AND THYROGLOBULIN MEASURED BY HIGHLY SENSITIVE RIA IN PATIENTS WITH JAPANESE DIABETES MELLITUS.

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We studied frequency of autoantibodies to thyroid peroxidase (TPO-Ab) and thyroglobulin (Tg-Ab), and examined the relationship between these antibodies and antibodies to glutamic acid decarboxylase (GAD-Ab) in japanese patients with type 1 and type 2 diabetes mellitus. TPO-Ab and Tg-Ab were measured by highly sensitive radioimmunoassey (RIA), and GAD-Ab by Anti GAD RIA Kit of Hoechst Japan. The subjects of our study was 70 patients of type 1 diadetes and 138 patients of type 2 diabetes.

The frequency of GAD-Ab was observed in type 1 diabetes (47%) and in type 2 diabetes (11%). The frequency of TPO-Ab and Tg-Ab was observed in type 1 diabetes (48%, 24%) and in type 2 diabetes (19%, 23%). The frequency of GAD-Ab was observed in type 1 diabetes with Tg-Ab (negative 54%, positive 50%) and with TPO-Ab (negative 47%, positive 56%), but the frequency of GAD-Ab was observed in type 2 diabetes with Tg-Ab (negative 9%, positive 21%) and with TPO-Ab (negative 9%, positive 37%). We concluded that 48% of type 1 japanese diabetes has relationship with thyroid autoimmunity and, 20-30% of type 2 diabetes with GAD-Ab positive has relationship with thyroid autoimmunity.

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Combined analysis of GADAs, IAAs, IA-2As and ICAs in a general population of schoolchildren

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Identification of subjects at risk for developing IDDM is based on detection of AAbs to ß-cell antigens. In order to identify high risk individuals for developing IDDM sera from 8,592 schoolchildren (6-17 years) were screened for GADA, IAA and IA-2A using 125I-labelled antigens and ICA measured by indirect immunofluorescence. 5% (433/8,592) exceeded the cut-offs of 99, 99 and 98 percentiles for GADA, IA-2A and IAA, respectively and 5 JDFU for ICA. Up to now 351 children initial positive for at least one AAb were re-examined after 8-12 weeks. For 69% (242/351) the AAb positivity was confirmed. We found 78% (189/242) to be positive for only 1 AAb, 13.2% (32/242) for only 2 AAbs, 6.1% (15/242) for 3 AAbs and 2.5% (6/242) positive for all 4 AAbs as follows:

1 AAb:	*prevalence*	*2 AAbs:*	*prevalence*	*3 AAbs:*	*prevalence*
GADA:	46.7%	GADA+IA-2A:	2.06%	GADA+IAA+IA-2A:	0%
IA-2A:	1.2%	GADA+IAA:	1.65%	GADA+IA-2A+ICA:	4.1%
IAA:	11.5%	GADA+ICA:	4.95%	GADA+IAA+ICA:	1.6%
ICA:	18.5%	IA-2A +ICA:	2.00%	IAA+ICA+IA-2A:	0.4%
		IA-2A+IAA:	0%		
		IAA+ICA:	2.47%		

64.8% (157/242) of the re-examined children were positive for GADA and/or IA-2A and only 27.2% (66/242) for IAA and/or IA-2A. 19.4% (47/242) were positive for at least 2 AAbs. Among these children the most frequent combination of AAbs measured by 125I-antigen binding assays was the combination GADA+IA-2A (8.7%) followed by GADA+IAA (5.7%) and IAA+IA-2A (2.9%). 45/242 were found to be of high risk for developing IDDM (defined by 1. positive for ≥3 AAbs; 2. ICA≥20 JDFU and GADA or IAA positive, or IA-2A and 1 further AAb is positive; 3. ICA≥40 JDFU, or GADA≥29xcut-off, or IA-2A≥2xcut-off). 76% (34/45) of them were positive for at least 2 AAbs, 53% (18/34) for GADA+IA-2A, 26% (9/34) for GADA+IAA and 20% (7/34) for IAA+IA-2A. From our preliminary results we conclude, for initial screening of large populations for risk for developing IDDM the easily measured AAbs GADA and IA-2A should be used, followed by the measurement of IAA and ICA in subjects positive for one or both of these.

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LONGITUDINAL STUDY OF ANTICARDIOLIPIN AUTOANTIBODIES IN CHILDREN WITH INSULIN DEPENDENT DIABETES MELLITUS

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Insulin dependent diabetes mellitus (IDDM), like other endocrinopathies, is characterized by an increased prevalence of organ and non-organ-specific autoantibodies. Recently, high levels of anticardiolipin antibodies (aCL) have been found in IDDM, but there are no reports on the follow-up of autoantibodies in these patients. We evaluated aCL-IgG and aCL-IgM (by ELISA assay) in 77 children and adolescents with IDDM (31 m, 46 f; age 9.6±4.6 yrs; duration of disease from onset up to 12 yrs; HbA1c 8.2±1.5%). In 51/77 diabetic patients aCL were periodically checked (every 3-4 months) for a period of 2.8±1.7 years. 102 age and sex-matched healthy subjects served as control group. ACL-IgG >20GPL and aCL-IgM >10MPL values were considered positive. ACL IgG or IgM were positive (at least once) in 28 patients (36%) ($p<0.0001$ vs control group), while aCL-IgG and IgM were positive respectively in 23 (30%) and 11 (14%) patients ($p<0.0001$ and $p<0.005$ vs control group). Only in 6 out of 77 (8%) IDDM patients aCL IgG and IgM were both positive. During the follow-up aCL-IgG were confirmed positive in 9/51 (18%) while aCL-IgM were positive in 4/51 (8%). The frequencies of aCL (IgG and IgM), in diabetic patients during the follow-up and in controls are reported in the table:

	n. 77	**n. 51** (follow-up 2.8±1.7 yrs)			*n.102*
aCL	once pos.	twice pos.	3 times pos.	>3 times pos.	*Controls*
IgG(>20GPL)	23 (30%)	4 (8%)	3 (6%)	2 (4%)	*8 (8%)*
IgM(>10MPL)	11 (14%)	2 (4%)	2 (4%)	0	*2 (2%)*

ACL (IgG and IgM), when positive, were always at lower levels and no correlation was found between aCL and sex, chronological age, metabolic control, and duration of the disease. Most of the patients had occasionally positive aCL (IgG or IgM), and in some of them they were fluctuating in time. Only 2 patients (males) had positive aCL-IgG during the whole follow-up tending to increase, but not significantly, with the duration of IDDM. In conclusion, aCL are significantly more frequent in young diabetic population fluctuating with time without clinical evidence of any specific autoimmune disorder.

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SCREENING OF RISK FOR DEVELOPING IDDM IN SCHOOLCHILDREN BY AUTOANTIBODY ANALYSIS IN COMBINATION WITH GENOTYPING

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Islet cell autoantibodies precede the onset of IDDM. The aim of our study is, to assess as an alternative to ICA the predictive diagnostic power of risk for developing type I diabetes of AAbs against the three ß-cell antigens, namely glutamic acid decarboxylase (GADA), protein tyrosine phosphatase (IA-2A) and insulin (IAA) by screening of a general schoolchildren population of an age range from 6 to 17 years. Up to now, sera from 8,592 children were screened for GADA, IA-2A and IAA by the use of 125I-antigen binding assays. The cut-offs used were 99, 99 and 98 percentiles, respectively. ICA (cut-off = 5JDFU) were detected by immunofluorescence technique. In the last AAb proficiency programs the sensitivity/specificity of these assays were 100%/100% for GADA and IAA and 100%/87% for ICA. The AAb screening of GADA, IA-2A, IAA and ICA revealed 5% (433/8592) AAb positive children. 397 (4.6%) were positive for only one AAb, 25 (0.29%) for two, 8 (0.09%) for three and 3 (0.03%) for four AAbs. By re-examination of primary positive children the AAb positivity was confirmed by 69%. By AAb determination, 0.52% (45/8592) of schoolchildren studied were found to be of high risk for developing IDDM. Four out of them - all positive for GADA and ICA, 3 additionally positive for IA-2A and 1 for IAA - followed already to IDDM. 9 out of the 45 (20%) high risk children are only positive for ICA (40 - 160 JDFU). 25 of 26 (96%) high risk individuals and 13 of 14 (93%) IA-2 antibody positive children bear the HLA DRQB1 genetic IDDM markers 0201 and 0302, and but not any child was positive for the protective genetic marker HLA DRQB1 0602. From our preliminary results we conclude, that the combined AAb detection by 125I-antigen binding assays is useful for initial screening of large populations and offers an alternative to the ICA screening assay. However, ICA is further needed to recognize individuals of risk for developing IDDM with highest diagnostic sensitivity. The predictive diagnostic power increases with the number of AAb species occurring and with their titres. The risk for developing IDDM is additionally increased by the absence of protective genetic markers and by the occurrence of HLA DRQB1 genetic markers of type 1 diabetes.

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SELECTED MARKERS OF INFLAMMATORY RESPONSE IN NEWLY DIAGNOSED TYPE I DIABETES MELLITUS

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Evidence suggest that mediators of inflammatory response play an important role in pathology of diabetes and diabetic vascular complications. The aim of our study was to estimate some markers of the acute-phase reaction in newly diagnosed type I diabetes. The study was conducted in a group of 10 patients aged 31.3±8.5 years, with duration of diabetes 19.2±20.7 days. We estimated serum concentration of CRP, alpha-1 acid glycoprotein (AGP) and alpha-1 antychymotrypsin (ACT) and glycosylation profile of AGP and ACT (microheterogeneity). Microheterogeneity of AGP and ACT were studied by affinity electrophoresis with concanavalin A as the ligand. The results were expressed as reactivity coefficients (RC). CRP, AGP and ACT serum concentrations were similar to noticed in healthy subjects. We observed significantly lower values of AGP-RC and ACT-RC in patients with type 1 diabetes (1.08±0.10 and 2.99±0.71) in comparison with healthy individuals (1.27±0.08 and 4.12±0.89) ($p<0.05$) ($p<0.05$). In conclusion, the AGP and ACT glycosylation profile noticed in our study is characteristic for persistant inflammatory process.

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LESSONS FROM ISTANBUL FAMILY STUDY: ARE RELATIVES OF YOUNGER ONSET DIABETES MORE RISKY?

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In this study we aimed to determine the prevalence of islet cell antibody (ICA) in first degree relatives of Type 1 diabetes patients. The study included 382 first degree relatives from 209 families (parenths; 134 -mother/father 86/48, siblings; 192 -female/male 100/92, and offsprings; 55 -female/male 27/28). Positive ICA was defined as ≥10 JDF u. and overall prevalence was 14.4% (55 relatives); however, only 15 subjects (3.9%) had a high titre ICA (20 JDF u.). Based on age at onset in the index case, family members were divided into two groups. 322 relatives of patients diagnosed ≤20 years of age composed group I, and 60 relatives of patients diagnosed > 20 years composed group II. Comparison of the two groups have shown that high titre ICA in group I was more frequent than in the group II (4.3% vs. 1.7%, $p<0.005$). Low titre ICA was also frequent in this group, but did not reach to a significant level. Although no difference was observed for ICA among parenths and siblings between two groups, frequency of ICA was found to be higher in offsprings of group I than in the group II (22.2% vs. 8.7%, $p<0.05$). Moreover, ICA was more prevalent among mothers, male siblings and female offsprings of group I (ICA in the same order; 16.7% vs. 0.0%, $p<0.001$, 13.6% vs. 0.0%, $p<0.001$, and 20.0% vs. 4.5%, $p<0.005$). In contrast, only fathers of group II were noticed to be more frequently ICA positive (33.3% vs. 11.1%, $p<0.001$). Our results have indicated that compared to relatives of older onset patients, ICA is more prevalent among first degree relatives of younger onset Type 1 diabetes patients. Particularly, mothers, male siblings, and female offsprings are seems to be utmost risk.

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EVIDENCE FOR IMMUNOREGULATORY DEFECTS OF GLYCATED LYMPHOCYTES IN DIABETES

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Various *in vivo* and *in vitro* data indicate that, nonenzymatic glycosylation of membrane proteins may be implicated in the changes observed in intrinsic and exposed group properties in diabetic state. Similarly, glycosylation of lymphocyte membrane proteins may have far reaching immunological implications, considering the role of membrane receptors in immunological activity. To evaluate the role of glycation in the immunoregulatory defects of diabetic lymphocytes, we measured both humoral and cell-mediated immune responses in diabetic and nondiabetic subjects. For humoral response, Widal test was used to estimate somatic and flagellar antibody levels in 30 diabetic and 20 nondiabetic individuals. To evaluate cell-mediated immune response, tuberculin test was performed on 32 and 20 diabetic and nondiabetic subjects respectively. The role of glycosylation in the compromised immune response was ascertained by colorimetric assay of lymphocyte membrane protein glycosylation. The impaired humoral and cell-mediated immune responses with glycosylation was reflected in the significant ($P<0.01$) negative correlation coefficient ($r = -0.62$ and $r = -0.65$, respectively) but not in the control group. This glycosylation - induced immunoregulatory defects may bring to light reasons why lymphocytes of diabetic subjects were observed to exhibit decreased immunoregulatory factors like interleukin, HLA among others.

PS 4

Cellular Markers and Inflammatory Mediators in IDDM

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PROLIFERATIVE T CELL RESPONSES TO PEPTIDES OF IA-2 BY IDENTICAL TWIN PAIRS DISCORDANT FOR IDDM

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To define T lymphocyte reactivity to an islet antigen IA-2 associated with IDDM we tested identical twins discordant for IDDM. Ten peptides were selected from the IA-2 molecule due to their predicted ability to bind HLA-DR4. We demonstrate that 18 IDDM twins, their non-diabetic twins and 15 control subjects can respond to IA-2 peptides. Two peptides were of interest: peptide 11 and peptide 16. The majority (72%) of 51 subjects (both with and without IDDM) responded to peptide 11, unrelated to disease duration in IDDM twins. Responses to peptide 16 were detected in 81% of twins, both diabetic and non-diabetic, within 6 years of the onset in the index twin but after 12 or more years in only 15% of twins, both diabetic and non-diabetic ($p<0.001$). Magnitude of T cell responses correlated with disease duration in the index twin in both IDDM twins ($r^2= 0.34$; $p < 0.01$) and their non-diabetic twins ($r^2= 0.35$; $p< 0.05$) but not with age, HLA status or IA-2 antibodies. T cell reactivity to peptides 11 or 16 did not correlate between identical twins consistent with the changes being non-genetically determined. Thus, we demonstrate in IDDM patients an epitope-specific down-regulation of T cell responses to an autoantigen that is disease-related but not disease-dependent and associated in non-diabetic twins with low-disease risk. Identification for the first time that an altered T cell response to an epitope of a disease-related antigen, peptide 16 of IA-2, is associated with protection from developing IDDM raises the possibility of designing therapeutic agents to prevent IDDM.

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BETA-CELL DIFFERENTIATION IS ASSOCIATED WITH AN INCREASED SENSITIVITY TO STREPTOZOTOCIN AND INTERLEUKIN 1-BETA

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Interleukin 1-beta (IL-1β) and streptozotocin (STZ) are toxic to the beta- cells, partly through the induction of the inducible nitric oxide synthase (iNOS). Increased sensitivity to STZ is caused by release of NO after uptake by GLUT-2. **AIM:** To determine the effect of STZ and IL-1β on the cytotoxicity and NO production in a pluripotent MSL-tumour cell-line, which express the GLUT-2, and a low GLUT-2 expressing MSL-AN cell-line transfected with Insulin Promotor Factor 1 (IPF-1). **METHODS:** Specific cytotoxicity was measured by the release of LDH (% of controls) and NO as accumulated nitrite (μM). The MSL-cell line obtain a pre-beta and a beta-cell phenotype dependent upon culture conditions. The glucagon producing MSL-AN cell-line was transfected with IPF-1, inducing insulin-secretion. **RESULTS:** Table 1: Dose dependent cytotoxicity to STZ in the beta-cells, and no effect on the pre-beta cells, despite PCR analysis shows GLUT-2 expression in both phenotypes. (N=3, in triplicates).

Table 1	1,0 mM STZ	2,5 mM STZ	5,0 mM STZ	7,5 mM STZ
MSL beta	16,98+/-6,03	20,56+/-8,74	35,86+/-4,63	57,14+/-11,73
MSL pre-beta	0,44+/-0,76	0,00+/-0,00	1,00+/-1,74	1,83+/-1,72
	p<0,02	p<0,03	p<0,01	p<0,01

In contrast after exposure of MSL-AN cells +/- IPF-1 expression to STZ, no significant differences were found. Table 2 shows increased cytotoxicity in the IPF-1+ cells, despite equal amounts of induced NO. (N=6, in triplicates).

Table 2	150 pg/ml IL-1	1500 pg/ml IL-1	3000 pg/ml IL-1
MSL-AN + IPF-1	5,06+/-1,23	37,38+/-1,19	39,24+/-5,04
MSL-AN - IPF-1	0,71+/-0,00	12,43+/-9,83	15,90+/-9,03
	p<0,02	p<0,02	p<0,01

CONCLUSION: Maturation from a pre-beta to a beta-cell phenotype is associated with acquired sensitivity to STZ, dependent upon the number of GLUT-2 receptors. The IPF-1 induced change from an alpha- to a beta-cell-like phenotype results in increased sensitivity to the toxic effect of IL-1.

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RELATIONSHIP BETWEEN HUMORAL AND CELLULAR IMMUNITY TO IA-2 IN INSULIN DEPENDENT DIABETES

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Autoantibodies to the neuroendocrine molecule IA-2, a 105kD transmembrane protein of the tyrosine phosphatase family, have been observed in individuals with IDD. We determined the levels of humoral and cellular immune reactivity to this autoantigen in order to further define its role in the pathogenesis of IDD. Peripheral blood mononuclear cells (PBMC) from individuals with newly-diagnosed IDD and healthy controls were stimulated in vitro with recombinant IA-2, a series of control antigens (glutathionine-S-transferase, tetanus toxoid, *Candida albicans*, mumps, bovine serum albumin) and a mitogen (phytohemagglutinin). Whereas proliferation of PBMC to IA-2 was significantly higher (mean stimulation index; frequency of response) in newly-diagnosed IDD subjects (3.5 + 3.3 @ 10μg/ml ; 11 of 26 (42%)) compared to controls (1.5 + 1.1, $P = 0.02$; 1 of 12 (8%), $P = 0.03$), reactivities to all other antigens were remarkably similar (all P= not significant). Autoantibody frequencies in newly-diagnosed subjects (when sera were available) were 11 of 19 (58%) for IA-2, ICA 65% (13/20), IAA 20% (3/15), and GADA 50% (10/20); frequencies similar to the reported literature: Despite anecdotal cases suggesting an inverse association between humoral and cellular immune reactivities against IA-2, no such overall relationship was observed (r_s = 0.18, P = 0.39). These studies support the autoantigenic nature of IA-2 in IDD and suggest the inclusion of cellular immune responses as a marker for IDD.

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More pronounced activation of mitogen activated protein kinases (MAPK) by IL-1 in β- than in pre-β-cell lines.

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IL-1 causes destruction of rat β-cells partly by inducing expression of inducible nitric oxide (NO) synthase and NO synthesis in β-cells. Continous cytokine mediated activation of the JNK and p38 subgroups of MAPKs signals programmed cell-death in other cells. In rat β-cells IL-1 induces activation of the MAPKs. A subclone of the MSL islet stem-cell clone, AN #697C1, has two derived cell lines #2.9-AN (pre-β-cell phenotype) and after transfection with the transcription factor (TF) STF-1, #1.10AN/IN (β-cell phenotype). These cell lines produce similar amounts of NO in response to IL-1, but only 1.10 is susceptible to IL-1 toxicity. The aim of this study was to investigate if the two cell lines exhibited differential IL-1 induced MAPK activation with the hypothesis that MAPK activation is a necessary second signal for IL-1 induced cell-death in the β-cell phenotype. 150.000 cells of either cell line were incubated with 0-3500 pg/ml of recombinant human IL-1 for 20 minutes after which the cells were lysed. The lysates were investigated for the phosphorylation of the substrates Elk-1, ATF-2 and the heat shock protein HSP-25 by [^{32}P] incorporation in the substrate proteins. The TF Elk-1 is phosphorylated by the MAP Kinases ERK 1/2 and JNK, the TF ATF-2 by ERK 1/2, JNK 1 and p38 whereas HSP-25 is phosphorylated only by p38 via a down-stream kinase MAPKAP-2 The proteins were separated by SDS-PAGE and visualized by autoradiography. IL-1 caused a marked dose dependent biphasic phosphorylation of ATF-2 and HSP-25 in 1.10 cells starting at 50-150 pg/ml and peaking at 2500-3000pg/ml of IL-1 with a 12-14 fold increase. In contrast, IL-1 (150-3000pg/ml) only caused a 2-3 fold increase in ATF-2 and HSP-25 phosphorylation in 2.9 cells. These data suggest that IL-1 signalling in islet cells depends upon the differentiation stage and that JNK/p38 activation may provide a necessary second signal which together with NO causes IL-1 mediated β-cell apoptosis.

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TYROSINE PHOSPHATASE IA-2 SPECIFIC CYTOTOXIC T CELLS FROM HIGH RISK IDDM RELATIVES

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Cytotoxic T lymphocytes (CTL) are proposed to play a key role in beta-cell destruction in insulin-dependent diabetes mellitus (IDDM). Beta-cell antigen-specific CTL activity could therefore reflect destructive islet autoimmunity and predict the development of IDDM. To facilitate the generation of human antigen-specific CTL, we derived dendritic cells (DC) from peripheral blood adherent cells from HLA-A2 positive high-risk IDDM relatives, in the presense of 600 U/ml GM-CSF and 400 U/ml IL-4. The DC were pulsed with a HLA-A2-restricted tyrosine phosphatase IA-2-peptide (MVWESGCTV) and cultured with autologous CD8 cells for 14 days. CTL specific for this peptide were generated in two out of six HLA-A2 positive at-risk IDDM first-degree relatives (Table, Subject 1: female, 11 y.o., HLA A2,2; DR4,4; DQ8,8. Subject2: female, 16 y.o., HLA A1,2; DR3,4; DQ2,8). In conclusion, this strategy may be a useful tool for investigating the diagnostic and predictive value of CTL in IDDM.

Table. Cytotoxic T lymphocyte activity against IA-2 peptide

	IAA	anti-GAD	anti-IA-2	% Specific killing		
	(-17- 35	(0-3	(0-5	Effector and target ratio		
	nU/ml)	units)	units)	50:1	10:1	2:1
Subject1	82	75	15	45±3.5*	29±4.2*	26±5.4*
Control 1	n.d	n.d.	n.d.	8.7±1.7	8.2±1.8	2.3±1.2
Subject 2	400	29	20	41±3.0*	31±5.9*	12±7.3
Control 2	n.d	0	-1.7	20±1.9	9.1±3.1	1.6±0.7

* p<0.02 vs HLA-, sex- and age- matched controls
n.d.: not done

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INHIBITION OF NITRIC OXIDE PRODUCTION AFTER INDUCTION OF HEAT SHOCK PROTEINS PREVENTS DNA STRAND BREAKS

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Nitric oxide (NO) is suggested to be a presumed mediator of interleukin 1ß (IL1)-induced ß-cell damage. Therefore it was the aim of this study (1) to prove whether the interruption of intracellular NO generation may prevent IL1-induced DNA damage, and (2) to elucidate the potential role of heat shock proteins (HSP) in protecting ß-cells from IL1-related injury. Study (1): RINm5F cells were treated for 18h either with human IL1 (25 U/ml) or N-nitro-L-arginine methylester (NAME, 1 mmol/l), a competitive inhibitor of NO synthase, alone or with a combination of both. Control cells remained untreated for the same period of time. Study (2): to attain maximal induction of HSP, RINm5F cells were maintained at 44°C for 30 min and transferred to 37°C for the following 5h. HSP expression was confirmed by means of autoradiography of SDS-PAGE separated [^{35}S]-labelled RINm5F proteins and Western blotting. After HSP induction, the cells were either incubated for 18h with IL1 (HS-IL1), or they were cultured without any treatment (HS-Controls). Results: IL1 exposure of RINm5F cells resulted in a more than 20-fold stimulation of NO generation measured by nitrite release into the culture medium (0.263±SEM0.016 vs. 0.012±0.002 μmol/10^5 cells in the controls, p<0.001). NO production was not altered by the addition of NAME or by the heat shock (HS) procedure alone. Both measures, however, totally prevented the IL1-stimulated NO generation. The abundance of cytoplasmic histone-associated DNA fragments (HADF) which were assessed by means of a sandwich ELISA was 2.5-fold increased in IL1-exposed cells as compared to controls (p<0.01). HADF were not influenced, neither by NAME alone nor by the HS procedure as such. Also, after these two treatments, no alteration of HADF was elicited by IL1. In conclusion, both the inhibition of IL1-stimulated NO production and the induction of HSP are effective in preventing IL1-induced DNA strand breaks. The mechanism underlying the protective effect of HSP seems to involve inhibition of intracellular NO generation.

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T CELL RESPONSES TO THE NOVEL AUTOANTIGEN IA2 IN IDDM PATIENTS AND HEALTHY CONTROLS.

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Insulin-dependent diabetes mellitus (IDDM) is probably mediated by T-lymphocytes recognizing critical β-cell autoantigens. Recently, a novel autoantigen in IDDM was described as the tyrosine phosphatase IA2. Antibodies to IA2 are detected at the peripheral blood of IDDM patients and prediabetic subjects. Nothing is known so far about epitope specific T cell reactivity to IA2. We studied T cell responses to the intracytoplasmatic domain of IA2 protein ($IA2_{ic}$) and predicted DR4- and DQ8-binding motifs (13mers) of the whole IA2-molecule in 12 IDDM patients less than 4 years from diagnosis and 5 HLA matched (DR4/ DQ8) healthy controls. Peripheral blood mononuclear cells of the probands were stimulated for 4 days by peptides and for 7 days by protein before measuring ^{3}H-Thymidine incorporation. Antibodies to $IA2_{ic}$ were measured by an immunoprecitation assay (sensitivity 72%). 10 out of 12 IDDM patients and 2/5 controls responded to $IA2_{ic}$ protein with stimulation indices (SI) >3. No correlation of T cell and antibody responses to $IA2_{ic}$ was seen. The HLA restriction (DR or DQ) of the T cell responses to $IA2_{ic}$ was investigated by antibody blocking experiments. 11 out of 12 IDDM patients and 3/5 controls responded to the selected IA2 peptides with SI>2. There were dominant responses to 2 DR4-motif and 1 DQ8-motif IA2 peptides. The DQ8-motiv peptide was recognised by 9/12 IDDM patients and 2/5 controls, one DR4-motiv peptide by 5/12 patients and 1/5 controls and the other DR4-motiv peptide by 2/12 patients and 0/5 controls. The binding of the peptides to either DR4 or DQ8 was confirmed by binding competition assays. Remarkable sequence homologies of all three IA2 peptides to a number of virus protein sequences were observed. The latter demonstration may be important for a molecular mimicry priming the immune response to IA2 following certain virus infections.

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INHIBITION OF NITRIC OXIDE SYNTHASE, GUANYLYL CYCLASE OR PROTEIN KINASE G ATTENUATES INTERLEUKIN-1β-INDUCED APOPTOSIS IN HIT-T15 CELLS.

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We have previously shown that the nitric oxide donor, GSNO, induces apoptosis in clonal pancreatic B-cells in a cGMP- and protein kinase G (PKG)-dependent manner. Since interleukin 1-β (IL-1β) is known to induce nitric oxide synthase in pancreatic B-cells we have now examined the possible dependence of interleukin-induced cytotoxicity on cGMP-signalling mechanisms. HIT-T15 cells were incubated for 24 hours in the absence or presence of 2pg/μl of human recombinant IL-1β. Attached cells were harvested, stained with acridine orange and scored as apoptotic or non-apoptotic, as judged by nuclear morphology, using fluorescence microscopy. IL-1β treatment resulted in a consistent increase in apoptosis in HIT-T15 cells and this effect was reversed by pre-incubation with the nitric oxide synthase inhibitor, L-NMMA (Number of apoptotic cells/ml: Control: 3032 ± 427; + 100μM L-NMMA: 1702 ± 146; + 2pg/μl IL-1β: 14245 ± 1661*; + IL-1β + L-NMMA: 4329 ± 780** (n=6).*=p<0.001, compared to controls,**=p<0.001, compared to IL-1β alone). Pre-treatment of HIT-T15 cells with the guanylyl cyclase inhibitor, ODQ, was also able to reverse IL-1β-induced apoptosis (Control: 2552 ± 245; + 10μM ODQ: 1237 ± 165; + 2pg/μl IL-1β: 10662 ± 1250*; + IL-1β + ODQ: 2046 ± 206** (n=6).*= p<0.001, compared to controls,**=p<0.001, compared to IL-1β alone). Furthermore, the specific PKG inhibitor, KT5823, significantly inhibited IL-1β-induced apoptosis (Control: 891 ± 116; + 0.5μM KT5823: 506 ± 64; + 2pg/μl IL-1β: 4949 ± 231*; + IL-1β + KT5823: 2225 ± 335** (n=6).*=p<0.001, compared to controls, **=p<0.001, compared to IL-1β alone). By contrast, the use of a protein kinase A-specific inhibitor, KT5720, had no effect on apoptosis (Control: 2593 ± 176; + 2μM KT5720: 2669 ± 218; + 2pg/μl IL-1β: 9696 ± 519*; + IL-1β + KT5720: 8295 ± 564** (n=6). *= p<0.001, compared to controls, **=p<0.001, compared to IL-1β alone). We conclude that interleukin-1β is able to induce apoptosis in HIT-T15 cells and that activation of the apoptotic mechanism is dependent on signalling pathways involving nitric oxide and cGMP generation and protein kinase G activation.

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T-CELL REACTIVITY AGAINST IA-2 PROTEIN AT DIAGNOSIS OF TYPE 1 DIABETES

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Several studies performed in animal models of autoimmune diabetes have indicated that the setective islet ß cell destruction is a T-lymphocyte mediated event. However, despite the importance of the T-cell response in the pathogenesis of type I diabetes, the target antigens of such a response remain largely uncharacterized. Among the diabetes-associated autoantigens identified so far starting from circulating autoantibodies, a specific T-cell reactivity has been reported against GAD65 and insulin, both in man and in the NOD mouse. In the present study we aimed to study the T cell response to the IA-2 islet tyrosine phosphatase autoantigen at the onset of type I diabetes. To this end, by participating to a T-cell workshop, we tested in blind a series of antigens (insulin, GAD65 and IA-2) for their reactivity with peripheral blood lymphocytes obtained from 8 newly diagnosed type I diabetic patients and from 9 age and sex-matched normal control individuals. A T-cell proliferation assay was performed in triplicate employing freshly isolated cells at a concentration of 150,000 cells/well, using RPMI containing 10% human autologous serum; after incubation for 72 h in presence or absence of the antigen to be tested, 3H-thymidine was added, left for 16h and plates were counted in a ß-counter. Among the antigens tested, a specific T-cell proliferation (defined as a Stimulation Index >3) was observed against IA-2 in 4/8 type I diabetic patients and in 0/9 control subjects ($p<0.01$ by Fisher extact test). A statistically significant difference ($p<0.01$ by Wileoxon test) was also found between the mean S.I. with IA-2 in patients (3.0±1.5) and that in controls (1.1±0.3). No difference was found between patients and controls in the T-cell proliferative response against insulin or GAD65. In conclusion these data show that a T-cell response against the IA-2 protein can be observed at type I diabetes diagnosis, suggesting that this islet molecule represents a target antigen not only at B-lymphocyte but also at T lymphocyte level.

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IS PGE2 RELATED TO THE PANCREATIC β CELL DAMAGE?: THE POSSIBLE INTERRELATIONSHIP BETWEEN IL-1-β, PGE2 AND NO.

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The aim of this study was to evaluate the possible interrelationship of PGE2 (prostaglandin E2) and NO (nitric oxide) on the isolated neonatal rat islets incubated with IL-1-β (Interleukin-1-β). Neonatal rat islets were isolated by collagenase digestion and cultured for six days in RPMI 1640 with 10 % of FCS. Islets were incubated with IL-1-β at 20, 60 and 200 U/mL concentration for 24 h and the isletys incubated with 60 U/mL were also incubated with NAME 100 μM for 18 h.. β-cell damage was evaluated by measuring insulin secretion rate after incubation in KRB with 16,7 mM glucose. Insulin (pmol/mL), NO (pmol/islet) and PGE2 ($pg/mL(isletxh)^{-1}$) were determined by RIA, Greiss reaction and ELISA respectively. Insulin secretion rate (S.R.) was calculated as the percentage of secreted insulin versus total insulin .IL-1-β promoted a significant and dose dependent decrease in the insulin S.R. (C: 4.84 ± 0.31, 20 U/mL: 1.51 ± 0.15*, 60 U/mL: 1.17 ± 0.11* and 200 U/mL: 0.98 ± 0.07* * = p< 0.001). NO levels rose in incubated islets at 60 and 200 U/mL of IL-1-β (C: 4.51 ± 1.21, 60 U/mL: 9.13 ± 2.73* and 200 U/mL: 11.23 ± 2.23* * = p<0.01). The PGE2 production was also increased after IL-1-β incubation at 60 U/mL (c: 10.62 ± 0.83 and 60 U/mL: 44.40 ± 4.61. NAME partially normalized insulin S.R., PGE2 (12.93 ± 1.39) and NO. PGE2 and NO presented a good correlation with insulin S.R. (PGE2: r = -0.654, p < 0.001 and NO: r = -0.478 ± 0.01. In conclusion , the IL-1-β induced inhibition of the insulin secretion rate could be related not only to the increase of NO but also to the PGE2 increase.

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ANALYSIS OF PEPTIDES ELUTED FROM IDDM-SUSCEPTIBILITY CLASS II MHC MOLECULES OF ANTIGEN PRESENTING CELLS PULSED WITH IA-2

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The nature of the mechanism by which class II MHC molecules such as DR4 and DQ8 confer susceptibility to IDDM is not known, but probably relates to presentation of key epitopes of autoantigens and/or microbes to T cells. In order to examine these events in detail, we have developed a system for the analysis of peptides eluted from antigen presenting cells (EBV-transformed B cells) pulsed with native autoantigen. EBV cells homozygous for DR4 and DQ8 grown *in vitro* were washed, resuspended in balanced salts and pulsed at 4^0C sequentially with lectin/avidin followed by recombinant biotinylated IA-2ic (intracellular domain). The lectin binds preferentially to disulphide-linked surface proteins with immunoglobulin-like domains (eg sIg, CR2). Cells were then incubated for 6 hours at 37^0C, by which time the complex was almost completely internalised, and harvested. HLA-DR4 was affinity purified from detergent extracts from approximately $2x10^{10}$ cells and α and β chains acid-dissociated. Peptides occupying the binding groove were separated from intact DR4 α and β chains by selective ultrafiltration and analysed by reverse-phase HPLC. IA-2 derived peptides were identified by comparing HPLC profiles of cells pulsed with lectin/avidin/IA-2 and lectin/avidin alone (control elution). In the control elution, six major discrete peptide peaks and numerous minor peaks were identified, spanning different degrees of peptide hydrophilicity and hydrophobicity. HPLC fractions were analysed by mass spectroscopy, and 85 novel peptide masses identified to high mass accuracy (0.01%) in the pulsed but not the control fractions. The 85 masses matched sequences in IA-2, often as "nested sets" of peptides around a core motif which was DR4 compatible. Our results suggest that it is possible to purify peptides naturally processed from IDDM-related autoantigens. The sequence analysis of these presented peptides will provide an insight into the role of HLA-DR and -DQ molecules in determining diabetes susceptibility and protection.

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The effects of TNFα and IFNγ on activation of the mitogen activated protein kinases (MAPK) in rat islets of Langerhans.

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IL-1 causes destruction of rat ß-cells partly by inducing expression of inducible nitric oxide (NO) synthase (iNOS) and thereby production of toxic NO radicals in the ß-cells. IL-1 activates the MAPKs JNK 1 and p38 through generation of ceramide. As for IL-1 TNF signalling leads to generation of ceramide, activation of $NF_{\kappa}B$ and MAPKs. IFN signalling is dependent upon activation of the JAKs and a downstream phosphorylation of the STAT proteins. The aim of this study was to investigate if TNFα and IFNγ activates MAPKs in rat islets of Langerhans with the hypothesis that stimulation with TNFα but not with IFNγ would lead to an activation of the MAPKs. Rat islets of Langerhans from 4-8 days old Wistar rats isolated by collagenase digestion and handpicking were precultured in RPMI 1640 with 10 % fetal calf serum for 4-7 days. 150 islets were wasched in RPMI 1640 with 0.5 % human serum and incubated with 0.5-1000 U/ml of human recombinant TNFα or rat recombinant IFNγ for 20 minutes after which the cells were lysed. The lysates were investigated for the phosphorylation of the substrates Elk-1, ATF-2 and HSP-25 by ^{32}P incorporation in the substrate proteins. The transcription factors Elk-1 and ATF-2 are phosphorylated respectively by the MAP kinases ERK and JNK 1, and by ERK, JNK 1 and p38 wheras HSP-25 is phosphorylated by p38 via a downstream kinase MAPKAP-2. The phosphorylated proteins were separated by SDS-PAGE and visualized by autoradiography. TNFα caused a marked dose dependent increase in the phosphorylation of both ATF-2 and HSP-25. IFN caused a significant dose dependent decrease in the phosphorylation of Elk-1 and ATF-2, but did not have any significant effect on the phosphorylation of HSP-25. These data suggest that TNFα signalling in islets of Langerhans is dependent on activation of JNK/p38 MAPKs but not on activation of ERK and that IFNγ inhibits JNK and ERK but not p38 MAPKs via unknown mechanisms.

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ENHANCED DETECTION OF T CELL PROLIFERATION TO IA-2 BY TARGETING ANTIGEN PRESENTING CELLS

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The pivotal role of the T cell in the pathogenesis of IDDM has led to an increased interest in the detection of autoantigen-specific T cells. The First International Workshop on Autoreactive T Cells (Canberra, 1996) indicates that conventional proliferation assays remain hampered by poor sensitivity and specificity. Our aim was to examine whether targeting of test antigens directly onto the antigen presenting cell surface enhances the detection of T cell proliferation. We developed an antigen delivery system (ADS) comprised of biotinylated polyclonal goat anti-human IgG, streptavidin and biotinylated test antigen at a range of concentrations (recombinant human biotinylated IA-2ic at 0.4-200 µg/ml or biotinylated tetanus toxoid at 0.05-10 µg/ml). By FACS analysis, anti-IgG binds almost 100% of monocytes, presumably through occupied FcgammaR, and B cells via surface immunoglobulin. Freshly isolated PBMC were obtained from newly diagnosed IDDM patients and age- and sex-matched controls. Cells were pulsed on ice for 30 minutes sequentially with each ADS component prior to incubation at 37^0C, 5% CO_2 for 5 days. Assays with the ADS were performed in parallel with conventional T cell assays using antigen added directly to PBMC in culture medium. Peak proliferation to tetanus toxoid was at least doubled using the ADS (range of stimulation indices 5-56) compared with the conventional assay (15-235). In patients with IDDM (n=3 to date) peak proliferation responses to IA-2ic were increased 2.4 to 7 fold in the assays using the ADS (range of SIs 4-29) compared to conventional assays (1-10). In all subjects use of the ADS gave enhanced proliferation compared to the conventional assay. The increased responses via this system were specific as only minimal proliferation was detected with either the ADS alone or an irrelevant biotinylated antibody. These results suggest this novel ADS method offers a greatly enhanced sensitivity of detection of autoreactive T cell proliferation, which may be of value in the diagnostic use of these assays.

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ISLET CELL CYTOTOXICITY AND DISEASE PROGRESSION IN THE BB RAT

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β-cell response to autoimmune attack determines subsequent progression to diabetes in genetically susceptible individuals. The extent to which this response is genetically predetermined or directly modified by the disease process is not known. We have isolated neonatal islets from diabetes prone (DP- BB/OK/RT1u) and diabetes resistant (DR - Lewis/RT1a) rat strains and monitored islet function (nitrite production, insulin release, DNA synthesis) after 20 hr treatment with a combination of cytokines (IL-1β, TNF-α, IFN-γ). Control islets were cultured (20 hr) in the absence of cytokines. Media accumulation of nitrite, in the absence and presence of cytokines, was lower in DP vs DR islets (control, 2.5±0.61 vs. 3.9±0.09pmol/islet; treated, 7.5±0.9vs. 13.7±0.8pmol/islet: mean±SE) and islet DNA synthesis was similarly reduced in DP compared to DR rats (control 470.1±43.2 vs. 1460.3±194.9 DPM/µg DNA; treated, 374.2±50.0 vs. 997.5±157.4 DPM/µg DNA: mean±SE). Cytokine treatment stimulated nitrite production and inhibited insulin release and DNA synthesis to the same degree, relative to control values, in both DP and DR islets. Reg gene expression is upregulated during diabetogenesis in BB rats and NOD mice. We have determined Reg mRNA levels (RT-PCR) in untreated and cytokine-exposed neonatal DP and DR rat islets. No significant differences in Reg gene expression were observed between any of the DP or DR islet groups studied. Results suggest that islet functional state, response to cytotoxic attack and disease progression may depend both on genetic predisposition to IDDM and modification by an active insulitis process.

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T CELL RESPONSES TO IDDM AUTOANTIGEN PEPTIDES SHARING HOMOLOGOUS REGIONS.

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Glutamic acid decarboxylase (GAD) is a major autoantigen in IDDM. Regions of homology exist between GAD (residues 250-273) and the Coxackie P2-C protein (residues 28-50) and between GAD (residues 506-518) and proinsulin (residues 24-36). The demonstration of T cell responses against these regions have substantiated postulates of molecular mimicry acting in the autoimmunity associated with IDDM. The aim of this study was to determine whether these homologous regions are shared targets of T lymphocyte reactivity in individual IDDM patients. Proliferative responses against two sets of peptides (50 μg/ml): $GAD_{254-276}$ and Coxackie P2-C_{32-54}, and $GAD_{506-518}$ and $proinsulin_{24-36}$ were measured in peripheral blood mononuclear cells from 17 newly diagnosed IDDM patients and 11 control subjects. Responses (SI>3) were found against each of the peptides in both patients and control subjects, and no differences were observed between groups. A strong positive correlation was found between responses to $GAD_{254-276}$ and Coxackie P2-C_{32-54} ($r^2 = 0.85$, $p<0.0001$), and between responses to $GAD_{506-518}$ and $proinsulin_{24-36}$ ($r^2 = 0.87$, $p<0.0001$), suggesting shared reactivity to the homologous regions in individual subjects. However, a similar correlation was also observed between responses to Coxackie P2-C_{32-54} and $proinsulin_{24-36}$, ($r^2 = 0.97$, $p<0.0001$) which do not share homology, suggesting that reactivity may not be due to mimicry. Examination of HLA DR and GAD antibody status in subjects showed responses in those with either DR3 or DR4, and in patients with and without GAD antibodies. These data suggest that 1. T cell responses to peptides containing putative autoreactive epitopes of GAD and proinsulin are not specific for IDDM, 2. correlation between T cell reactivity to peptides is not restricted to those containing homologous regions, and 3. non-antigen specific factors are important determinants of in vitro measurements of T cell reactivity.

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DIFFERENTIAL CYTOKINE EFFECTS IN INS-1 AND RIN1046-38 INSULINOMA CELLS:

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We are interested in engineering of cell lines that might serve as surrogates for islets in IDDM therapy. A possible problem for *in vivo* use is the well documented cytotoxic effect of cytokines such as IL-1b and γ-IFN on islet β-cells. We have therefore investigated the cytotoxic effects of these cytokines on two insulinoma cell lines, RIN1046-38 and INS-1. Whereas exposure of INS-1 cells to IL-1b for 48 h killed 50% of cells, no cytotoxic effect of this cytokine was observed on RIN1046-38 cells, even though both cell lines produced large quantities of nitrite (10 and 30 μM/mg protein/24 h, respectively). Northern blot analysis revealed that mRNA for inducible nitric oxide synthase (iNOS) was induced by IL-1b treatment in both lines within 4 h, but while expression faded quickly in RIN 1046-38 cells, it was sustained in INS-1 cells out to 18 h. Inhibition of iNOS by N-monomethyl-arginine (NMMA) blocked the cytotoxic effect of IL-1b on INS-1 cells. Opposite to its effects on iNOS expression, IL-1b treatment caused an induction of Mn superoxide dismutase (MnSOD) mRNA in RIN1046-38 cells that persisted for 18 h whereas in INS-1 cells MnSOD mRNA waned by 12 h. Rat γ-IFN had a cytotoxic effect on both insulinoma lines, but nitrite and iNOS mRNA could only be detected in the RIN1046-38 cells. NMMA did not block the cytotoxic effect of γ-IFN on the latter cell line. These data suggest that: 1) The higher MnSOD:iNOS ratio in RIN 1046-38 cells may explain their resistance to the cytotoxic effects of IL-1b; 2. The cytotoxic effect of γ-IFN is independent of iNOS induction and NO production in both cell lines.

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HIGH T-CELL RESPONSES TO THE GLUTAMIC ACID DECARBOXYLASE (GAD) ISOFORM 67 REFLECT A HYPER-IMMUNE STATE THAT PRECEDES THE ONSET OF IDDM

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Pancreatic β-cell destruction leading to insulin-dependent diabetes mellitus (IDDM) is an autoimmune T cell-mediated process. Peripheral blood T cells which proliferate to islet antigens such as glutamic acid decarboxylase (GAD), (pro)insulin or tyrosine phosphatase IA-2, can be detected in at-risk, first-degree relatives of people with IDDM. However, cross-sectional studies cannot define the relationship between T-cell responses and progression to IDDM. Longitudinal studies were therefore undertaken on 50 at-risk, first-degree relatives tested at least yearly for up to four years, during which time five developed IDDM. Peripheral blood T-cell responses to GAD67(aa 208-404)-glutathione-S-transferase (GST) fusion protein, GST, insulin and tetanus toxoid were measured, together with antibodies to islet cells, GAD, insulin and IA-2. High levels of antibodies to GAD or insulin were generally associated with low T-cell responses to these antigens. Relatives who developed IDDM were characterised by high levels of antibodies to insulin and/or islet cells, and high T-cell responses to GAD67-GST and tetanus, but not insulin, in the 24 months before clinical diagnosis. Cross-sectionally, T-cell responses to GAD67(aa208-404)-GST and to full-length GAD65-GST were highly correlated ($R=0.75$, $p<0.002$). In conclusion, increased cellular immunity to the mid-region of GAD67 was a marker of late pre-clinical IDDM but appears to reflect a more general, transient state of cellular immune hyper-responsiveness.

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INTERFERON-γ SIGNAL-TRANSDUCTION IN RINm5F CELLS AND HUMAN PANCREATIC ISLETS

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The radical nitric oxide (NO) may be a mediator of β-cell damage in IDDM. The cytokines IFN-γ and IL-1β are required for expression of the enzyme nitric oxide synthase (iNOS) and NO production by human pancreatic islets. Possible mechanisms by which IFN-γ potentiates IL-1β-induced iNOS mRNA expression are: a. stabilization of iNOS mRNA; b. activation of the nuclear factor-κB (NF-κB); c. increased expression of interferon regulatory factor-1 (IRF-1). Addition of IFN-g (1000 U/ml), before or after arrest of IL-1β-induced iNOS gene transcription, did not prolong iNOS mRNA half life in the rat insulin-producing cell line RINm5F (50-60% decline in iNOS mRNA after 2-4 h, with or without the presence of IFN-g). IFN-g also failed to modify IL-1(25 U/ml)-induced NF-kB activation in RINm5F cells, as determined by electrophoretic mobility shift assay (optical density; n = 2-3): control, 82 ± 33; IFN-γ, 101 ± 50; IL-1β, 4531 489; IL-1β + IFN-γ, 4844 ± 633. However, IFN-γ induced a 19-fold ($P<0.01$ vs control) increase in nuclear IRF-1 protein content in RIN cells, an effect further potentiated by IL-1β (37-fold increase; $P<0.01$ vs control). The total cellular content of IRF-1 protein increased by 30-50-fold ($P<0.05$ vs control at 2h) in human islets exposed for 2, 4 or 8h to IFN-γ or IFN-γ + IL-1β. IL-1β alone induced a marginal and transient increase in IRF-1. It has been previously described that nicotinamide prevents IL-1β-induced IRF-1 expression in rat pancreatic islets. However, nicotinamide (20 mM) presently failed to prevent IL-1β + IFN-γ-induced IRF-1 protein expression in human pancreatic islets. In conclusion, the effects of IFN-γ on iNOS expression can neither be explained by iNOS mRNA stabilization nor increased NF-kB activation. However, IFN-γ induces an early increase in cellular IRF-1 content, and this may contribute to increased iNOS mRNA expression.

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PROBABLE RECOGNITION OF HUMAN INSULIN IN THE CONTEXT OF HLA-DRB1*0406 MOLECULE BY Vβ8 T-CELLS

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Insulin autoimmune syndrome (IAS, Hirata's disease) is thought to be an ideal model for investigating autoimmune mechanisms, especially human insulin autoantibody production. Glu74 in HLA-DRB1*0406 molecule revealed a crucial amino acid residue for the presentation of a human insulin epitope, and a posterior part of human insulin α chain after disulfide-bond cleavages were crucial for the T cell presentation. Here, we analyzed T-cell receptor (TCR) Vβ usages in cloned T cells which proliferated in autologous mixed lymphocytes reaction (MLR) of peripheral blood mononuclear cells (PBMC) with DRB1*0406 in the presence of human insulin. Human insulin-specific T cell clones by limiting dilution technique using PBMC from 2 donors with DRB1*0406 were established from blast cells in autologous MLR in the presence of 40 μM human insulin for the first week and 40 μM human insulin and 100 U/ml of rIL-2 for the following weeks. mRNA was isolated from the T cell clone and was analyzed by RT-PCR with 22 TCR Vβ specific primers. We found that there was a striking monoclonal increase in the amount of Vβ8 TCR gene product in the different T cell clones. It suggests that in this model, a limited number of T-cells cause the insulin autoantibody production.

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IL-1 AND TGFβ_1 AFFECT THE EXPRESSION OF TYPE I AND II IL-1 RECEPTORS IN THE RAT INSULINOMA CELL LINE RIN-5AH.

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Earlier studies from this laboratory had shown that the combination of IL-1β and TGFβ_1 was inhibitory to the logarithmic growth of RIN-5AH insulinoma cells, while either cytokine alone was without any effect. The aim of this study was first to document the presence of IL-1 receptor (types I and II) expression and then to investigate the effect of these cytokines on receptor expression at the mRNA level. RIN-5AH cells were grown logarithmically in high glucose-Dulbecco's MEM, with TGFβ_1 (80 pM) and IL-1 (5 nM) added, 2 or 26 hrs respectively after seeding of the cells (added in same order in combination experiments). Cells were harvested at given times and polyA+ cellular RNA was isolated. The effect on the mRNA IL-1 receptor levels was monitored by northern blotting of polyA+ RNA probed with specific probes. The RIN-5AH cells showed a gradual increase of RI mRNA with a doubling of the signal after 72 hrs of culture (near confluence). IL-1β showed a dramatic decrease in RI mRNA, that was evident at 6 hrs post-addition and maintained until 72 hrs of culture. TGFβ_1 showed a complex pattern of RI mRNA expression, with a substantial reduction in message by 8 hrs, an increase above controls at 24 hrs and a decrease well below background levels by 72 hrs. By contrast, the combination of the two cytokines leads to a very rapid (3 hrs) increase in RI mRNA. This is followed by severe down regulation of the message, making it undetectable by 72 hrs of culture. The cytokines, alone or in combination, have the same effect on RII mRNA for the first 24 hrs of incubation, yet by 72 hrs TGFβ and the combination of IL-1β and TGFβ_1 lead to an increase in RII message, compared to untreated cells. However, IL-1β incubation results in a severe downregulation of RII mRNA, making it undetectable at 72 hrs. In summary IL-1β causes severe down-regulation of RI and RII mRNA in RIN-5AH cells, while TGFβ_1 incubation leads to a more complex pattern that eventually results in down regulation of RI and upregulation of RII. The combination of the two cytokines leads to final down regulation of RI and upregulation of RII. We conclude that these two cytokines have quite complex pattern of interactive signal transduction that must be further investigated to determine the cause of the resulting inhibition of cellular growth.

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CELLULAR IMMUNE REACTIVITIES AGAINST PROINSULIN IN INSULIN DEPENDENT DIABETES.

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Investigations of animal models for insulin dependent diabetes (IDD) suggest that insulin may serve as a key autoantigen in the pathogenic events which culminate in this autoimmune disorder. In addition, recent studies of humans have suggested that proinsulin and/or a fragment from the region spanning C-peptide and the B-chain of insulin may serve as an autoantigen of IDD. Therefore, we analyzed cellular immune reactivities to proinsulin in order to clarify the role of this molecule in the pathogenesis of IDD. In vitro peripheral blood mononuclear cell (PBMC) responses (7 day, 10^5 PBMC/well) against proinsulin, insulin, control antigens (tetanus toxoid, *Candida albicans*, mumps, bovine serum albumin, and a mitogen (i.e., PHA) were determined in 32 individuals with newly-diagnosed IDD ($\leq$ one day from diagnosis) and 17 healthy non-diabetic individuals. Unlike previous reports by ourselves and others suggesting IDD associated elevations in cellular immunity to β cell antigens (e.g., glutamic acid decarboxylase, IA-2, etc.), we observed equivalent levels of PBMC proliferation against every antigen in this panel including proinsulin (all P = not significant). Specifically, the mean stimulation index ($\pm$ SD) and frequency of reactivity to proinsulin for controls and IDD patients (respectively) was: 1μg/ml (1.6 $\pm$ 1.1, 1/17(6%); 2.8 $\pm$ 3.2, 6/32 (18%)); 10μg/ml (1.7 $\pm$ 1.3, 1/17 (6%); 1.5 $\pm$ 0.8, 1/27 (4%)); and 50μg/ml (1.1 $\pm$ 0.7, 1/14 (7%); 1.4 $\pm$ 1.4, 1/25 (4%). Taken together with our previous studies reporting only rare occurrences of autoantibodies to proinsulin, the role of immunity to this molecule in the pathogenesis of IDD in humans is unclear.

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CHARACTERIZATION OF DIABETOGENIC, GAD65-SPECIFIC T CELL CLONES ESTABLISHED FROM NOD MICE.

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The autoimmune destruction of beta cells which leads to insulin dependent diabetes in NOD mice and human subjects appears to be mediated primarily by T cells. Recent reports have implicated glutamic acid decarboxylase 65 (GAD65) as an important antigen in the pathogenic T cell response to beta cells in NOD mice. The goal of these investigations was to determine the role of GAD65-specific T cells in beta cell destruction in NOD mice. Beta cell destruction in human subjects and in NOD mice is preceded by lymphocytic infiltration of islets and it is presumably these infiltrating cells that are directly responsible for this destruction. Therefor, the approach taken was to establish panels of GAD65-specific T cell clones from the islet infiltrates of NOD mice. Isolated islets from prediabetic NOD mice were trypsin digested to release the infiltrating cells an these cells were used to establish GAD65 specific T cell lines and clones by in vitro stimulation with antigen and APCs. A mixture of three synthetic peptides of GAD 65 identified as dominant epitopes in this response by and designated 17 (residues 247-266), 34 (residues 427-446), and 35 (residues 435-456) was used as antigen. Thus far we have established GAD65-specific T cell lines from individual mice 7, 8, and 9 weeks old at the time of islet isolation, and have cloned the line from the 8 week mouse and analyzed the resultant clones. These clones responded to peptide 17, were predominantly of the TH1-like phenotype, and responded to islet cells in vitro. More importantly, the clones that have been analyzed thus far are capable of acceleration of diabetes in young NOD recipient mice in adoptive transfer experiments. These finding suggest that GAD65 specific T cells participate in beta cell destruction in the spontaneous disease process.

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SULPHATIDE AND ITS PRECURSOR, GALACTOSYLCERAMIDE, INFLUENCE THE PRODUCTION OF CYTOKINES IN HUMAN MONONUCLEAR CELLS

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Sulphatide is expressed in the central and peripheral neural system, in islets of Langerhans, and in tissues affected by late diabetic complications. Autoantibodies to sulphatide are present in patients with insulin-dependent diabetes and the Guillain-Barré syndrome. Cytokines influence these disease processes, and we therefore studied whether sulphatide and its precursor galactosylceramide (gal-cer) influence the *in vitro* production of cytokines by blood mononuclear cells (MNC) originating from 15 healthy persons. Using lipopolysaccharide (LPS)-stimulated cells sulphatide increased the IL-2 production (163±17% of controls without sulphatide, p=0.02), and gal-cer increased the IL-1α production (145±13%, p=0.006), whereas neither gal-cer nor sulphatide had effect on the production of IL-6, IL-10 or TNFα. When stimulating cells with phytohemagglutinin (PHA), sulphatide decreased the production of IL-6 (88±5%, p=0.009), IL-10 (66±3%, p=0.000003), and TNFα (75±9%, p=0.02). Gal-cer, however, increased the production of IL-6 (188±13%, p=0.000006), and decreased the production of TNFβ (80±6%, p=0.007). Neither gal-cer nor sulphatide had effect on the production of IL-2 or IFNγ from PHA stimulated cells. Northern blot analysis using an IL-6 probe showed similarly an increased amount of IL-6 mRNA after gal-cer incubation (range 469%-150%, n=3, of PHA-stimulated control). Thus, sulphatide and gal-cer influence the production of several cytokines thought to be involved in immunoinflammatory disease processes.

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A REDUCED *IN VITRO* GENERATION OF CD8$^+$ T CELLS BY SPLENIC DENDRITIC CELLS FROM THE BB RAT, BUT NOT FROM THE NOD MOUSE

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Development of type 1 diabetes results from an autoimmune attack of, primarily, effector T cells towards the β cells of the islets of Langerhans. Antigen presenting cells (APC), including dendritic cells (DC) and macrophages, play an important role in the regulation of T cell function. Intrinsic disturbances in DC function have been suggested in both human and animal (NOD mouse, BB rat) type 1 diabetes. Suboptimal activity of DC may lead to an insufficient activation of suppressor T cells whereas autoimmune T cells are fully activated.

In this study we investigated (1) the accessory cell function in syngeneic MLR (sMLR) of DCs from BB rats and NOD mice, (2) the *in vitro* generation of CD4$^+$ and CD8$^+$ T cell subsets, and (3) the expression of MHC class I, II and ICAM-1 on the DCs.

Splenic DCs (enriched by Nycodenz density gradient centrifugation) were cocultured with nylon wool-purified splenic T cells for 5 days at a radio 1:5. Proliferation of T cells was measured after adding tritiated thymidine for 16 hrs. Expression of surface markers on T cells and DCs were studied with FACS.

The stimulator capacity of DCs from control Wistar rats (age 307 wks) cocultured with either Wistar or BB T cells was 142029 ± 59091 resp. 9862 ± 4456 cpm. DCs from BB rats (age 3-7 wks) had a significant lower stimulatory capacity (35108 ± 57617 resp. 3333 ± 2330; n=6, p<0.05). DCs from the BB rat were unable to maintain particularly CD8$^+$ T cells in culture (n=6), resulting in an increased CD4/CD8 ratio. The expression of MHC class I, II and ICAM-1 on DCs from both rat strains did not differ. Regarding the NOD, DC from both spleen and lymph nodes, were as good stimulators of syngeneic MLR as control C57BL/10 DC. Moreover NOD DC as well as C57BL/10 DC stimulation of T cells induced a similar increase of the CD4/CD8 ratio in syngeneic MLR.

In conclusion, accessory cell function of splenic DCs from the BB rat is abnormal in young animals (before disease). This abnormality is not due to differences in MHC class I, class II and ICAM-1 expression on the DCs. Since DC function in the sMLR is related to the induction of regulatory or suppressive T cells, the reduction in the number of CD8$^+$ T cells in the MLR is suggestive for a defect in the generation of such T cell populations by DCs from the BB rat *in vivo*. The accessory cell function of DCs from the NOD mouse was normal, indicating that the immune disturbances in both models of spontaneous type 1 diabetes are dissimilar.

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IN VITRO RELATIONSHIP OF LYMPHOCYTES FROM TYPE I DIABETIC CHILDREN AND RAT ISLET CELLS

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In order to investigate the cell mediated mechanisms of immune damage of beta cells we used a xenogeneic model of human peripheral blood lymphocytes (PBL) and rat islet cells. It is known that PBL form Type I diabetics inhibit the insulin secretion of cultured rat beta cells. Islet cell function was assessed by net insulin release in the presence of basal or stimulatory media and was expressed as the insulin release index-IR. After coculturing PBL from Type I diabetic children with rat islet cells, the islet cells lost their ability to repsond to stimulatory medium. The IR index in the group of diabetic children was 1.04±0.05, whilst the IR index for the nondiabetic control subjects was 1.8±0.05 (p<0.01). To elucidate the nature of the effector cells responsible for inhibition of in vitro insulin secretion PBL were treated with anti-CD3, anti-gamma-delta T, anti-CD25 or anti-LFA-1 antibodies. All suppressed the inhibition of insulin secretion by rat islet cells. However, treatment with antibodies to MHC Class I or II failed to suppress insulin secretion. The effects of anti-CD3 (IR index: 1.75±0.07), anti-gamma-delta T (IR index: 1.82±0.09), and anti-CD25 (IR index: 1.50±0.10), antibodies indicated a role for activated T cells expressing the CD3 associated gamma-delta receptor. In addition, the effect of anti-LFA-1 antibodies (IR index: 1.92±0.11), suggests that direct contact between PBL and rat cells is of importance. To examine this possibility we studied rosettes formed between PBL of diabetic children and rat islet cells (no rosettes form with PBL from control subjects and rat islet cells). Electron microscopy revealed a close association of PBL with the rat islet cells. In conclusion, our data suggest that at least one mechanism for the damage of beta cells is via their direct contract with non-MHC restricted activated gamma delta T lymphocytes.

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SKIN TEST FOR DETECTION OF CELLULAR AUTOIMMUNITY TO GAD65 IN IDDM: A STUDY IN NOD MOUSE.

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Cellular autoimmunity to GAD65 is closely associated with development of IDDM in human and NOD mouse. The cellular immunity has been detected by radioactive thymidine uptake into T-cells in presence of GAD65. To simply detect the cellular autoimmunity to GAD65, we devised a novel method by a skin test and examined its reliability and usefulness in NOD mouse. For an antigen of the skin test, a mouse GAD65 C-terminal peptide (86 amino acids) was expressed in E. coli and purified. Ten micrograms of the peptide was injected into the mouse footpad together with the crystal of alum potassium. The delayed type hypersensitivity reaction measured by footpad swelling was observed with peak at 48 h after the injection in NOD mice, but not in five normal mouse strains. Two recombinant peptides unrelated to the IDDM autoantigens, rat cyclophiline and bacterial carboxyl carrier protein, did not elicit the footpad reaction. Polymorphonuclear and mononuclear cell infiltrations were observed in the intradermal tissues injected with the GAD65 peptide, but not with the alum. Spleen cell proliferation to the GAD65 peptide measured by thymidine uptake was significantly higher in the NOD mouse than that in the other normal mouse strains. There was a significant correlation between the footpad swelling and thymidine incorporation (r=0.89, p<0.01). These results suggest the feasibility of detection of anti-GAD65 cellular immunity by the skin test and the potential clinical application of the GAD65 skin test for prediction and diagnosis of IDDM.

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CD4$^+$ T LYMPHOCYTE SUBSETS DETERMINE DURATION OF CLINICAL REMISSION IN RECENT-ONSET INSULIN-DEPENDENT DIABETES

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Previous studies have suggested that changes in CD4$^+$ T lymphocyte subsets might influence the clinical course of the disease in patients with recent-onset insulin-dependent diabetes mellitus (IDDM). The aim of this follow-up study was to compare changes in CD4$^+$ T cell subsets between two groups of recent-onset IDDM patients, exhibiting a long-term (> 6 months)(group A, N=16) or a short-term (< 6 months) clinical remission (CR) (optimal metabolic control without insulin lasting >30 days) (group B, N=18) and with those in age-matched healthy controls (group C, N=10). The percentage of CD4$^+$ T cell subsets was analysed in the peripheral blood by using two-color immunofluorescence staining with monoclonal antibodies defining helper-inducer (CD45R0$^+$) and suppressor-inducer (CD45RA$^+$) subsets of CD4$^+$ T cells and flowcytometry analysis. In each IDDM patient, the analysis was done sequentially (a) in initial insulin-requiring state (IRS I); (b) in CR and (c) in relapse of IRS after CR (IRS II). We found that percentage of CD4$^+$CD45R0$^+$ T cells in the state of CR was higher in group A than in group B (32.7$^+$/-2.2 vs 28.4$^+$/-2.0%, p<0.05), while simultaneously CD4$^+$CD45RA$^+$ T cells did not differ between the groups (25.8$^+$/-1.9 vs 24.2$^+$/-2.1%,p=NS). In both groups, the percentage of CD4$^+$CD45R0$^+$ T cells was lower in CR than in IRS I and IRS II (group A: IRS I: 35.7$^+$/-1.9, IRS II: 34.7$^+$/-2.2 %, p<0.05; group B: IRS I: 34.9$^+$/-1.8, IRS II: 35.3$^+$/-1.6 %, p<0.05; A vs B = NS) and it always remained higher than in controls (C:24.9$^+$/-1.4%;p<0.05). However, the percentage of CD4$^+$CD45RA$^+$ T cells was higher in CR than in IRS I and IRS II (group A: IRS I: 22.8$^+$/-1.6, IRS II: 23.1$^+$/-2.1 %, p<0.05; group B: IRS I: 23.2$^+$/-1.9, IRS II: 22.9$^+$/-1.9 %, p<0.05; A vs B = NS) and did not differ from controls. Our results signify that increased number of CD4$^+$CD45R0$^+$ T cells inversely correlates with shorter duration of CR, while the correlation between the number of CD4$^+$CD45RA$^+$ T cells and the duration of CR has not been found. The results imply that duration of CR could be modulated on the level of T helper-inducer activity.

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DIABETOGENICITY OF INSULIN SPECIFIC, TH2 T CELL CLONES IN NOD MICE.

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Insulin-specific T cells are a predominant component of the infiltrates that accumulate around pancreatic islets of NOD mice during the pathogenesis of insulin dependent diabetes. The great majority of nominally insulin specific T cell clones derived from these infiltrates (290/312, 93%) recognize epitopes contained on residues 9 - 23 of the B chain (B:9-23) of mouse insulin. Administration of B:9-23 to NOD mice by either subcutaneous or intranasal routes confers potent protection from diabetes. The aim of these investigations was to determine the mechanism by which B:9-23 protects NOD mice from diabetes. The cervical lymph nodes of NOD mice treated with intranasal B:9-23 were harvested and the resultant cell suspensions were cultured in vitro with B:9-23 to establish B:9-23 specific lines from four individual mice. All four lines produced the TH2 cytokines IL-4 and IL-10 upon stimulation with antigen and syngeneic splenic APCs. One of these lines was cloned and a panel of B:9-23 specific clones with TH2 cytokine production profiles was obtained. Many lines of evidence imply that TH2 cytokines protect NOD mice from diabetes, however, adoptive transfer experiments indicated that the TH2 clones were as potent as TH1 clones in transfer of diabetes to young NOD mice. In order to determine the fate of TH1 and TH2 clones in vivo, the cell tracking dye CFSE was used to label these clones. Mice were injected with CFSE labeled clones and total pancreatic infiltrating cells were isolated after the desired interval of time and analyzed by flow cytometry for various surface markers and cytokine production. The results of these experiments indicated that both TH1 and TH2 clones recruited large populations of other cells to the pancreas which outnumbered the injected clones by 10 to 200 fold. The recruited cells included: CD8 T cells, B cells, macrophages, and of interest, both TH1 and TH2 CD4 T cells. These results suggest beta cell destruction by TH2 cells is mediated by the recruited cells and that the recruited cells also contribute to pathogenicity when TH1 clones are injected.

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INCREASED CD69 AND HLA-DR EXPRESSION IN IDDM OF LONG STANDING

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To better define activation of circulating T-cell subsets in IDDM of recent onset (N=30) and of long standing (N=27; duration of disease >36 months), CD4$^+$ and CD8$^+$ T-cells were differentiated as to naive and memory cells, and expression of HLA-DR, interleukin-2 receptor (IL-2R, CD25) and CD69 was analyzed by three-color flow cytometry. Twenty-six healthy subjects of similar age and sex served as controls. No significant deviation was seen in IDDM vs. controls in CD25 expression on CD4$^+$ or CD8$^+$ cells, nor in their CD45RA$^+$ or CD45RA$^-$ subsets. CD8$^+$ cells, however, showed significantly increased amounts of HLA-DR molecules in newly manifested IDDM, both on CD45RA$^+$ CD8$^+$ cells (+ 45%) and the total population (+ 39%), but not on CD45RA$^-$ CD8$^+$ cells (+ 34%, NS). The same observation could be made in IDDM of long standing (CD45RA$^+$, + 76%; total CD8$^+$ cells, + 57%), where also CD45RA$^+$ CD8$^+$ cells exhibited increased HLA-DR expression (+ 40%, p<0.04). These patients also expressed increased amounts of HLA-DR molecules on CD4$^+$ cells (+ 29%), with CD45RA$^-$ cells being largely responsible for that elevation (+ 32%). CD69 expression in IDDM was not different from controls, but was higher in IDDM of long standing (p<0.05) for CD69 expression on CD4$^+$ (+ 48%), CD8$^+$ (+ 40%), and CD45RA$^-$ CD4$^+$ (+ 50%) cells than in IDDM of recent onset. Enhanced HLA-DR expression on T-cells was mainly due to their expression on naive CD8$^+$ T lymphocytes and was similar in both longstanding and newly manifested IDDM, whereas CD69 was slightly decreased at disease manifestation, but increased in longstanding disease. The observed peripheral T cell activation suggests an ongoing immune process in IDDM both at the time of its clinical manifestation and after 136 $\pm$ 109 months of duration.

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MADCAM-1 IS REQUIRED FOR THE DEVELOPMENT OF DIABETES IN NONOBESE DIABETIC MICE

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Environmental antigens may trigger the activation of self-destructive T cells in insulin-dependent diabetes via molecular mimicry. Many of the proposed antigens enter the body via gastrointestinal tract and are therefore presented to T cells in the gut-associated lymphoid tissue (GALT) wherein lymphocytes enter by interacting with the mucosal vascular addressin MAdCAM-1. The aim of this study was to investigate if the development of diabetes in nonobese diabetic (NOD) mice can be prevented by blocking lymphocyte homing to GALT. We show that an early blockade of MAdCAM-1 function prevents diabetes. While ineffective if started later, blockade started at 3 weeks age resulted in a near-complete reduction in diabetes incidence ($p < 0.01$). The blockade affected GALT by depleting Peyer's patches of naive (CD44^{low},CD45RB^{high}) T cells, inhibited the development of diabetogenic cells and also selectively diminished pancreatic homing of lymphocytes derived from GALT of young donors. These results show that MAdCAM-1 is required at two distinct steps of diabetes pathogenesis in the NOD mouse: for the development of diabetogenic lymphocytes and for the homing of the early-developing effector cells to the pancreas. Thus, MAdCAM-1-mediated homing of lymphocytes to, and activation in the GALT is critical for the development of early diabetogenic lymphocytes which use MAdCAM-1 also for their homing to the pancreas.

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EXPRESSION OF SURFACE CD8 AND CD23 AND PRODUCTION OF SOLUBLE FORMS IN IDDM PATIENTS WITH ANTIBODIES TO GAD

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Autoreactive T cells and anti-GAD antibodies may participate in the autoimmune-mediated process in IDDM. T cell activation markers are increased in IDDM, but B cell activation remains uncertain. To investigate the roles of B cell activation the production of soluble (s)CD8 and B cell-derived autocrine growth factor, sCD23, and expression of surface T and B cell antigens were examined. Peripheral mononuclear cells were obtained from 5 patients with anti-GAD antibody(+) IDDM, 8 patients with Graves' disease, and 12 normal controls. The cells were incubated with or without anti-CD40 monoclonal antibody and IL-4 for 7 days. The sCD8 and sCD23 in the supernatant were measured by enzyme immunoassay and cell surface antigens were measured by FACS using monoclonal antibodies. Anti-GAD antibodies were measured by radioimmunoassay. The production of sCD 8 and sCD23 were increased after 7 days of incubation with anti-CD40 and IL-4 in IDDM (sCD8:95±38→142±49U/ml; sCD23:2.4±0.4→12.5±2.1ng/ml) as well as in other groups. The percentages of CD8(+) cells were significantly decreased in patients with Graves' disease(21.6±2.4→12.0±2.7%), and slightly decreased in the normal controls (23.7±1.6→20.0±1.5%), but was not changed in anti-GAD(+)IDDM (24.7±3.6→24.9±4.3%). The percentages of CD4(+) and CD23(+)cells remained unchanged during the incubation. Anti-GAD antibodies were not detected in the supernatant after the incubation. Thus, B cell activation mediated by CD40 and IL-4 is involved in the increase of soluble forms of CD antigens in autoimmune endocrine disease but differently in IDDM and Graves' disease.

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ROLE OF MACROPHAGE-DERIVED CYTOKINES IN THE PATHOGENESIS OF KRV-INDUCED AUTOIMMUNE DIABETES IN DR-BB RATS.

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The diabetes-resistant BioBreeding (DR-BB) rat, derived from DP-BB forebears, does not normally develop spontaneous insulitis or diabetes, but when infected with Kilham rat virus (KRV) this animal develops autoimmune diabetes similar to the DP-BB rat. In this study, we attempted to determine whether macrophages and macrophage-derived cytokines play any role in the development of KRV-induced diabetes in DR-BB rats. 78% of DR-BB rats treated with KRV and Poly I:C developed diabetes; whereas, depletion of macrophages with liposome- encapsulated dichloromethylene diphosphonate (Cl_2-MDP) in KRV and Poly I:C-treated DR-BB rats resulted in the near complete prevention of insulitis and diabetes. Measurement of the macrophage-derived cytokines IL-12, TNF-α, and IL-1β revealed a selective increase of expression, after KRV infection, in the splenic lymphocytes and the pancreatic islets. Measurement of CD4+ T cell-derived cytokines revealed that IL-2 and IFN-γ cytokine gene expression closely correlates with an elevation of IL-12, but IL-4 and IL-10 did not change. Inactivation of CD8+ T cells using anti-CD8 antibody resulted in a significant decrease in the incidence of diabetes and severity of insulitis. Depletion of macrophages before the isolation of splenic lymphocytes from DR-BB rats treated with KRV and Poly I:C resulted in the loss of ability to transfer diabetes to young DP-BB rats. On the basis of these observations, we conclude that macrophages and macrophage-derived cytokines play a critical role in the cascade of events leading to the destruction of pancreatic β-cells, culminating in the development of IDDM.

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GAD-PEPTIDE STIMULATES TH1 LYMPHOCYTES IN DIABETIC CHILDREN, WHILE ABBOS STIMULATES BOTH TH1 AND TH2.

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Background: The insulitis at the clinical onset of IDDM involves CD4+ lymphocytes or Th-lymphocytes with at least two subpopulations: Th1-lymphocytes which produce e.g. IFN-γ and Th2-lymphocytes which produce e.g. IL-4. Glutamic Acid Decarboxylase (GAD), and especially a peptide with similar amino acid sequence as the Coxsackie B virus, may trigger the autoimmune process. Other studies have suggested the ABBOS-peptide from bovine serum albumin (BSA) in cow´s milk.

Aim: To identify if Th1 and/or Th2-lymphocytes are activated by the Coxsackie-virus associated GAD-peptide or by the ABBOS-peptide in children with recent onset of IDDM.

Patients and Methods: Human peripheral blood mononuclear cells (PBMC) from 20 children with recent onset of IDDM and from ten healthy HLA-matched children and adults (DR3, DR4 or DR3/4) were isolated by Ficoll Paque and stimulated with the specific GAD-peptide a.s. 247-279 or the ABBOS-peptide a.s. 152-169. The first-strand cDNA, achieved by reversed transcription, was diluted in different concentrations. Reversed transcriptase coupled to the polymerase chain reaction (RT-PCR) was used to give a semi-quantitative mRNA expression of IL-4 and IFN-γ. C-peptide was determined by radioimmunoassay (RIA).

Results: No increased mRNA for GAD and only weak IL-4 mRNA for ABBOS could be seen in healthy controls, while the GAD-peptide caused mRNA for IFN-γ in 8/20 patients ($p<0.05$); of whom 5/10 at diagnoses and 3/10 after 3-15 months duration. No mRNA for IL-4 was noticed except in 2/4 after 9-15 months duration. A high C-peptide at three months of duration seems to be correlated to a low IFN−γ mRNA response ($p=0.07$). The ABBOS-peptide produced IFN-γ mRNA alone in 4 patients, IL-4 mRNA alone in 2 patients and both in 7 patients.

Conclusion: The specific GAD-peptide causes a Th1 response which may damage the β-cells, while the ABBOS-peptide causes a mixed Th1/Th2 response which may just reflect either intolerance to cow´s milk or an activated immunesystem.

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ACTIVATION OF Syk TYROSINE KINASE PLAYS A KEY ROLE IN THE EXPRESSION OF THE IL-1β GENE IN CY-TREATED NOD MICE.

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We and others have previously shown that activation of macrophages with cyclophosphamide (CY) resulted in the upregulation of IL-1β gene expression, which mediates inducible nitric oxide synthase (iNOS) and nitric oxide (NO) production. iNOS and NO are known to be associated with the destruction of β-cells and the inhibition of β-cell function, leading to the acceleration of diabetes in NOD mice. This investigation was initiated to determine whether the enhanced expression of the IL-1β gene by CY is a result of the activation of Syk tyrosine kinase in NOD mice. First, we examined the cytokine gene expression (IL-1β, IL-12, IFN-γ, IL-4 and IL-10) of the splenocytes, peritoneal macrophages and pancreatic islets containing immunocytes. We found that IL-1β was significantly increased in the splenocytes, peritoneal macrophages, and pancreatic islets containing immunocytes in CY-treated NOD mice. In addition, IL-12 and IFN-γ , but not IL-4 or IL-10, increased in the pancreatic islets containing immunocytes. Second, we examined whether Syk tyrosine kinase is increased in Mac1+ cells in CY-treated NOD mice. We found that Syk tyrosine kinase clearly increased in Mac1+ cells prepared from splenocytes and peritoneal macrophages of CY-treated NOD mice. On the basis of these observations, we conclude that CY may activate macrophages, resulting in an increase of IL-1β gene expression due to the activation of Syk tyrosine kinase.

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DECREASED IN VITRO IL-4 AND IL-10 PRODUCTION BY PERIPHERAL BLOOD IN FIRST DEGREE RELATIVES AT HIGH RISK OF IDDM

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There is an accumulating evidence that the imbalance between Th1 and Th2 lymphocyte subsets plays a key role in the development of autoimmune diabetes in NOD mice, but it is possible also in humans. The aim of the present study was the estimation of in vitro production of Th1-(INF-γ, IL-2) and Th2-derived (Il-4, IL-10) cytokines by peripheral blood in high risk of insulin-dependent diabetes subjects (preclinical stage), since alterations in their secretion could represent early events in the immune-mediated islets destruction. The study was performed in 25 first degree relatives of type 1 diabetes patients with high (>20JDF), persistent ICA titres and 21 age and sex-matched healthy controls. Cytokines concentrations in supernatants of whole blood cultures with PHA (10ug/ml) were quantified by ELISA. We observed a lower concentrations of IL-4 in culture supernatants in ICA positive relatives as compared with the control group, both at 48h ($p<0.05$) and 72h of incubation ($p<0.05$). Similary in the prediabetic group lower IL-10 levels at 48 and 72h of culture ($p<0.05$) was found. We did not observe statistical differences in *in vitro* production of IL-2 and INF-γ by peripheral blood in high risk diabetes mellitus subjects and healthy controls. In subjects at increased risk of type 1 diabetes levels IL-4 positively correlated with IL-10 ($R=0.342$, $p<0.03$). There were negative correlation between IL-10 concentrations after 48 h of incubation and levels of HbA1c ($R=-0.437$, $p<0,05$). In conclusion our study has shown decreased IL-4 and IL-10 production, but normal secretion of Th1-derived cytokines by peripheral blood of prediabetic humans. It could suggest that the early stage of autoimmune process in type 1 diabetes in humans is associated with decreased function of Th1-cells rather than overactivation of Th2 subset in the peripheral blood.

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SPECIFIC REG II GENE OVEREXPRESSION IN THE NOD MOUSE PANCREAS DURING ACTIVE DIABETOGENESIS.

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The reg genes, normally expressed by acinar cells, have been shown to be associated with beta cell regeneration. We have described an overexpression of reg genes in the Non Obese Diabetic (NOD) female mice pancreas suggesting that the regenerative process may be involved in autoimmune aggressed beta cells (Diabetes, 1996, 45, 67-70). Since two reg genes have been characterized in the mouse genome, we have quantified the respective pancreatic reg I and reg II mRNA using specific probes and the Dot blot method. We firstly found a significant higher level of both reg genes before 130 days of age in non diabetic NOD mice compared to older animals. In addition reg II expression was prevalent to reg I (females: 4.97 ± 1.34 A.U. vs 2.39 ± 0.4 A.U., $p<0.05$, $n=11$; males: 3.41 ± 0.79 A.U. vs 1.55 ± 0.27 A.U., $p<0.05$, $n=11$) which is the opposite pattern to that found in OF1 control mice of the same age (reg II/reg I= 0.24). Secondly a much higher expression of reg II gene, but not of reg I, was observed in other animals at high risk of diabetes (cyclophosphamide injected males, n=6, reg II : 21.6 ± 6.86 A.I., reg I : 1.38 ± 0.47 A.U. or overtly diabetic females, n=13, reg II : 8.44 ± 2.4 A.U., reg I : 2.07 ± 0.43 A.U.). In diabetic animals, reg II overexpression was present despite undetectable insulin mRNA suggesting that this phenomenom does not depend on the persistance of beta cells. In conclusion, this observation confirms that autoimmune diabetogenesis is associated with reg gene overexpression in the exocrine pancreas and also demonstrates that the two reg genes are modulated by different regulations.

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DECREASED CD3-MEDIATED PROLIFERATION IN TYPE 1 DIABETES COULD BE DUE TO A CONSTITUTIVE DEFECT OF THE TRANSDUCTION SIGNALING PATHWAY

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CD3- mediated proliferative response of PBMC of IDDM adults patients (newly diagnosed patients n= 25, long-standing patients n= 19) was studied and compared to healthy control subjects (n= 36) , type II diabetic patients (n= 12) and non diabetic first degree relatives of IDD patients (n= 19). The effect of addition of costimulative signals (i-e PMA and exogenous cytokines IL-2 or IL-4) was evaluated. Proliferative response to anti-CD3 mAb was significantly lower in IDDM patients (mean SI 4.21± 2.55 in newly-diagnosed IDDM and 2.75± 3.03 in long-standing IDDM) compared to other groups (mean SI 5.85± 3.51 in control subjects, 5.96± 5.80 in type II diabetic patients, 5.26± 4.47 in first degree relatives). Addition of either PMA or exogenous cytokines was unable to overcome the defective proliferative response. Production of IL-2, IFNγ and IL-4 of CD3- stimulated PBMC was not different in IDDM patients and controls. Furthermore, there was no correlation between the stimulation index and degree of metabolic control (HbA_1C), genetic background (MHC class II DR or DQ), persistence of autoantigenic stimulation (C-peptide, ICA, GADA, IA2A).
In conclusion, because this low proliferative response was found whatever duration of symptoms, a constitutive defect of the CD3-TcR signaling pathway is suspected and is currently studying.

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BETA-CELL REPLICATION IN INSULIN-INDUCED REMISSION OF IDDM IN BB/WOR//TKY RATS

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In this study, we investigated the biochemical and histological characteristics of remission in diabetic BB/Wor//Tky rats. Plasma glucose levels (PG), pancreatic insulin content (PIC), histological severity of insulitis (i.e. area of islet occupied by mononuclear cells; score 0: 0%, 1: 1-24%, 2: 25-49%, 3: 50-100%, 4:atrophied), and islet beta-cell volume (%/pancreas) were determined in non-diabetic rats (aged 7-10 weeks), newly diabetic rats (onset day; aged 11-12 weeks), rats in remission (aged 12-15 weeks), and non-remission diabetic rats (> 7 days after the onset; aged 11-12 weeks). Beta-cell replication was also estimated using the 5-bromo-2'-deoxyuridine (BrdU) pulse labeling method. In rats in remission, PIC were higher than in newly diabetic rats, as evidenced by the near normalization of PG. Mean insulitis scores were significantly lower and beta-cell volume was significantly higher in rats in remission than in newly diabetic rats (mean insulitis score: 0.93 ± 0.64 vs. 3.02 ± 0.39, beta-cell volume: 0.46 ± 0.12 vs. 0.12 ± 0.08 %/pancreas, respectively). In newly diabetic rats, both the mean insulitis score and beta-cell volume were heterogeneous (insulitis score range: 2.38-3.87; beta-cell volume: 0.02-0.36 %/pancreas). BrdU labeling index significantly increased 3- to 4- fold in newly diabetic rats and also in rats in remission, compared with young adult non-diabetic rats (aged 9-10 weeks). Beta-cells may therefore still be present even in newly diabetic animals, and at the very early stage of insulitis the suppression of insulitis by insulin treatment and the compensatory replication of beta-cells might possibly contribute to IDDM remission in BB/Wor//Tky rats.

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INCREASED IN VITRO INTERLEUKIN-12 PRODUCTION BY PERIPHERAL BLOOD IN HIGH RISK IDDM FIRST DEGREE RELATIVES

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Cytokines secreted by antigen presenting cells, lymphocytes T and pancreatic β cells are considered as the major mediators in pathogenesis of IDDM. It is suggested that cytokines released by macrophages/monocytes could have initial role in β-cell damage. The aim of the present study was the estimation of in vitro production of macrophage-derived cytokines:IL-1β, TNF-α, IL-12 by peripheral blood in high risk IDDM first degree relatives, since it could reflect early events leading to the development of type 1 diabetes in humans. The study was performed in 25 high risk IDDM subjects and 21 age and sex-matched healthy controls.IL-1β, TNF-α and IL-12 concentrations in supernatants of whole blood cultures with PHA(10ug/ml) were quantified by ELISA. In the ICA positive relatives of IDDM subjects levels of IL-12 were significantly higher as compared with the control group, both at 48h ($p<0.02$) and 72h ($p<0.05$) of incubation and positively correlated with TNF-α and IL-1β ($R=0.46$, $p<0.002$ and $R=0.32$, $p<0.05$). We did not observe statisitcal differences in *in vitro* production of TNF-α and IL-1β between the study groups. In conclusion we suggest that our finding support the hypothesis, that IL-12 is involved in the pathogenesis of human IDDM. If the involvement of Th1 cells is confirmed in the destruction of islet β-cells, it is possible that IL-12 antagonists will have a role in the future prevention of insulin dependent diabetes mellitus.

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RESTORATION IN VITRO OF INSULIN SECRETION FROM NOD MOUSE ISLETS WITH SEVERE INSULITIS

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Pancreatic islets isolated from 12 wk old prediabetic NOD mice have a suppressed glucose stimulated insulin release. This can be be reversed by culture of the islets. In this study we have examined whether islets isolated from much older animals with advanced insulitis also can recover. We also studied whether supplementing the culture medium whith the cytokine IL-12 (10 ng/ml), which accelerates diabetes development in vivo in NOD mice, alone or in combination with the T-cell stimulating cytokine IL-2 (4 ng/ml) affected this recovery. Islets were isolated from 5, 12, 20 and 26 wk old female NOD mice. Some islets were used immediately after the isolation process for insulin release determination while others were cultured for 7 days with or without cytokines. Medium was changed on day 2, 4 and 6. In the age groups above 5 wk the glucose stimulated insulin release (ng/10 islets x 1 h) was lower in freshly isolated compared to cultured islets (5wk:4.1±0.9 vs 7.3±1.2, NS; 12wk:6.9±1.6 vs 14.3±3.6, p=0.014; 20wk:9.3±1.6 vs 17.0±3.4, p=0.045; 26wk:5.0±1.1 vs 14.9±1.1, p=0.003). Culture with IL-2 + IL-12 induced a minor decrease in glucose stimulated insulin release in islets from 12 wk old animals (10.9±3.2 vs control 16.4±5.6, p=0.025 paired t-test). With increasing age islet DNA content in freshly isolated islets increased due to immune cell infiltration. Islet DNA content in cultured islets was decreased by 40-60% ($p<0.01$) compared to freshly isolated islets in the age groups over 5 wk. This study shows that islets from NOD mice, as old as 26 wk, have an ability to recover from suppressed islet function when removed from the autoimmune in vivo environment.

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Is the assumed TH1/TH2 imbalance at diabetes onset reflected by serum total and specific IgE-levels?

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Introduction The development of type I diabetes mellitus (IDDM) has been assumed to be mediated by T helper 1 (TH1) cells, while TH2 cells have been considered to possess some protective potency. In contrast, atopic diseases are TH2 mediated, and IgE-production is regulated by TH2 cytokines. Therefore, a relative TH2 deficit, as assumed in IDDM, could well be reflected by low serum IgE-levels at the time of IDDM onset. ***Material and Methods*** Total IgE and specific IgE to seven frequent inhalative allergens (Phadiatop®, Pharmacia, Uppsala, Sweden) were measured in sera of 59 children [26 boys, 33 girls; median age 9.6 years (1.8-14.6)] at IDDM onset by the Pharmacia CAP system. Furthermore, glutamic acid decarboxylase-antibodies (GADA) and insulin-auto-antibodies (IAA) were simultaneously determined by radioimmuno-assays (Brahms Diagnostica GmbH, Berlin, Germany). ***Results*** Total IgE was elevated in 11 patients (19%). Specific IgE was found to be elevated in 25% of the children (see table), which is not markedly different from its prevalence in the age-related general population. Moreover, GADA- and IAA-positivity was as frequent in patients with normal and elevated specific IgE-levels.

Age group (years)	Total IgE(kU/l) mean±SD	Upper normal values	Elevated total IgE	Elevated specific IgE
< 4.0	75.9 ± 85.7	66 kU/l	2 / 5 (40%)	0 / 5 (0%)
4.0-6.9	94.7 ± 77.6	118 kU/l	4 / 15 (26.6%)	4 / 15 (26.6%)
7.0-10.9	137.4 ± 313.8	330 kU/l	1 / 18 (5%)	3 / 18 (16.6%)
≥ 11.0	115 ± 135.3	240 kU/l	4 / 21 (19%)	8 / 21 (38%)

Message We conclude that patients at IDDM onset have the potency to produce specific IgE-antibodies indicating appropriate TH2 mediated immunity.

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A REDUCTION IN THYMIC B-LYMPHOCYTE NUMBER IS ASSOCIATED WITH AUTOIMMUNITY IN THE RAT.

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A thymic B-cell deficiency (TBD) was originally found in the diabetes-prone BB-rat (DP) by differential screening using thymic cDNA libraries from DP and NEDH rats. TBD is inherited in a recessive mode, and the gene responsible for the trait, *Tbd*, was found to be tightly linked to the diabetes susceptibility gene *Lyp*. To investigate whether *Tbd* is identical to *Lyp*, we have analyzed several rat strains for presence of TBD using immunolabelling of single cell suspensions followed by FCM and immunohistochemistry. Brown Norway (BN) and NEDH rats was used as control strains. Four rat strains, diabetes-resistant BB, Lewis, PVG and Wistar Furth rats showed thymic B-cell numbers significantly lower than the control strains ($p < 0.05$ for all strains) - although higher than DP rats. The first three of these are known to be susceptible to induction of autoimmune diseases such as type I diabetes. This is in agreement with the role of thymic B-cells in negative selection. We speculate that *Tbd* may be a new diabetes (or autoimmune) susceptibility gene of the rat, since none of the four strains carries the DP allele of the *Lyp* gene, indicating that *Tbd* is not identical to *Lyp*.

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TROGLITAZONE ALTERS THE CYTOKINE PRODUCTION OF ACTIVATED LYMPHOCYTES FROM HUMAN DONORS.

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Troglitazone (TGL) is a thiazolidinedione compound which increases sensitivity to insulin and is used to treat insulin resistance in NIDDM. This compound also has anti-inflammatory properties which make it of interest as an immunomodulator. In vivo experiments have shown that the compound reduces diabetes incidence in NOD mice, an animal model of IDDM. This study aimed to investigate the ability of TGL to interfere with pathological processes mediated by cytokines which play a critical role in TH1/TH2 switching (IL-4, IFNγ and GM-CSF). Lymphocytes were collected from healthy human donors and cultured with different concentrations of TGL (0-62500 ng/ml). The cells were then stimulated with PHA and the resulting culture supernatant assayed by determining its ability to support the growth of a GM-CSF/IL-4 dependent cell line (TF-1). The presence of IFNγ was determined by ELISA technique. The results indicate that IL-4 levels were unchanged when lymphocytes were cultured in presence of different TGL concentrations. However IFNγ and GM-CSF levels were reduced in the presence of high TGL concentrations (12500-62500 ng/ml). IFNγ and GM-CSF are secreted by TH1 lymphocytes, therefore we suggest that TGL could reduce diabetes incidence in vivo by reducing TH1 cytokine production.

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ANTI-CD38 AUTOANTIBODY: A NEW MARKER FOR POTENTIAL IDDM - A STUDY WITH NON-OBESE DIABETIC(NOD) MOUSE.

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We have shown that pretreatment of pancreatic B cell with anti-CD38 antibody inhibited D-glucose-induced insulin release and $[Ca^{2+}]i$ rise, indicating the involvement of CD38 antigen, an ecto-enzyme ADP-ribosyl cyclase, in insulin secretion. The present study was aimed to know the possible existence of autoantibody against CD38 in diabetics.

A method to detect CD38 antibody(CD38-AB) was developed in which two fragment peptides(287-297;Ag-1), 241-255;Ag-2) as antigen and a biotin-avidin method using ABTS as detection system were employed. NOD mice aged 5-12 weeks were sacrificed to obtain sera and pancreases. Higher values of OD than mean +2SD of control samples(sera from B6 mice) was judged as "positive".

Sera of 5-week old mice, whose pancreatic islets were normal, were CD38-AB negative. After 7 weeks, varying degree of lymphocytic infiltration around the islets were observed in all subjects tested, and 84 % of mice were shown to be positive for CD38-AB. A majority of CD38-AB exhibited stronger binding to Ag-1 than to Ag-2. Most of mice older than 11 weeks became diabetic, and remained CD38-AB positive.

These results clearly indicate a diagnostic value of CD38 autoantibody to predict the onset of IDDM.

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PLASMA INSULIN AND PROINSULIN CONCENTRATIONS IN IGT AND NIDDM

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Disproportionate hyperproinsulinemia was observed in non-insulin-dependent diabetes mellitus (NIDDM). Diminished proinsulin (PI) and insulin (IRI) responses to a glucose load (75 g OGTT) are also predictive of worsening to diabetes. In the present study, we performed a study in 72 subjects with varying degrees of glucose tolerance to clarify β-cell dysfunction. Proinsulin and insulin were measured by a radioimmunoassay. The antibody against proinsulin was cross-reactive with 65-66 split proinsulin. At baseline, NIDDM (12) subjects had disproportionate hyperproinsulinemia (PI:IRI ratio; $16.2 \pm 1.3\%$) as compared to that in subjects with normal glucose tolerance NGT ($13; 9.0 \pm 1.1\%$) and with IGT ($45; 9.7 \pm 0.9\%$). Proinsulin and insulin index (Δ IPI:Δ BS30; Δ IRI:Δ BS30) in NIDDM ($4.7 \pm 1.2; 12.5 \pm 3.4$) were significantly lower than those who have NGT ($8.2 \pm 1.6, p<0.05; 56.0 \pm 10.8, p<0.01$) and IGT ($10.8 \pm 3.4$; $66.5 \pm 17.2, p<0.01$) in OGTT. Although OGTT in NIDDM showed a significant correlation between both Δ IPI:Δ BS30 ($r=-0.6, p<0.05$) and Δ IRI:Δ BS30 ($r=-0.7, p<0.01$) and 120-min glucose levels, this not the case in NGT nor in IGT. Both proinsulin and insulin responses to OGTT revealed a gradual decrease according to the degrees of glucose tolerance. The present findings suggest that an intracellular abnormality of pancreatic B-cell function, which reduces the conversion of PI to insulin and the release of disproportionately large amounts of PI relative to insulin.

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HEPATIC INSULIN CLEARANCE AND B CELL SECRETION DURING THE PROCESS OF AGING IN MAN.

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The aim of the study was to evaluate the secretory activity of pancreatic beta cell and the hepatic clearance of insulin in healthy subjects during aging. In 100 subjects (males and females) in age range 17 to 92 years with BMI <27 kg/m^2, divided in four groups according to age: (I: 18 aged 17 - 59 years, II: 23 aged 60 -69 years, III: 33 aged 70 - 79 years, IV: 26 aged from 80 to 92 years) oral glucose tolerance test (75g) and the i.v. glucagon test (1 mg) were carried out, and the blood glucose, serum insulin (IRI) and serum C-peptide (CP) were measured in fasting state and over 2 h after oral glucose and after glucagon. The hepatic insulin clearance was calculated from CP/IRI ratio. With advanced age the increase in fasting blood glucose and in blood glucose area under the curve after both stimuli, a decrease in fasting and stimulated serum CP, with no differences in fasting and stimulated serum IRI concentrations was observed. Consequently, the serum CP/IRI ratio decreased from 10±3.8 (SD) in group I to 5.4±1.7 in group IV, $p<0.05$. These results indicate that process of aging is associated with reduced hepatic clearance of insulin probably expressing a compensatory adaptation to the age-dependent decrease in pancreatic beta cell secretory activity.

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PLASMA PROINSULIN RESPONSE IN OBESE INDIVIDUALS WITH VARYING DEGREES OF GLUCOSE TOLERANCE IN KOREA.

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Controversy continues as to the role of β-cell function in the etiology of noninsulin-dependent diabetes mellitus(NIDDM). The β-cell function of NIDDM includes alterations in proinsulin levels, an observation made in several ethnic groups. We have performed a cross-sectional study evaluating proinsulin response after 75g oral glucose tolerance test(OGTT) in obese individuals(>27 kg/m^2 in body mass index, BMI). ALL 37 subjects were classified as 3 groups(obesity with normal glucose tolerance;12, obesity with IGT;10, obese NIDDM;15 cases). The patients were all new-onset diabetics. We measured proinsulin, specifc insulin, and c-peptide by radioimmunometric assay. The age and BMI were similar between groups but NIDDM group had lesser in basal c-peptide and insulinogenic index(p=0.01). The basal proinsulin level did not have difference (0.14, 0.13, 0.19 pM)between 3 groups, but the plasma proinsulin response in NIDDM group at 30 minutes after OGTT had greater than in other groups(p=0.05). And the ratio of proinsulin/(proinsulin+insulin) in NIDDM group at 30 minutes after OGTT also had greater than of other groups(0.07±0.03, 0.08±0.01, 0.15±0.06, p=0.01). These results show that the β-cell dysfunction of new-onset obese NIDDM includes increased proinsulin response after OGTT. And the proinsulin response at 30 minute after OGTT may be an indicator of early onset NIDDM.

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DIABETES MELLITUS IN THE ELDERLY: CLINICAL ASPECTS, BETA CELL FUNCTION AND IMMUNOLOGY PROFILE. Nasri, F.; Sá, J.R.; Pimenta,W.; Russo, E.K and Dib, S.A. UNIFESP-EPM São Paulo Brazil

The prevalence of Diabetes Mellitus (DM) are high in the elderly. Although the NIDDM is the most common type of DM in this population 30-40% of these patients are non obese and 5-1% are IDDM. Also in this group we have a less family history of DM and chronic complications. The aim of this study its to promote a better characterization of DM with diagnostic after 65 y.o. We studied 2 groups: Group I- normal elderly (10 (9 F/1M) - 76.1±5.7 y.o) Group 2- Elderly patients with 1 year or less of clinical DM, without retinopathy (15(11F/ 4 M) - 72.0 ± 4.9 y.o). In these 2 groups we studied: HbA1c, Anti-Islet cell (ICA-protein A) antibody, C-peptide(RIA) before and 60' (In 8 diabetic patients and 8 normal we measured the C pep at 90' and 120') after Sustacal^R (360Kcal/dose), also we used the C-peptide index (C-Pep/glucose). We stopped the oral hypoglycemic agents 3 months before the Sustacal^R Test. We used the non-parametric tests: Mann-Whitney and Wilcoxon and values of $p < 0,05$ were considered to statistic significance. Results: There were not difference between age, family history of diabetes (I:40%;II:53.3%),BMI (I:27.2±3.3; II:29.3±4.5Kg/m^2), HbA1c(I:5.8±0.3; II:6.43±1.7%), ICA (positive I:20% ;II:26.6%),were similar in the 2 groups. The C-peptide levels also were similar between the 2 groups in the basal condition and after Sustacal^R : basal - I: 0.21 ± 0.15 and II: 0.23 ± 0.24 pmol/ml and high value- I: 0.47 ± 0.39 and II: 0.41 ± 0.49 Before x after : $p < 0.05$ in I and II. The C-peptide index increased in 1.64 (90') fold in the control group and in 2.84 (90') in the diabetic group, after Sustacal^R . After 3 months on diet treatment we observed a group(IIA) of diabetic patients that answered to diet with glucose levels ≤ 7 mmol/l (8 patients: HbA1c: 5.6±1.8) and another group (IIB) with no answer to diet (gluc.>7.0),(7 patients: HbA1c: 7.1 ± 1.8). The basal (IIA: 0.32±0.24; IIB: 0.19± 0.22 pmol/ml) and highest value (IIA: 0.45±0.47 and 0.37±0.38 pmol/ml) were similar but the increase in C-peptide index after SustacalR was higher in the group that answered to diet (IIA: 3.2 fold and IIB: 2.2 fold). Our data did not shown differences between clinical, HbA1c and C peptide secretion between normal control and elderly diabetic patients. Also a high prevalence of ICA in this age group. So others studies are need to verify the difference pathogeneses of hyperglycemia and the real significance of autoantibodies in the elderly.

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INTACT PROINSULIN CONVERSION IN PATIENTS WITH INSULINOMA.

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The nature of proinsulin processing and secretion in insulinoma patients is incompletely understood. Levels of intact proinsulin (IPI) and total proinsulin immunoreactivity (PIM)(intact proinsulin and conversion intermediates) were measured by specific ELISA methods in the fasting state and under exogenous hyperinsulinaemia with and without hypoglycaemia. Nine insulinoma patients were compared with 10 healthy control subjects matched for body adiposity (BMI 26.7±3.9 vs. 25.5±4.8 kg/m^2). In the fasting state plasma (P) glucose levels were lower in insulinoma patients: 3.2±0.9 vs. 5 4±0.3 mmol/l in controls, p<0.001. However, both IPI and PIM were increased 20-30-fold in insulinoma patients: 76±47 vs. 2.4±0.3 pmol/l and 168±102 vs. 7.8±0.8 pmol/l in controls, respectively, p<0.001. The fasting ratio of IPI/PIM was also significantly elevated in insulinoma: 45±3 vs. 31±2% in controls, p<0.001. During a 180-min hyperinsulinaemic euglycaemic clamp (P-glucose 4.4-5.0 mmol/l) levels of both IPI and PIM were remarkably constant in the insulinoma patients. Thus, at the end of the clamp (180 min) IPI/PIM was unchanged 44±4%. In contrast in the controls both IPI and PIM decreased as expected: 180-min: 1.3±0.2 and 4.0±0.7 pmol/l, respectively, p<0.01, IPI/PIM 35±3%. Hypoglycaemia (P-glucose 2.2-2.5 mmol/l) was then introduced and kept constant for 60 min (210-270-min). During this part both IPI and PIM were still unchanged in insulinoma, thus IPI/PIM at 270 min was 47±5%. In controls there was a further suppression of IPI and PIM levels: 0.6±0.1 and 1.5±0.2 pmol/l, IPI/PIM was 42±7%.

We conclude, that since intact proinsulin is disproportionately increased in insulinoma patients, conversion of intact proinsulin is impaired. However a significantly proportion is further converted to proinsulin conversion intermediates. The study support the autonomous nature of intact proinsulin secretion in insulinoma.

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HOW MUCH IS POSSIBLE RECOVERY OF INSULIN SECRETION IN INSULIN DEPENDENT DIABETICS AT TIME OF CLINICAL REMISSION?

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It was known that lost of first phase of Insulin secretion precede onset of IDDM.At time of clinical remission patients have not got recovery of this phase.The aim of this study was to estimate how much we can expect recovery of beta cells secretors capacity at time of remission.IVGTT was performed to estimate Acute Insulin Response-AIR. Glucose 0,5g/kg BW as 30% solution was fast infused and blood samples were drawn at 0,1,3,6,10, 15,30 and 60 minutes.We measured C-peptide in daily profile also.Investigations were performed in 21 newly diagnosed IDDM patients(group A), treated by intensified insulin Pen therapy. In 11 patients IVGTT was repeated at time of clinical remission (group B).Results were compared with10 patients who did not have clinical remission (Group C),one month after IDDM diagnosis(8h after last regular insulin doses).At time of IDDM onset,first phase was impaired, as at time of remission.No significant improve of both phases of insulin secretion at time of remission(differences for all values were no significant (p>0,05).Patients without remission had very similar insulin levels also.Values of Insulin(mIU/l) are on table:

Groups	0'	1'	3'	6'	10'	15'	30'	60'
A	16,2+ 2,2	18,4+3,5	20,6+5,4	15,9+4,4	14,8+3,2	23,3+4,5	18,6+5,6	7,6+4,2
B	16,8+ 3,3	19,2+2,3	21,2+4,4	18,3+4,7	10,4+2,2	20,4+4,5	20,5+3,5	18,5+2,5
C	17,2+3,4	17,4+3,5	17,4+2,7	13,4+3,5	14,2+3,5	19,6+3,7	19,6+2,9	19,3+4,3

We found significant differences in C-peptide(nmol/l) values during daily profile at time of IDDM onset and at time of remission($p<0,01$),except for values at 13h.

Time	8h	10h	13h	15h	18h	20h	24h
Onset	0,43+0,02	0,69+0,03	0,42+0,02	0,59+0,04	0,61+0,05	0,71+0,10	0.59+0,1
Remiss.	0,17+0,03	0,36+0,02	0,48+0,10	0,29+0,01	0,24+0,03	0,33+0,02	0,22+0,1

Our results suggest that AIR during clinical remission is very weak and satisfied metabolic control at that time probably dependent of beta cells stimulation with other stimulators such as maybe amino acids.

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LEVELS OF DES 64, 65 PROINSULIN IN SUBJECTS WITH HISTOLOGICALLY PROVEN INSULINOMAS

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We investigated the relative contribution of the proinsulin-like molecules, intact, des 31, 32 proinsulin and des 64, 65 proinsulin, to the hyperinsulinaemia in 20 subjects with histologically proven insulinomas (male:female, 4:16; age median (range) 58.0(32 - 82)yrs; and BMI 25.5(20.1 - 38.4)kg.m^{-2}). Insulin, intact and des 31, 32 proinsulin were determined by in-house immunometric assays. Des 64, 65 proinsulin was assayed with a novel in-house two-site immunoradiometric assay based on a guinea-pig polyclonal antibody against des 64, 65 proinsulin and an anti-proinsulin monoclonal antibody. The assay showed less than 5% cross reaction with intact proinsulin (at 250pmol.l^{-1}), and no cross-reaction with insulin (up to 2500pmol.l^{-1}) or des 31, 32 proinsulin (up to 250pmol.l^{-1}). The sensitivity of the assay was 0.12 to 0.06pmol.l^{-1}. Recovery of the des 64, 65 proinsulin added to human plasma or 5% BSA/Tris buffer at 2.5pmol.l^{-1} was 98.4% range 92 -105%, at 7.6pmol.l^{-1} was 89.0 range 84 - 95% and at 16.0pmol.l^{-1} was 102.8 range 98 to 104%. The inter and intra-assay CVs at 3.0pmol.l^{-1} were 12.3 and 9.3%, at 8.5pmol.l^{-1} were 7.4 and 8.2% and at 15.6pmol.l^{-1} were 6.9 and 11.0% respectively. The proinsulin-like molecules as a proportion of total immunoreactive insulin ranged from 7.5 to 89.8%. Intact proinsulin was the predominant insulin precursor in 9 of the subjects and in 11 of the subjects the des 31, 32 species was predominant. While des 64, 65 proinsulin was detected in all the samples assayed it was not the predominant precursor in any of them. Des 64,65 proinsulin only comprised 4% (range 1 -11%) of immunoreactive insulin in these subjects.These results support the findings that 1) proinsulin to insulin conversion occurs primarily via the des 31,32 branch , 2) the des 64,65 proinsulin form may be of little physiological significance and 3) assays for insulin that show only cross-reaction with the des 64,65 species may be considered largely specific for insulin.

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LESSONS SHOULD BE TAKEN FROM 60 COMPLETE REMISSION PATIENTS WITH TYPE 1 DIABETES

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In this study we aimed to define clinical and laboratory characteristics of 60 complete remission cases with Type I diabetes (F/M 13/46, age 17.4±6.1 yrs, BMI 19.5±2.6 kg.m^{-2} and ICA +/- ; 36/18). Remission was induced by multiple subcutaneous insulin injections (MSCII) and defined as no insulin requirement with daytime normoglyceamia for at least 15 days. Results were compared with data of 10 incomplete remission [ICR: ≤0.25 IU.kg^{-1}.day^{-1} insulin requirement, (F/M 6/4, age 18.0±5.6 yrs, and BMI 19.1±2.2 kg.m^{-2} , ICA +/- 4/6) and 19 non-remitted (F/M 13/6, age 15.4±5.0 yrs, and BMI 18.3±2.0 kg.m^{-2}, ICA +/- 12/7)] cases. Compared to florid period, mean fasting blood glucose (FBG) and HbA_{1c} levels were decreased (p=0.000), while basal C-peptide (BCP) and insulin autoantibody (IAA) levels were increased significantly at clinical remission (p=0.000). Stimulated C-peptide (SCP) was also increased but did not reached to a significant level. Mean time to starting of remission was 53±29 days. Compared to remission values, mean FBG, HbAıc, and BCP did not change at 3rd month, and no change was observed in HbAıc and BCP at 6th month (p=0.007). However, at 12th month mean FBG increased (p=0.036) and BCP decreased (p=0.04), HbAıc did not changed from remission values. Insulin requirement in IU.kg^{-1}.day^{-1} have shown a slow trend from 0.6±0.3 at entry to 0.1±0.1 at 3rd month and 0.3±0.2 at 6th and 12th month (p=0.000 , p=0.007, and p=0.03 respectively). Results of insulin induced CR patients were also compared to 25 spontaneously occured CR patients while under conventional insulin therapy (F/M 4/21, age 21.1± 7.6 year, BMI 21.0 ±3.7 kg.m^{-2}, ICA+/-17/3 and time to remission; 211.4± 128.1 days, florid and remission BCP/SCP in ng.ml^{-1} were respectively 0.5±0.4 /1.7±0.3 and 1.3±0.5/2.3±0.7). Both BCP and SCP in spontaneous CR were increased more remarkably at remission. Trends in decreasing insulin requirement was slower than insulin induced CR patients. We conclude that male gender , older age at onset, normal BMI, lesser insulin requirement at onset are predictors of CR. Moreover, there is at least another form of CR occured spontaneously, the parameters mentioned above are even more remarkable along with better beta cell reserve than insulin induced CR.

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FIRST-DEGREE RELATIVES OF NIDDM SUBJECTS HAVE REDUCED INSULIN SECRETION.

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We aimed to study insulin release in first degree relatives of NIDDM patients. Twenty-one normo-glucose tolerant relatives (mean± SEM age 45.4±1.3 yr, BMI 26.4±0.8kg/M^2, waist-hip ratio 0.803±0.015, aerobic capacity ($\dot{V}O_2$Max) 29.1±1.4 ml/kg/min) of NIDDM patients, and 21 normo-glucose tolerant controls matched individually for gender, age, BMI, waist-hip ratio and $\dot{V}O_2$Max underwent a hyperglycemic clamp (10 mMol/L, 180 min). First phase insulin release (0-10 min) was not significantly lower (logarithmic transformation, MANOVA, p=0.11). Second phase insulin release was lower in relatives (MANOVA, F=4.18, p=0.047). ANOVA showed lower plasma insulin levels from 120 minutes onwards in the relatives (all p<0.05); at 180 min geometric mean (95 %CI) plasma insulin levels were 55 (45 to 67) and 77 (61 to 97) mU/L. Insulin action assessed as Glucose Infusion Rate (GIR) divided by mean plasma insulin (I) during 2nd and 3rd hour of the clamp (GIR/I) was not different (both p>0.20); GIR/I_3 was 0.17-±0.01 vs 0.18±0.02 mg/kg/min/mU/L.

Conclusion: Normo-glucose tolerant first-degree relatives of NIDDM subjects have a decreased second phase insulin release. Insulin action is not markedly decreased.

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INSULIN SECRETION AND RESISTANCE IN URBAN SOUTH INDIANS: ASSESSMENT BY HOMEOSTASIS MODEL ASSESSMENT (HOMA)

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A house to house survey was done in 3 areas in Madras city. A total of 613 subjects were studied. Investigations included fasting and post glucose (2 Hr) plasma glucose estimations and fasting and 2Hr plasma insulin levels. Insulin resistance and insulin sensitivity were derived by HOMA model. Fasting insulin levels were highest in those with Impaired Glucose Tolerance (IGT) (n = 98) 11.2 ± 20 Uu/ml followed by diabetics (n = 144) 7.0 ± 5.6 Uu/ml and those with Normal Glucose Tolerance (NGT) (n = 371) 6.7 ± 6.6 Uu/ml. However insulin secretion as assessed by HOMA was lower in diabetics compared to subjects with NGT 0.06 ± 0.05 vs 0.09 ± 0.08 but IGT subjects had higher values 0.14 ± 0.25. Insulin resistance was significantly higher in diabetic and IGT subjects 46.1 ± 45.6 and 47.5 ± 87.4 respectively compared to NGT subjects 26.1 ± 27.8 (P <0.001). Use of HOMA model provides reliable estimates of insulin secretion and resistance in epidemiological studies.

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INSULIN SENSITIVITY, INSULIN SECRETION AND GLUCOSE EFFECTIVENESS IN SUBJECTS WITH NIDDM IN KOREA: A MINIMAL MODEL ANALYSIS

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The clinical characteristics of patients with non-onsulin dependent diabetes mellitus(NIDDM) in Korea are different from that of western countries and most of them are non-obese. Obesity is well known cause of insulin resistance. So insulin secretory defect would be more important role for the developement of diabetes in Korean NIDDM patients. Twenty subjects with NIDDM(8 male, 12 female: mean age 38.4 yr) were performed the 75 gram oral glucose tolerance test(oGTT) and insulin administration-modified frequently sampled intravenous glucose tolerance test (FSIGT). The insulin and glucose dynamics were analyzed by MINMOD computer program. The peak insulin levels after i.v. glucose load(PI) were significantly decreased in diabetic patients(98±77 pmol/L vs 361±290 pmol/L, $p<0.05$). S_I and S_G of diabetic patients were significantly lower than those of control subjects(SI : 1.84±1.13 vs 6.51±4.24 x 10^{-4} min^{-1}/ (uU/ml), $p<0.05$, S_G: 0.0191±0.0066 vs 0.0445 ± 0.0136 per min., p=0.0002) There was significant inverse correlation between S_I and waist/hip ratio($p<0.05$, r=-0.4). These results suggest that insulin resistance may be an important risk factor for obese old age onset NIDDM, while impairment of insulin secretion could be a major risk factor for non-obese young age onset NIDDM in Korea. Exogenous insulin administration-modified FSIGT is a useful tools for the assessment of insulin secretory capacity and resistance in normal and NIDDM patents with well controlled blood glucose levels.

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QUANTIFICATION OF PANCREATIC RESPONSIVENESS DURING A STANDARDISED MEAL TOLERANCE TEST

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We have quantified pancreatic responsiveness employing a novel model of C-peptide secretion during a standardised meal tolerance test (MTT). The model relates C-peptide secretion in a linear fashion to glucose concentration during MTT and defines (i) pancreatic sensitivity M_1 as an increase in C-peptide secretion per unit increment in plasma glucose concentration and (ii) basal pancreatic sensitivity M_0 as C-peptide secretion per unit plasma glucose concentration at fasting plasma glucose level. A population (standard) was used to derive values of transfer rate constants of a two compartmental model of C-peptide kinetics. MTT (75g CHO, 500kcal) was performed after overnight fast in 60 normal subjects (M:F: 27:33; age: 50±10 yrs; BMI: 26.4±5.0 kg/m^2; fasting plasma glucose: 5.2±0.5 mmol/L; mean±SD) and 26 subjects with newly diagnosed NIDDM (M:F: 20:6; age: 49±10 yrs; BMI: 31.0±4.0 kg/m^2; FPG: 11.9±3.0 mmol/L). Plasma C-peptide and glucose were measured every 10 to 30min over 180 min and employed to estimate indices M_1 and M_0. Subjects with newly diagnosed NIDDM showed reduced pancreatic sensitivity M_1 (21.8 ± 12.9 vs 87.2 ± 37.0 x 10^{-9}/min; NIDDM vs normal; $p < 0.001$) and basal pancreatic sensitivity M_0 (6.6 ± 3.1 vs 10.7± 4.0 x 10^{-9}/min, $p < 0.001$). M_1 and M_0 were estimated with excellent precision (CV < 15%) and were significantly correlated (r=0.50, p<0.001). Additional model-independent calculations confirmed that C-peptide secretion is linearly related to glucose concentration during MTT. We conclude that pancreatic responsiveness can be quantified with *precision* during MTT in both normal subjects and subjects with newly diagnosed NIDDM.

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LEUCINE IS A BETTER PRIMING AGENT THAN ARGININE FOR INSULIN SECRETION IN GLICLAZIDE TREATED NIDDM

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Both branch chain amino acids and charged ionic amino acids stimulate insulin scretion from the beta-cell, but the mechanisms are different: we wished to examine synergistic effects with sulphonylurea therapy. Eight gliclazide-treated non-insulin-dependent diabetic patients were studied on two occasions with a protocol of basal observation for 30min, 60 min infusion of randomised leucine or arginine and a further 90 min hyperglycaemic clamp. Basal glucose concentration was the same on both occasions (mean 7.82mmol/l leucine study vs 7.79 mmol/l arginine study; p=ns.) and the glucose declined to 7.50 mmol/l and 7.25mmol/l respectively by 30min. After leucine infusion the declination of glucose continued, but levelled out or reversed with arginine, such that by the end of the infusions, glucose levels were 6.63±0.69mmol/l (leucine) and 7.62±0.67mmol/l (arginine);$p<0.02$. Arginine caused a sharp rise in insulin secretion (17.8.mU/l to 43.8mU/l in six minutes) at the onset of the infusion and thereafter insulin secretion was not significantly different throughout either the amino acid or hyperglycaemic clamp periods (mean 42.1 mU/l, vs. 44.7 mU/l respectively; p=ns). By contrast the leucine infusion caused little acute change in secretion but augmented with time from basal 17.2 mU/l to end of infusion 29.4mU/l.. During the hyperglycaemic clamp period there was significant further augmentation of insulin secretion rising to 81.6±16mU/l at the end of the study. Leucine significantly augmented insulin secretion compared to arginine (81.6±16 vs. 54.0±8.4 mU/l respectively $p<0.002$). These data suggest that leucine is a better priming agent for sulphonylurea than arginine. Additive effects on insulin secretion may allow one to use combinations of branch chain amino acids and sulphonylureas to augment insulin secretion in the presence of hyperglycaemia.

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INTERIM REPORT FROM AN INTERNATIONAL WORKSHOP FOR THE COMPARISON OF AMYLIN ASSAYS

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Amylin is a 37 amino acid peptide that is cosecreted with insulin by pancreatic β-cells in response to nutrients. Since amylin's discovery in 1987 many researchers have initiated studies to investigate its potential biological functions and physiological roles. Several laboratories have reported plasma amylin concentrations using different immunoassay techniques. We therefore established a workshop to compare the amylin assays since it is important to know to what extent they produce comparable results. A total of 10 amylin assays were compared from 8 separate participating laboratories. The represented methodologies included both ELISA and RIA with either solid phase, liquid phase, or no pre-assay extraction requirement. Six different primary antibodies or antibody pairs were represented. Blinded plasma samples including pools from Type I diabetic, fasting non-diabetic (F), glucose stimulated non-diabetic (S) and glucose stimulated IGT subjects were analyzed by each laboratory using both the individual laboratory's standard curve and a supplied common standard (F and S were each submitted as 3 blinded individual samples in order to assess intra-lab imprecision). The median (interquartile range) intra-lab %C.V was 11.1% (4.6%-38.3%) and 8.4% (4.2%-16.6%) for F and S respectively when the common standard was used. Results between labs, however, had several fold differences in values for samples in the quantitative range of the assays (see table below).

Sample	Median (pM)	Interquartile Range (pM)	Min - Max (pM)
Type I	1.6	0.3 - 3.6	0.1 - 5.4
F (mean of 3)	4.4	2.1 - 6.9	1.4 - 14.3
S (mean of 3)	8.9	5.6 - 11.8	4.1 - 18.5
IGT	13.3	8.6 - 25.3	3.5 - 33.3

We conclude that variation between individual laboratories makes it currently difficult tc compare plasma amylin results between labs. Since it has been reported that circulating amylin is heterogeneous, it is possible that the variation seen in this comparison is due, at least in part, to differences in assay specificity. Further investigation, including additional sample comparisons, will be completed before recommendations are made.

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THE EFFECT OF ONE MONTH CORTICOTHERAPY ON GLUCOSE TOLERANCE AND INSULIN SECRETION

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Chronic corticotherapy deteriorates glucose tolerance and induces insulin resistance. Earlier reports suggested that chronic effects of corticotherapy may be less pronounced than the acute ones. The aim of the sudy was to compare the acute (one week) and chronic (one month) effects of corticotherapy on glucose tolerance and insulin secretion. Three glucose tolerance tests were done in a group of 12 euthyroid patients with Graves' ophthalmopathy: before therapy, after one week and after one month of prednisone administration in a daily dose of 60mg. Basal glucose levels did not change during therapy. Glucose levels at 60 and 120 min were higher than pretreatment values but the differences were not significant. Glucose levels at one week and at one month were not different. In 5 patients glucose tolerance was unchanged during therapy, in 5 patients it deteriorated, in one patient glucose tolerance improved after prednisone and in one patient diabetic curve was noted. Basal insulin (IRI) levels increased from 24.3 μU/ml to 42.2 μU/ml ($p<0.001$) after a week and to 35.8 μU/ml ($p<0.01$) after a month. IRI levels at 60 and 120 min were significantly higher during treatment when compared to pretreatment IRI levels but again there was no difference in IRI levels at one week and at one month. The analysis of variance (ANOVA) showed significant difference in insulin response during treatment ($F=17.62481, p<0.00001$),while this cannot be demonstrated for glucose levels. It is concluded that 1) the administration of prednisone 60 mg/d for one month markedly increases insulin response while only slightly deteriorating glucose tolerance and 2) glucose tolerance and insulin response at one week and at one month are not different.

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MEASURING INSULIN SECRETION: ACCURACY AND EFFECT OF SAMPLING SCHEDULE

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We have evaluated the accuracy of calculations of pre-hepatic insulin secretion during a simulated meal tolerance test (MTT) using the standard population model of C-peptide kinetics (POP-MOD). Five normal male subjects (N) (age: 36±2 yrs; BMI: 22.7±0.8 kg/m^2; mean±SE) and five male diet-treated patients with NIDDM (D) (age: 49±4 yrs; BMI: 26.8±2.1 kg/m^2) received a variable infusion of biosynthetic human C-peptide (I-BHCP) mimicking pre-hepatic insulin secretion during MTT while endogenous C-peptide secretion was suppressed. Plasma C-peptide was measured every 5 min for 240 min. POP-MOD and a deconvolution method were used to reconstruct I-BHCP from the measurements. Total infusion rate was estimated with clinically acceptable bias (N: 13±9; D: 19±4%; mean±SE) which was not significantly different from zero. Similar bias (p=NS, ANOVA) was observed when calculations were based on samples taken at 15 min intervals (N: 8±8; D: 20±4%) and 30 min intervals (N: 13±9; D: 21±4%). Root mean square error measuring the average deviation between the infused and calculated BHCP was also independent of the sampling frequency (N: 1.2±0.2; D: 1.3±0.2 pmol/kg/min). We conclude that this methodology can accurately estimate post-prandial total C-peptide secretion both in normal subjects and subjects with NIDDM thus obviating the need for individual estimates of C-peptide kinetics. Plasma C-peptide samples need only be drawn at 30 minute intervals.

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INSULIN SECRETORY CAPACITY AND EXOCRINE PANCREATIC FUNCTION IN PATIENTS WITH MALNUTRITION-RELATED DIABETES MELLITUS

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The relationship between endocrine and exocrine pancreatic functions in patients with Protein Deficient Diabetes Mellitus (PDDM) and Fibrocalculus Pancreatic Diabetes (FCPD), the two subtypes of Malnutrition-Related Diabetes Mellitus (MRDM), is still unclear. Recent ERCP data of cases with FCPD and TCP (non-alcoholic Tropical Calcific Pancreatitis without diabetes) suggest that the severity of pancreatic damage does not follow a straightline relationship with the development of diabetes. The present study was undertaken to explore this relationship in greater detail using arginine stimulation test as a measure of insulin secretory capacity and secretin stimulation test as an indicator of exocrine pancreatic function. Six patients with PDDM (5male/1female; BMI 17±1 (M±SD); HbA1c 15.6±3.1%) and 3 patients with FCPD (3m; 16±3; 15.2±3.9%) along with 6 nondiabetic controls (6m; 18±1; 5.1±0.4%) and 3 patients with TCP (2m/1f; 20±3; 5.4±0.5%) were studied. Arginine HCl (1.4 mval/kg/30 min) was infused i.v. and blood taken at 15 min. intervals to assess plasma C-peptide (EIA) and glucose. Secretin (1 CU/kg/60 min) was infused i.v. and duodenal juice collected at 15 min. intervals via a duodenal tube using marker perfusion technique. Bicarbonate output was measured and is presented as area under the curve. The results (M±SD) are as follows:

	BIC (AUC)	C-PEPTIDE pmol/l		GLUCOSE mmol/l	
	mmol/60min	0 min	30 min	0 min	30 min
Control (n=6)	248 ± 79	636 ± 113	1173 ± 257†	4.5 ± 1.2	5.1 ± 0.7
PDDM (n=6)	180 ± 129	301 ± 138	604 ± 315‡	15.2 ± 2.1	16.3 ± 3.4
FCPD (n=3)	37 ± 17*	361 ± 104	607 ± 203	12.3 ± 4.5	13.6 ± 3.5
TCP (n=3)	28 ± 16*	753 ± 351	1754 ± 829	4.5 ± 0.5	5.2 ± 0.1

* $P < 0.01$ vs. Control. † $P < 0.01$ vs. 0 min. ‡ $P < 0.05$ vs. 0 min.

The data shows that exocrine pancreatic function is fairly well-preserved in PDDM cases, and insulin secretory capacity may be highly dissimilar in FCPD and TCP groups inspite of similar levels of exocrine dysfunction. Although the patient numbers are very low the present data reinforces the earlier impression that factors other than generalized pancreatic damage are also involved in the development of diabetes in FCPD.

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INTERFERENCE BY ANTI-MURINE ANTIBODIES IN C-PEPTIDE ASSAY KITS

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Anti-murine antibodies (HAMA) are known to interfere immunoassay using mouse monoclonal antibodies (mmab). We have studied the interference by HAMA in three commercial C-peptide assay kits sold in Japan: Daiichi C-peptide kit III(method 1), Tosoh IIC-peptide (method 2) and Shionogi C-peptide RIA (method 3). Method 1 is a two antibody competitive RIA and uses mmab for the second antibody. Method 2 is a two-site immunometric assay and uses mmab for detection antibody. Method 3 is a one antibody competitive RIA and uses rabbit polyclonal antibody. Serum HAMA level was assayed using Immunostrip HAMA IgG(Immunomedics, USA). Case 1 was a 3-year-old DM and case 2 was a 74-year-old DM. Both cases were HAMA (+). Interference was noticed in method 1. In case 2, peak of the interference came 16 months after that of HAMA. No interference was observed in method 2. Treatment of serum by polyethyleneglycol or protein A eliminated interference in method 1. When the second antibody of method 1 was changed to rabbit polyclonal antibody, CPR values were close to those by method 3. Addition of mouse immunoglobulin (mIg, ~2000 μg/ml) to assay buffer decreased interference, although weak interference still remained. Pretreatment of serum with magnetic particle bound mIg (150μg/ml) completely eliminated interference, showing that all interference was due to HAMA. We conclude that method 1 is vulnerable to interference by HAMA.

	[case 1]	[case 2] 0	5	21	29	42 Months
R1	4.1	1.0	4.2	14	8.0	2.7
R2	0.91	-	-	0.94	0.94	0.97
HAMA	45	3	460	287	65	12 (ng/ml)

R1 and R2 denote the ratio of CPR values by method 1 and by method 2 to those by method 3, respectively.

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ALTERED ANTIOXIDANT/PRO-OXIDANT HOMEOSTASIS AND INSULIN SECRETORY RESPONSE IN DEVELOPMENT OF DIABETES FROM OBESITY

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The study was performed with the aim: to obtain the evidence whether decreased antioxidative defence mechanism, accompained with increased oxidative stress, can reflect an evolution of diabetes from obesity; to establish the possible relationship between insulin secretory response and antioxidant/pro-oxidant homeostasis, as well as the extent of changes in their responsivity during glucagon stimulation. The study included 24 obese healthy subjects(BMI=35.81±4.33) and 12 obese patients considered as diabetics (BMI=33.66±5.67), as well as 20 control normal-weight, age-matched subjects (BMI=20.03±3.12). Total plasma antioxidative capacity (TPAC) progressivelly decreased from obesity toward diabetes (66.05±5.21% $p<0.001$ in obese diabetics vs 72.3±2.45% $p<0.05$ in obese and 84.8±5.09% in control subjects), together with plasma GSH (4.08±0.81 $p<0.05$ in obese diabetics vs 4.96±2.07 in obese and 6.3±1.12 nmol/l in control) and marked decrease of Er GSH (0.72±0.12 $p<0.001$ in obese diabetics vs 1.05±0.03 $p<0.05$ in obese and 1.25±µmol/mlRBC in controls).The increase of plasma MDA was significant in obese diabetics (6.28±1.56 $p<0.001$) compared with obese (4.12±0.76) or controls (3.67±1.02µmol/l). The increase of Er lipid peroxidation was shown to occur in basal state (3.53±0.28 $p<0.05$ in obese diabetics vs 3.23±0.36 $p<0.05$ in obese and 2.75±0.34nmolMDA/ml in control) and increased susceptibility to oxidative stress (7.92±0.44 $p<0.05$ in obese diabetics vs 7.66±0.28 in obese and 7.12±0.83nmolMDA/ml RBC). All parameters were measured simultaneously in basal state and after glucagon stimulation. Glucagon-stimulated insulin release was accompained with the increase of plasma GSH (29.64±7.05% $p<0.05$ in obese vs 6.13±1.95% in obese diabetics) and decrease of plasma and Er MDA (-13.26±-7.93 in obese vs -7.94±5.33% in obese diabetics). Positive correlation was found between the percent of rise of C-peptide and plasma GSH ($r=0.58$ $p<0.05$). Obtained results suggest that the insulin-secretory potential could be functionaly dependent on the plasma and Er redox state. The progressive deterioration of TPAC, plasma GSH and Er GSH from obese subjects to obese diabetics, could point to evolution of diabetes from obesity and progressive loss of insulin secretory reserve due to increased oxidative stress.

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FASTING GLUCOSE CONCENTRATION HAS A GREATER EFFECT THAN BMI ON INSULIN PULSATILITY IN NORMAL SUBJECTS

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Basal insulin pulsatility, which is found in normal individuals, is known to be reversibly diminished in both mild hyperglycaemia and obesity. To address the question of the importance of obesity or glucose concentration on this effect we have studied 23 normoglycaemic subjects (11F,12M) for 120 minutes in the basal state taking 1-min samples assayed for glucose and insulin. Data were analysed by mean concentration and by Fourier Transform analysis to examine for insulin pulsatility. Overall insulin pulsatility could be detected with a peak oscillatory periodicity between 9 and 15 min. When split by mean fasting glucose level of 5.1mmol/l (range 4.51 to 5.87mmol/l; group size 11 and 12 subjects) there was less oscillatory activity in those with the higher basal glucose concentration which was significantly different (ANOVA 3-20 mins: $p=0.037$) and most significant at 15min oscillatory period $p<0.025$ (paired t). This was reproduced by sub-group analysis where the changes were more apparent in a best-six v worst-six comparison. By contrast, when the data were divided by median Body Mass index 25 (BMI range 19.3 to 37.53kg/m^2; group size n=11 & 12) there was no differences in the FT profile (p NS). Thus we conclude that even within the normal range, those with a higher fasting plasma glucose have less oscillatory insulin activity. It is not possible from these data to decide whether the reduction of oscillations is the cause or the effect of the elevated glucose concentration.

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THIS ABSTRACT HAS BEEN WITHDRAWN BY THE AUTHOR.

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Insulin Secretion in Vitro: Ion Channels and Oscillations

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ß-CELL DYSFUNCTION IN THE 'FINNISH TYPE' OF NEONATAL HYPERINSULINISM WITH NO IDENTIFIED MUTATIONS IN K_{ATP} CHANNEL GENES.

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Mutations in the genes encoding subunits of the ß-cell K_{ATP} channel (i.e. SUR1 and $K_{IR}6.2$) cause familial persistent hyperinsulinaemic hypoglycaemia of infancy (PHHI). In Finland, the phenotype of the disease is generally severe, necessitating pancreatectomy in the affected infants. Other unique clinical features include high frequency of premature birth (47%) and gestational diabetes (28%). The majority of the known cases (n=19 since 1983) originate from a limited area in central Finland, suggesting a common recessive gene. Two cases were analysed by direct sequence analysis of the SUR1 and $K_{IR}6.2$ genes. None of the known mutations were found, and 19 (of 39) SUR1 exons and the entire coding sequence of $K_{IR}6.2$ revealed wild-type sequence. We have now examined the function of insulin-secreting cells in a familial Finnish patient with a severe form of PHHI. The patient did not respond to treatment with diazoxide, and responded poorly to octreotide. Near-total pancreatectomy was performed, and islet-like cell clusters (containing ~15% ß-cells) isolated for in vitro studies of ion channels and intracellular Ca^{2+} homeostasis. In situ patch-clamp recordings, whole-cells and inside-out patch studies consistently revealed the absence of functional K_{ATP} channels in the presence of the channel agonists diazoxide (0.5mM, n=10), and somatostatin (100nM, n=8) and under 'ATP-free' conditions (n=8). The loss of K_{ATP} channel function was associated with the appearance of action potentials in intact cells, consistent with an inability of PHHI ß-cells to maintain a normal resting membrane potential. $[Ca^{2+}]_i$ responses to glucose (15mM), KCl (40mM), tolbutamide (0.1mM), and diazoxide (0.5mM), all of which affect ß-cells through depolarization-response coupling, were not seen in PHHI ß-cells. Islets were, however, responsive to agonists that are not strictly dependent upon voltage-gated Ca^{2+} influx, such as acetylcholine. In summary, isolated ß-cells from patients with the 'Finnish' PHHI phenotype lack functional K_{ATP} channels and this is associated with generation of Ca^{2+} action potentials.

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DEMONSTRATION OF TETRODOTOXIN-INSENSITIVE Na^+ OSCILLATIONS IN GLUCOSE-STIMULATED β-CELLS

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Glucose promotes the turnover of Na^+ in β-cells both by enhancing the entry of the ion and providing the ATP required for the extrusion by the Na/K pump. Recent studies have demonstrated glucose-induced oscillations of the cytoplasmic Na^+ concentration ($[Na^+]_i$), which were amplified by opening of the voltage-dependent Na^+ channels by veratridine. In the present study it was analyzed if glucose-induced $[Na^+]_i$ oscillations are affected by cyclic AMP, which has been proposed to facilitate entry of Na^+ through an unspecific cation channel. $[Na^+]_i$ was measured in individual mouse β-cells with dual wave-length fluorometry using the indicator SBFI. Small oscillations of $[Na^+]_i$ (amplitude 1-4 mmol/l; frequency 0.2-0.5/min) were observed in the presence of 11 mmol/l glucose during perifusion with media containing either 1.3 mmol/l Ca^{2+} or 5 mmol/l Sr^{2+}. The amplitudes of the oscillations increased to 5-15 mmol/l after introduction of 10 nmol/l glucagon or 1 mmol/l 8-Br cAMP. These oscillations remained unaffected by 3 µmol/l tetrodotoxin. However, the oscillations disappeared when lowering glucose to 3 mmol/l or adding 10 µmol/l methoxyverapamil, 400 µmol/l diazoxide or 0.1 µmol/l clonidine. It is concluded that glucose-stimulated pancreatic β-cells exhibit oscillations of $[Na^+]_i$ also in the presence of tetrodotoxin and that this rhythmicity will be particularly pronounced after raising cyclic AMP.

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REGULATION OF PHOSPHOLIPASE C - LINKED CA^{2+}-SIGNALING AND INSULIN SECRETION BY K_{ATP}-CHANNELS IN CLONAL BETA CELLS

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Modulation of the ATP-sensitive K^+ current (I_{KATP}) is central to glucose-induced Ca^{2+} signaling and insulin secretion. Agonists coupled to phospholipase C (PLC), like arginine-vasopressin (AVP) and bombesin, cause frequency-modulated Ca^{2+} transients and enhance insulin release under conditions where glucose is elevated. Thus, modulation of I_{KATP} activity by glucose may interact with PLC-linked Ca^{2+} signaling and insulin secretion. To test this hypothesis we investigated the effects of I_{KATP} modulation on AVP or bombesin-induced Ca^{2+} signals and insulin secretion. Intracellular free Ca^{2+} ($[Ca^{2+}]_i$) was measured in single fura-2 loaded HIT cells and insulin secretion was determined from cell populations. In the presence of glucose (10 mM) tolbutamide (3-300 μM) which inhibits I_{KATP} by binding to the sulfonylurea receptor increased the frequency and sometimes the amplitude of the AVP- and bombesin-induced Ca^{2+} transients, or switched the Ca^{2+} transients to a plateau-like rise in $[Ca^{2+}]_i$. Diazoxide (10-100 μM), which activates I_{KATP}, lowered $[Ca^{2+}]_i$ in the presence of glucose (10 mM), reduced the frequency and sometimes the amplitude of the Ca^{2+} transients, or stopped them in a subset of cells. Glucose deprivation decreased the frequency and amplitude of AVP-induced Ca^{2+} transients and lead to their complete cessation, which was partly antagonized by tolbutamide. In the presence of glucose (10 mM) AVP or bombesin-induced insulin secretion from populations of HIT cells was potentiated by tolbutamide and inhibited by diazoxide. In glucose-free medium, AVP or bombesin-stimulated insulin release was reduced, but was potentiated by tolbutamide. Thus, modulation of I_{KATP} by glucose or pharmacological interventions critically regulates PLC-linked Ca^{2+} signaling and insulin secretion from HIT cells presumably by modulating voltage-gated Ca^{2+} influx. (Supported by DFG Scho 466/1-3)

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GLUCOSE-INDUCED PULSATILE RELEASE OF INSULIN INDEPENDENT OF ATP-SENSITIVE K^+ CHANNELS

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Glucose-induced cyclic variations in the permeability of the ATP-sensitive K^+ channel in pancreatic β-cells result in periodic depolarization and entry of Ca^{2+} and oscillations in the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$). These oscillations occur in parallel with the pulsatile release of insulin and have been suggested to regulate the secretory pulses. However, pulsatile release of insulin has also been observed under conditions with a stable $[Ca^{2+}]_i$ such as at non-stimulatory glucose concentrations or after depolarization with tolbutamide or K^+. We have now investigated whether variations in the ATP-sensitive K^+ channel activity are required for the generation of glucose-induced pulsatile insulin release from individual islets of Langerhans using a sensitive ELISA for determination of insulin. When the islet β-cells were depolarized by 30.9 mM K^+ and their ATP-sensitive K^+ channels were continously opened by 400 µM diazoxide, insulin release was oscillatory with a frequency of 0.41 ± 0.04 per min whereas $[Ca^{2+}]_i$ was stable and elevated in the presence of 3 mM glucose. When the glucose concentration was increased to 11 or 20 mM glucose, the amplitude of these insulin oscillations increased whereas their frequency was unaffected. The results show that glucose-induced pulsatile release of insulin can occur independently of variations in the ATP-sensitive K^+ channel activity or $[Ca^{2+}]_i$ of islet β-cells and indicate that other factors, possibly an oscillatory metabolism, are important for the generation of pulsatile release of insulin.

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PROPERTIES OF ION CHANNELS AND THE REGULATION OF INSULIN SECRETION IN THE NOVEL GLUCOSE-RESPONSIVE β-CELL LINE, BRIN-BD11.

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We recently cloned a β-cell line by the electrofusion of normal rodent β-cells and the immortal RINm5F insulin-secreting cells. These cells - BRIN-BD11, display a normal concentration-related glucose-responsiveness. In this study we have extensively investigated the properties of K^+ channels in BRIN-BD11 cells using patch-clamp techniques, and also characterised the effects of pharmacological regulators of ion channels on insulin release. At sub-stimulatory concentration of glucose (2mM), openings from K_{ATP} channels were routinely observed in the intact cell-attached patch configuration (n=40). These channels were inhibited by the presence of higher glucose concentrations (15mM) and by tolbutamide (TOL, 0.2mM) and the imidazolines efaroxan (EF, 0.2mM) and phentolamine (PHE, 0.2mM). At equivalent concentrations TOL, EF and PHE were all able to initiate insulin secretion from BRIN-BD11 cells (2mM glucose) and potentiate the actions of higher glucose concentrations (n=6). This is consistent with the inhibition of K_{ATP} channels. In addition, K_{ATP} channels were activated by both diazoxide and pinacidil (0.2-0.4mM), and both compounds inhibited glucose-, TOL-, EF- and PHE-induced insulin secretion in parallel experiments (n=6 replicates each). Whole-cell studies revealed the presence of inward currents (carried by Ca^{2+} and/or Na^+) and outward K^+ currents resulting from voltage-gated K_{Ca} and delayed rectifier K^+ channels; these currents were sensitive to TEA^+ and quinine (n=6). Finally, inside-out patches (n=42) were used to assess the biophysical properties and nucleotide-dependent regulation of K_{ATP} channels. Current-voltage relationship plots revealed an inward rectification to the I-V profile with a linear inward conductance of approximately 60pS. In isolated patches K_{ATP} channels underwent 'run-down', they were inhibited by ATP (10μM-1mM; IC_{50}=~30μM), and were sensitive to ADP (0.01-1mM) added alone and in combination with ATP. In summary, BRIN-BD11 cells show many features of ion channel function that resemble native β-cells indicating that they are an important model cell for *in vitro* studies of the regulation of insulin secretion.

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VOLTAGE–INDEPENDENT Ca^{2+} CHANNELS DO NOT MEDIATE THE SLOW Ca^{2+} OSCILLATIONS IN β-CELLS

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Single pancreatic β-cells respond to stimulating concentrations of glucose with 2-5 min oscillations of the cytoplasmic Ca^{2+} concentration. It is generally believed that these oscillations reflect periodic entry of Ca^{2+} into the β-cells. There is extensive evidence that repetitive depolarization with opening of voltage-dependent Ca^{2+} channels (VDCCs) is the underlying phenomenon. However, it has also been claimed that the oscillations are mediated by specific voltage-independent channels (VICCs). The latter conclusion was based on lack of effect of the L-type VDCC blocker nifedipine and the observation that Mn^{2+}, which supposedly does not enter VDCCs, permeates into glucose-stimulated β-cells. We have now used these tools to evaluate the possible involvement of VICCs in the generation of glucose–induced slow Ca^{2+} oscillations. It is demonstrated that rapid influx of Mn^{2+} into mouse β-cells requires depolarization and is blocked by 5 μmol/l nifedipine. This concentration also abolished the glucose–induced Ca^{2+} oscillations. Moreover, parallel measurements of cytoplasmic Ca^{2+} and electrical activity demonstrated that the oscillations occur in synchrony with bursts of action potentials. We conclude that the slow oscillations of Ca^{2+} in glucose–stimulated β–cells reflect periodic openings of VDCCs.

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HIGH EXPRESSION OF *TRP*, A PUTATIVE CALCIUM-RELEASE ACTIVATED CHANNEL IN MOUSE INSULINOMA CELLS

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Insulin secretion is stimulated by glucose, hormones and neurotransmitters. Both activation of a non-selective cation current and activation of a Ca^{2+} current in response to depletion of intracellular Ca^{2+} stores have been suggested to play a role in this stimulation. The properties of these currents resemble those reported for the Drosophila gene *trp*. The aim of this study was to determine whether *trp*-related genes are expressed in pancreatic β-cells. We used RT-PCR and Northern blot analysis to examine expression of *trp* genes in the mouse insulinoma cell line (MIN6) and in pancreatic islets. Of the six known mammalian *trp*-related genes (*trp*1-6), only *trp*1 was expressed at high levels in MIN6 cells. We cloned the murine *trp*1 cDNA from a MIN6 cDNA library and identified four variants (α, β, γ and δ), generated by alternative splicing near the N-terminus. In vitro translation showed that only the α and β splicing variants are efficiently expressed. The β variant is the dominant form in MIN6 cells and mouse pancreatic islets, whereas the α variant is the major type in the mouse brain. Functional studies suggest that the human *trp*1 β-variant encodes a non-selective cation channel, activated by depletion of intenal Ca^{2+} stores. This suggests that murine *trp*1 may also encode a non-selective cation channel in β-cells.

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POTENTIATION OF SULPHONYLUREA-INDUCED INSULIN SECRETION BY EFAROXAN INVOLVES BLOCKADE OF α2-ADRENOCEPTORS.

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Imidazoline compounds and sulphonylurea drugs each stimulate insulin secretion from the pancreatic B-cell and can also interact functionally to generate an enhanced secretory response *in vivo* and *in vitro*. In the present study we have used the two enantiomers of the imidazoline efaroxan (efx) to investigate whether potentiation of the secretory response to glibenclamide (glib) reflects binding to the islet imidazoline receptor or whether α2-adrenoceptor antagonism is more important. (+)Efx is a potent α2-antagonist and when administered to Wistar rats (3.5mg/kg) together with glib (2.5mg/kg), it caused a marked increase in circulating insulin beyond that in response to glibenclamide alone. (-)Efx (the more potent imidazoline ligand) failed to potentiate the insulin response to glib. Equivalent data were obtained with isolated rat islets incubated *in* vitro where (+)efx did not alter insulin secretion itself at concentrations up to 50μM but dose-dependently enhanced the response to 1μM glib ((+)Efx: 0.7±0.06 ng/islet/h; glib: 1.2±0.15; (+)Efx&glib: 2.3±0.2; P<0.001) . By contrast, (-)efaroxan (25μM) provoked an insulin secretory response but did not further enhance the response to glibenclamide ((-)Efx: 1.4±0.15 ng/islet/h; glib: 1.25±0.15; (-)Efx&glib: 1.5±0.18). When isolated islets were treated with 1μM phenoxybenzamine to block the α2-adrenoceptors, (+)efaroxan failed to potentiate the secretory response to glibenclamide ((+)Efx: 1.3±0.10 ng/islet/h; glib: 2.3±0.2; (+)Efx&glib: 2.1±0.2). Similar results were obtained when the α2-adrenoceptors were down-regulated by exposure to the agonist UK14304 (10μM) or when they were uncoupled by treatment with 200ng/ml pertussis toxin. None of these treatments prevented the stimulation of insulin secretion by (-)efaroxan or glibenclamide, alone. Thus, the results indicate that the ability of efaroxan to enhance the insulin secretory response to glibenclamide does not result from interaction with imidazoline receptors but suggest that it occurs as a consequence of the blockade of islet α2-adrenoceptors.

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EXPRESSION OF CALCIUM CHANNEL SUBUNITS IN PANCREATIC β-CELLS OF OLD RATS

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Age in men is often associated with the development of NIDDM (non insulin dependent diabetes mellitus). One of the major pathological effects in NIDDM is a reduction in the ability of pancreatic β-cells to respond appropriately to an increase in the blood glucose. Ca^{2+} influx through voltage dependent L-type calcium channels and consequent increase in cytosolic calcium are accepted as crucial steps in insulin secretion. Islets of old rats have been shown to exhibit decreased Ca^{2+} influx and impaired insulin secretion. The aim of our study is to investigate the influence of age per se on the subunit expression of the L-type calcium channel. We characterized in islets of 2 year old rats the α_{1C}, α_{1D}, β_3 and $\alpha_2\delta$ subunit messages of the L-type calcium channel representing the subtypes which have been shown to be mainly expressed in the pancreas. Oligonucleotide primers were designed to amplify by RT-PCR regions specific to the subunits. The amplification products were verified by southern blotting and non-radioactive hybridization. Compared to young rats the cardiac isoform α_{1C} message was found in islets of old rats at much lower levels. The expression of the neuroendocrine subtype α_{1D} was in age also significantly reduced. The levels of the $\alpha_2\delta$-complex detected in old rats were slightly different compared to young rats. But the expression was also reduced. In contrast to the other subunits we observed for the β_3 in both tissues equal expression. This down-regulation of L-type calcium channel subunits suggests that the changed expression may contribute to the impaired insulin secretion associated with age. Our results indicate that this defect may be caused by decreased transcription of genes encoding β-cell L-type calcium channel subunits. These alterations in the stimulus secretions coupling process during aging could therefore have important implications for the development of NIDDM.

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IMPAIRED EFFECT OF SULFONYLUREA ON ATP SENSITIVE K^+ CHANNEL BY INHIBITION OF METABOLISM IN β CELLS

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Sulfonylureas becomes less potent to reduce blood glucose levels according to the severity of NIDDM. We have reported that glucose metabolism in pancreatic β cells is impaired under NIDDM. Using dinitrophenol (DNP) which inhibits mitochondrial metabolism, we examined insulin secretory capacity, K_{ATP} channel activity and binding for sulfonylurea receptor (SUR) or K_{ATP} channel protein (BIR) using glibenclamide and cibenzoline. The augmentation of insulin secretion by glibenclamide was disappeard under 100μM DNP, but that by cibenzoline was intact. In experiments using patch-clamp techniques, the dose dependency of glibenclamide for K_{ATP} channel inhibition was observed to shift the right from IC_{50} of 3.04nM to 51.3nM under 100μM DNP in cell-attached mode. In inside-out mode, dose-dependent inhibition of K_{ATP} channel was observed under 10μM ATP, but under 0.1μM ATP K_{ATP} channels were not closed even at high concentration of glibenclamide (1μM). On the other hand, cibenzoline also inhibited K_{ATP} channels dose-dependently in cell-attached mode, but IC_{50} was not changed under 100μM DNP (41.5μM vs 40.7μM). The binding of [^{3}H]-glibenclamide to β cells was equally displaced by unlabelled glibenclamide with and without 100μM DNP. In bindng experiment using expressed protein of SUR or BIR, glibenclamide bound to SUR only, but cibenzoline also bound to BIR. These data indicate that uncoupling between SUR and BIR is involved in impaired inhibition of K_{ATP} channels and dissappearance of insulin secretory augmentation by glibenclamide under inhibiton of metabolism in pancreatic β cells.

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THIS ABSTRACT HAS BEEN WITHDRAWN BY THE AUTHOR.

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NA^+ ENTRY THROUGH VOLTAGE-GATED CHANNELS IS ESSENTIAL FOR SULFONYLUREA-INDUCED INSULIN RELEASE FROM PANCREATIC B CELL.

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Freshly isolated pancreatic islets from Wister rats (male,2-3 mo) were batch-incubated to assess the role of Na^+ in insulin secretion by estimating IRI (μU/islet/30 min) using RIA.Glucose stimulated insulin secretion at all concentrations beyond the basal supply of 5.5 mM (G5.5).Neither TTX(4μM), the Na^+ channel blocker, nor its activator veratridine (VT, 100μM), altered the glucose effect on insulin secretion (G16.7=40±12, G16.7+TTX=60±11, G16.7+VT=43±8; p= ns). However, when Na^+ was removed from outside of islets (Na^+0), the glucose effect disappeared by about 80%: the residual 20% stimulatory effect (4.7± 0.5 at basal vs 11±2 at G16.7;p=0.002) could be ascribed to stimulation of Ca^{2+} entry through reversely operated Na^+/Ca^{2+} exchanger that occurs under Na^+0 conditions. In the absence of TTX , both glibenclamide (10μM) and gliclazide (100μM) stimulated insulin release (for glibenclamide IRI being 6±0.7, for gliclazide 7±0.4 at p=0.02 and 0.01 respectively vs IRI 3.4±0.5 at G 5.5).When the toxin was added in presence of the hypoglycemic agents, the latter became completely ineffective (glibenclamide + TTX=4.3±0.7, gliclazide+TTX =2.88 ±0.4; p=ns both vs G5.5 as well as G5.5+TTX). Na^+0 mimicked the effect of TTX on glibenclamide but unlike glucose it had no residual effect. Moreover, a dose-reponse experiment revealed a concentration-dependent decrease in insulin release by glibenclamide (at 100 nM 10±0.5, at 1 μM 7.5±0.8 and at 10 μM 6.7±0.6: a significant decrease between 100 nM and 10 μM at p=0.006 was observed). Vt not only counteracted this fall but also showed a tendency to significantly increase the effect of glibenclamide at 1 μM (7.5±0.9 to 10±0.9 at p=0.06).We conclude that Na^+ entry through voltage-dependent channels are essential for sulfonylurea-induced insulin release.

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INTRA-ISLET GLUCAGON IS A DETERMINANT FOR THE ELECTRICAL ACTIVITY OF PANCREATIC β-CELLS

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Pancreatic islets exposed to 11 mM glucose exhibit complex variations of the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) with slow (0.3-0.9 min^{-1}) or fast (2-7 min^{-1}) oscillations or with a mixed pattern. It is well established that the regular fast oscillations correspond to the electrical burst activity in islets, and we now show that the slow oscillations are explained by very long bursts of action potentials. Using confocal microscopy it is demonstrated that the mixed pattern with slow and superimposed fast oscillations is due to simultaneous presence of cell populations with the respective responses. In islets with mixed $[Ca^{2+}]_i$ oscillations, exposure to the intracellular Ca^{2+}-ATPase inhibitors thapsigargin or DTBHQ resulted in selective disappearance of the fast pattern and amplification of the slow one. Islets exhibiting only the slow oscillations reacted to low concentrations of glucagon with induction of the fast pattern and sometimes the typical mixed one. Also the glucagon-induced fast oscillations were counteracted by DTBHQ. In the absence of added glucagon the fast oscillations decreased with the duration of islet culture and were more common in large islets. Since glucagon-releasing α-cells are more abundant in large islets, the fast oscillations seem to require the presence of this cAMP-elevating hormone. We have recently demonstrated that cAMP amplifies intracellular Ca^{2+} mobilization evoked by inositol 1,4,5-trisphosphate. The data indicate that glucagon-facilitated mobilization of intracellular Ca^{2+}, with or without capacitative Ca^{2+} influx, is a determinant for the glucose-induced electrical activity of β-cells located within pancreatic islets.

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THE MECHANISM BY WHICH NALIDIXIC ACID INHIBITS INSULIN RELEASE FROM RAT PANCREATIC ISLETS.

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We previously reported that the hypoglycemic effects of new quinolone antibiotics might be ascribed to an increase in Ca influx. In the present study, we studied possible effects on insulin release of the oldest quinolone, nalidixic acid (NA), which was reported to induce hyperglycemia. Rat pancreatic islets were isolated by collagenase digestion and incubated for 1 h. When the effects of NA (1-1000 uM) on insulin release induced by 10 mM glucose was examined, the maximal inhibition (ca. 50 % of the control, $p<0.01$) was observed at 0.1 mM. Either an increase in glucose concentration from 10 to 20 mM or addition of forskolin (5 uM) or TPA (100 nM) completely antagonized the inhibitory effect of NA. At 3 mM glucose, TPA (100 nM) induced insulin release, which was not affected by 0.1 or 1 mM NA. In conrast, high K (25 mM) induced insulin release, which was augmented by NA (0.25-1 mM) dose-dependently. The oxidation of D-[U-^{14}C] glucose was not affected by 0.1 mM NA at 3 or 10 mM glucose. At 10 mM glucose, cyclic AMP accumulation was inhibited by 17 % ($p<0.01$) with 0.1 mM NA. This inhibition was disappeared when glucose concentration was increased to 20 mM. In the presence of 0.25 mM diazoxide and 25 mM K, insulin release induced by 10 mM glucose was doubled compared to that observed in the usual KRB buffer. The release was not affected with 0.1 mM NA but inhibited by 20% with 1 mM NA. These data suggest that NA inhibits insulin release at the step(s) distal to an increase in cytosolic Ca concentration and that it may be due to inhibition of adenylate cyclase and protein kinase C.

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SPECIFICITY OF CAPACITATIVE Ca^{2+} ENTRY INTO β-CELLS

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Capacitative influx of Ca^{2+} is believed to be important in the signal transduction of pancreatic β-cells. We have now used the fluorescent indicator fura–2 to characterize the specificity of this capacitative Ca^{2+} entry pathway. To avoid interference with voltage-dependent Ca^{2+} entry the cells were hyperpolarized with 400 μmol/l diazoxide and the channel blocker methoxyverapamil was also present in some experiments. The cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) of the hyperpolarized mouse β-cells was strikingly resistant to changes in external Ca^{2+}. In cells exposed to 20 mM glucose, stimulation with 100 μmol/l carbachol induced an initial $[Ca^{2+}]_i$ peak followed by a sustained increase due to capacitative influx of the cation. Capacitative influx was also permanently induced by the intracellular Ca^{2+}-ATPase inhibitor thapsigargin. In the presence of capacitative influx $[Ca^{2+}]_i$ became markedly sensitive to variations in external Ca^{2+}, but this sensitivity was blocked by La^{3+}. In β-cells exposed to both Ca^{2+} and Mn^{2+} there was slow Mn^{2+} quenching of the fura–2 fluorescence, which was accelerated upon stimulation of capacitative influx. In β-cells equilibrated in 5 mmol/l Sr^{2+}, carbachol exposure resulted in a pronounced $[Sr^{2+}]_i$ peak due to intracellular mobilization but little or no sustained elevation. Moreover, after activation of the capacitative pathway by exposure to thapsigargin, variations in extracellular Sr^{2+} in the 0–20 mM range had only marginal effects on $[Sr^{2+}]_i$. β-cells equilibrated with Sr^{2+} exhibit almost normal signaling due to influx through voltage-dependent channels and release from internal stores. However, since there are some intriguing differences compared to Ca^{2+}, Sr^{2+} may become an important experimental tool for elucidating the role of the capacitative pathway in β-cell signal transduction.

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THE ANTIMALARIAL DRUGS MEFLOQUINE AND HALOFANTRINE INHIBIT ATP-SENSITIVE POTASSIUM CHANNELS

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The cinchona alkaloids quinine and quinidine are known to cause hyperinsulinaemic hypoglycaemia. In a recently-published placebo-controlled volunteer study, the commonly-used quinoline methanol antimalarial drug mefloquine also reduced plasma glucose and increased serum insulin significantly (Davis TME *et al.*, B J Clin Pharm 1996;42:415-421). The aim of the present study was to investigate whether the cellular mechanism of action of mefloquine and the chemically related phenanthrene methanol drug halofantrine on pancreatic beta cells is through closure of ATP-sensitive potassium (K-ATP) channels. Experiments were carried out using the cloned beta cell K-ATP channel which comprises two subunits (Kir6.2 and the sulphonylurea receptor SUR1). Both subunits were co-expressed in *Xenopus* oocytes and K-ATP currents examined in giant inside out patches. The intracellular membrane surface was exposed to a range of drug concentrations and the vehicle alone. Mefloquine at 10 μM inhibited K-ATP channel currents by a mean ± SEM of 80 ± 4% (n=9). Halofantrine (10 μM) inhibited activity by 34 ± 2% (n=5). The chemically unrelated sequiterpene lactone antimalarial artesunate had little inhibitory effect at 10 μM (7 ± 2%, n=5). The drug vehicle was without effect. These data show that, consistent with the effects of cinchona alkaloids, mefloquine and halofantrine close pancreatic beta cell K-ATP channels. In certain clinical situations, this may predispose to hypoglycaemia through enhanced insulin secretion.

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INSULIN SECRETION IS DOMINATED BY COMPOUND EXOCYTOSIS IN MOUSE β-CELLS.

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The aim of this study was to correlate changes in cell capacitance with amperometric detection of secretion in mouse pancreatic β-cells. We used amperometry to detect 5-hydroxytryptamine (5-HT) which is taken up by the β-cell granules and co-secreted with insulin. Glucose induced electrical activity which was accompanied by spikes of 5-HT release. Voltage-clamp pulses initiated 5-HT release within 100-400 ms of the onset the depolarisation. Peak 5-HT levels were attained ≈200 ms after the commencement of the response although secretory events could be observed several seconds after the end of the stimulation. Capacitance increases occurred simultaneously with and were proportional to the 5-HT responses. The basic secretory event seen in conjunction with 5-HT release was a step increase followed by an mono-exponential decline. There was an easily detectable minimum size of these events which compares favourably to what can be estimated for the release of a single vesicle. A typical 5-HT transient had a jagged appearance resulting from the summation of several basic secretory events of various size and latency time. Both single and aggregates of vesicles appear to participate in the secretory response. The vesicle aggregates contributed with >90% to the response. In ≈25% of the cells, 5-HT release occurred without any simultaneous increase in cell capacitance thus indicating that endocytosis and exocytosis occur simultaneously. Regions of the cell which were rich in granules (as indicated by quinacrine fluorescence) exhibited, on average, a 40% larger 5-HT response than regions poor in granules. Our data suggests that amperometric detection of secretion, when combined with capacitance measurements, provides useful additional information on the processes involved in insulin secretion.

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Direct stimulation of insulin release from the MIN6 ß cell line by a new imidazoline compound, S-21663, via an original site

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The MIN6 cell line derived from *in vivo* immortalized insulin-secreting pancreatic ß cells was used to study the insulin-releasing capacity and the mode of action of S-21663, a newly synthesized imidazoline compound known for its hypoglycemic effect *in vivo* and its ability to release insulin from perfused pancreas. S-21663 (10^{-5} M to 10^{-3} M) released insulin from the MIN6 cells with a maximal effect observed at 10^{-4} M; a drop in the stimulation factor was noted between 10^{-4} and 10^{-3} M. Its efficacy, which did not differ whatever the glucose concentration (stimulatory or not), was higher than that of the other secretagogues tested (glucose, sulfonylureas or the peptide tGLP-1). In contrast to tGLP-1, S-21663 did not modify the cyclic AMP content. On the other hand, it increased Ca^{2+} influx *via* verapamil- and nifedipine-sensitive voltage-dependent calcium channels, the insulin release being a direct consequence of this Ca^{2+} entry. The S-21663-induced Ca^{2+} influx appears to be the consequence of a closure of potassium channels distinct from the K-ATP channels, as determined by measurement of ^{86}Rb efflux and use of a K-ATP channel opener. Considering the actions of S-21663 on insulin release and ion fluxes as compared to that of other secretagogues, including efaroxan (an imidazoline compound which was shown to act on insulin release in a glucose-dependent manner *via* a binding site distinct from the imidazoline I_1 and I_2 sites), we conclude that S-21663 acts through a novel site linked to an original intracellular pathway. The unique features displayed by S-21663 (an efficient secretagogue acting through a novel site linked to non-K-ATP K^+ channels) indicate that this compound, or new drugs derived from it, might be the basis for a new type of pharmacological management of NIDDM.

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OSCILLATORY SIGNALLING FOR INSULIN RELEASE IN MAN

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Single human islets are known to respond to glucose with oscillations of cytoplasmic Ca^{2+} and insulin release. So far there is no evidence for a corresponding rhythmicity in unequivocally identified human β-cells. In the attempts to characterize signalling of importance for pulsatile release of insulin in man advantage was taken of the Ca^{2+} analogue Sr^{2+}, which is particularly useful for demonstrating oscillatory activities in islet cells. Dual wavelength fluorometry with the indicator fura-2 was used for measuring cytoplasmic Sr^{2+} in β-cells taken from cadaveric organ donors and identified by immunostaining for insulin at the end of the experiments. Glucose-induced slow oscillations (frequency 0.1-0.5/min) were sometimes seen in individual β-cells already at a concentration of 3 mmol/l of the sugar. Addition of 10 nmol/l glucagon resulted in a broadening of the oscillations and sometimes in their transformation into sustained increase. Moreover, the presence of glucagon resulted in the appearance of short transients of Sr^{2+}, which disappeared after exposure to the intracellular Ca^{2+}-ATPase inhibitor thapsigargin. Digital image analyses indicated that the slow oscillations are synchronized among cells in small aggregates and entire islets. Measuring insulin in the perifusate from single islets with a sensitive ELISA technique the slow oscillations were found to correspond to pulsatile release of the hormone. It is concluded that the human β-cell has similar type of oscillatory signalling for insulin release as observed in experimental animals.

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THE SIGMA RECEPTOR LIGAND (+)-3PPP EXHIBITS IMIDAZOLINE-LIKE INSULIN SECRETAGOGUE ACTIVITY IN RAT ISLETS OF LANGERHANS

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The insulin secretagogue activity of imidazoline compounds is mediated by an imidazoline receptor, that is functionally associated with K-ATP channels. This receptor has eluded complete characterisation but recently, it has been suggested that it may be similar to the sigma-2 site found in brain. Thus, we have studied the effects of sigma receptor ligands on insulin secretion to investigate the involvement of sigma receptors in the insulin secretagogue activity of imidazolines. In contrast to imidazoline secretagogues, the potent sigma ligands haloperidol and ifenprodil markedly inhibited glucose-stimulated insulin release. Three other compounds, ketamine, phencyclidine and (+)-MK801, partially reversed the inhibitory action of diazoxide on glucose-induced insulin release (a characteristic of imidazoline agonists acting at the islet imidazoline receptor), despite exerting slight inhibitory effects on secretion alone. However, one compound, (+)-3PPP ((R)-3-(3-hydroxyphenyl)-N-propylpiperidine hydrochloride), reported to be a sigma receptor agonist, did not inhibit glucose-induced insulin release, but rather, it potentiated the insulin secretory response of islets incubated at 6mM glucose, in a dose-dependent manner. (+)-3PPP was also able to reverse the effects of diazoxide on insulin release. Significantly, both the direct stimulatory effect on secretion (6mM glucose: 2.38 ± 0.29 ng/islet/h; + 100μM (+)-3PPP: 5.46 ± 0.57; + (+)-3PPP + 50μM KU14R: 3.55 ± 0.37* (7); * P<0.01 relative to (+)-3PPP alone) and the reversal of diazoxide (20mM glucose plus 200μM diazoxide: 0.65 ± 0.07; + 100μM (+)-3PPP: 3.41 ± 0.35; + (+)-3PPP + 50μM KU14R: 2.33 ± 0.19* (12): *P<0.01 relative to (+)-3PPP alone) by (+)-3PPP were antagonised by the specific imidazoline secretagogue antagonist KU14R. Thus, (+)-3PPP appears to behave like an imidazoline secretagogue in isolated rat islets. The results with (+)-3PPP suggest that the islet imidazoline receptor may share certain pharmacological characteristics with the sigma receptor found in brain.

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UNMASKING OF GLUCOSE-REGULATED Ca^{2+} TRANSIENTS IN PANCREATIC β-CELLS BY REPLACING K^+ WITH Cs^+

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The glucose-induced movements of Ca^{2+} resulting in insulin release are known to depend on the K^+ permeability of the pancreatic β-cell. We have examined how equimolar replacement of 5.9 mmol/l K^+ with its less permeable analogue Cs^+ affects the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) in isolated mouse β-cells loaded with the fluorescent indicator fura-2. Whereas the presence of Cs^+ did not affect $[Ca^{2+}]_i$ in resting β-cells, it transformed the slow $[Ca^{2+}]_i$ oscillations seen at 11 mmol/l glucose into sustained elevation. When Cs^+ was allowed to equilibrate with the β-cells during 40 min in the presence of 3 mmol/l glucose, a subsequent rise of the sugar to 5-7 mmol/l resulted in sustained elevation of cytoplasmic Ca^{2+} with initial 5-10 min periods of superimposed transients (frequencies 3.4 ±0.4/min). These pronounced transients persisted after depletion of intracellular Ca^{2+} stores by thapsigargin. However, after elevation of glucose from 3 to 20 mmol/l there was sustained elevation of $[Ca^{2+}]_i$ without transients. Both the sustained elevation and the transients were immediately eliminated by blocking the L-type Ca^{2+} channels with 50 µmol/l methoxyverapamil. It is suggested that the short transients reflect the depolarisation-dependent entry of Ca^{2+} in response to the reduction of K^+ permeability obtained by Cs^+ and glucose. When sufficiently pronounced the depolarisation will counteract the Cs^+ blockage, explaining the gradual disappearance of the Ca^{2+} transients and their absence at 20 mmol/l glucose.

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THE IMIDAZOLINE RX871024 AND THE SECRETIN GLP-1 INDUCE GLUCOSE DEPENDENT INSULIN SECRETION IN βTC-6 CELLS

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The βTC-6 cell is a glucose sensitive insulin secreting clonal line of pancreatic islet ß-cells derived from transgenic mice. Modulators of insulin secretion like the imidazolines phentolamine and RX871024 (RX) as well as the glucagon-like peptide-1 (GLP-1) were studied with respect to their glucose dependent insulin secreting properties. In the absence of glucose 100 µM RX induced a 1.9-fold insulin secretion in βTC-6 cells ($p<0.05$). However, in the presence of 10 mM glucose the secretion of insulin was concentration-dependently enhanced by RX (EC_{50} = 14.2 µM) which was 2.8-fold ($p<0.05$) at 100 µM RX. This RX induced insulin secretion could be attenuated by the addition of α_2-adrenergic agonists like epinephrine and UK 14,304 ($p<0.05$). In the presence of 150 µM diazoxide the RX stimulated insulin secretion in the absence of glucose was reduced about 50% ($p<0.05$). In the presence of stimulatory concentrations of glucose, however, the RX induced insulin secretion was not influenced by diazoxide. GLP-1 (7-37) was more potent (EC_{50} = 0.9 nM) compared to RX with respect to insulin secretion in βTC-6 cells. In the presence of 10 mM glucose 30 nM GLP-1 increased the insulin secretion about 1.9-fold ($p<0.05$). Thus, the maximal stimulation obtained with GLP-1 was about 2-fold lower compared with the maximal effect obtained with the imidazolines phentolamine and RX ($p<0.05$). Insulin secretion stimulated by glucose, RX and GLP-1 was markedly increased in the presence of 25 µM of the PDE inhibitor IBMX. Thus, cAMP may be a common intracellular mediator in the process of insulin secretion of βTC-6 cells. In conclusion, the insulin secretion stimulated by RX is dependent on the glucose concentration used and shows striking similarities to GLP-1 induced insulin secretion. This indicates a second mechanism of action of RX despite blockade of ATP-sensitive K^+-channels. The data presented may provide further insight into the mechanism of the insulinotropic effect of RX and other imidazolines.

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OSCILLATIONS IN CYTOSOLIC CA^{2+} BY GASTRIN RELEASING PEPTIDE IN SINGLE INSULIN PRODUCING HIT-T15 CELLS

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Gastrin releasing peptide (GRP) occurs in islet nerves and stimulates insulin secretion by a phospholipase C-dependent mechanism. The effect of the neuropeptide on cytosolic Ca^{2+} ($[Ca^{2+}]_{cyt}$) is, however, not established. We therefore investigated the effect of GRP on $[Ca^{2+}]_{cyt}$ in FURA-2-loaded, superifused, single insulin-producing HIT-T15 cells at 3.3 mM glucose. GRP (100 pM) induced an initial peak in $[Ca^{2+}]_{cyt}$ followed by either a sustained increase (44% of cells) or a variable pattern of oscillations (56% of cells). GRP-induced oscillations had a frequency of 1.1±0.1 per min (range 0.63-1.9) and an amplitude of 113±11 nM (range 33-250 nM; n=31). When extracellular Ca^{2+} was chelated by EGTA (2 mM) during GRP-induced oscillations, one transient of $[Ca^{2+}]_{cyt}$ still appeared, whereafter oscillations stopped but reappeared promptly when extracellular Ca^{2+} was normalized. EGTA added to cells exhibiting a GRP-induced sustained increase in $[Ca^{2+}]_{cyt}$ reversibly lowered the $[Ca^{2+}]_{cyt}$. Depletion of intracellular Ca^{2+} stores by the Ca^{2+} ATPase-inhibitor thapsigargin (1 µM) stopped GRP-induced oscillations. Similarly, the protein kinase C activator 12-O-tetradecanoyl-phorbol-13-acetat (TPA; 20 nM) markedly reduced the frequency of GRP-induced oscillations (0.14±0.1 per min during TPA+GRP compared to 1.05±0.3 per min during GRP alone, $P<0.05$). It is concluded that **1)** GRP induces oscillations in $[Ca^{2+}]_{cyt}$ in a majority of HIT-T15-cells, whereas other cells respond with a sustained increase in $[Ca^{2+}]_{cyt}$ **2)** the oscillations are caused by repetitive mobilization of Ca^{2+} from intracellular stores but extracellular Ca^{2+} is required to maintain oscillations **3)** the GRP-induced sustained increase in $[Ca^{2+}]_{cyt}$ is dependent on Ca^{2+}-uptake **4)** the frequency of GRP-induced oscillations is negatively modulated by direct protein kinase C activation. Hence, two major separate actions are exerted by GRP on $[Ca^{2+}]_{cyt}$ in insulin producing cells, the nature of which is determined by the individual cell.

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Insulin Secretion in Vitro: Secretagogue Recognition and Signal Transduction

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INSULIN RELEASE AND GENE EXPRESSIONS OF INSULIN II AND GLUT2 ARE DIFFERENTLY MODULATED BY GLUCOSE EXPOSURE IN INS1 CELLS

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We have previously shown that long term exposure to high glucose down regulates the gene expressions of GLUT2 and insulin as well as the glucose stimulated insulin secretion in the glucose sensitive β cell-line INS-1. The aim of the present study was to detect if there was a differential impact on the gene expression of GLUT2 and insulin II as compared to insulin release of 1 and 4 days exposure to a graded range of glucose levels between 1.0 and 26.6 mM. Cells were cultured in a modified RPMI 1640 medium containing the different glucose levels. Total RNA was analysed by northern blotting and insulin release analysed by RIA. Students unpaired one-tailed t-test was used to test the degree of significance. After 1 day we found no difference in the GLUT2 gene expression in cells cultured at this broad range of glucose. The amount of insulin II mRNA was equally high at glucose levels between 1.0 and 13.3 mM whereas higher glucose caused a down regulation of insulin II gene expression.This contrasted to the uniform high insulin release at glucose concentrations at or above 6.6 mM and the reduced insulin release found in cells cultured at 3.3 mM glucose or less ($p<0.0002$). After 4 days GLUT2 gene expression was down regulated in cells cultured at 13.3 mM glucose or more. The amount of insulin II mRNA was lower at glucose levels below 6.6 mM or above 13.3 mM than to the expression found at concentrations in between. This is in agreement with the pattern of insulin release. Thus the maximal insulin release appeared at 6.6 mM glucose being reduced at lower ($p=0.0000$) as well as at 10 mM ($p<0.02$) or higher glucose concentrations ($p=0.0000$). **In conclusion:** The insulin release surprisingly correlates better to the amount of GLUT2 mRNA than to the amount of insulin II mRNA at glucose levels higher than 6.6 mM. In contrast, the insulin release corresponds better to the level of insulin II mRNA than to the GLUT2 mRNA level at low glucose concentrations as expected. This points to a putative importent role for GLUT2 in the development of glucose desensitization.

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FUNCTIONAL AND IMMUNOHISTOLOGICAL SPECIES SPECIFICITY OF $G\alpha_{olf}$ AND $G\alpha_i$ EXPRESSION IN PANCREAS, ISLETS AND ß-CELLS

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Proteins Gα are part of trimeric G proteins which transduce the regulatory signals of the pancreatic islet B cell function. Our aim was to evidence in different rodent species and B cell lines, the presence of $G\alpha_{olf}$ and of $G\alpha_i$ isotypes likely to be coupled to adenylate cyclase stimulation and inhibition. Immunodetection studies were coupled to western blot analysis and ADP-ribosylation by cholera toxin (CTX) or pertussis toxin (PTX). The presence of $G\alpha_{olf}$ was doubly checked by antibodies raised against 23-38 (Y27) or 100-118 (SC) sequences, and that of $G\alpha_{i3}$ by antibodies against 345-354 (SC) or 105-127 (Y45) sequences. Specific immunofluorescence of α_{olf} was localized in the B cell lines HIT-T15 and RINm5F and almost exclusively in B cells of rat, mice, hamster and human and in endothelial cells of small vessels. Some α-cells are slightly labeled. By western blot only one or two bands in the 43-48 kD zone were detected in islets and B cell lines, with differences according to the species of the animals or the passages of the cell lines. The same band was detectable in neonatal rat pancreatic membranes due to the abundance of islets. $G\alpha_s$ was present in both exocrine and endocrine pancreas. Specific bands were detected in hamster islets and rat islets at the expected place at 52 and 46 kD, with additional bands in the 28-30 and 18-20kD zones. After CTX the [^{32}P]-labeled bands corresponded to the major immunoexpression of $G\alpha_{olf}$ and α_s with some additional labeling above 80 Kd or around 28kD. Gαi was almost exclusively present in B cells and the $G\alpha_{i3}$ isotype was found in normal B cells and HIT cells. This study indicates that the diversity of the functional α subunits present in normal ß-cells is shared by different mammal species and that, their specific role in the answer of B cells remaining unknown.

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THE CALCIUM SIGNAL TRANSDUCTION PATHWAY REGULATES CRE-BP1 TRANS-ACTIVITY.

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We have previously shown that transcription factor CRE-BP1 activates insulin gene expression. In this study we investigate the regulatory mechanism of CRE-BP1 trans-activity. Fusion proteins of the GAL4 DNA-binding domain and the CRE-BP1 activation domain (amino acids 1-345) were introduced with expression plasmids encoding the catalytic domains of protein kinase A (PKA), calmodulin-dependent protein kinase II (CaMKII), or calmodulin-dependent protein kinase IV (CAMKIV). The 5xGAL4-luc reporter plasmid contains tandem repeats of the GAL4-binding site and the coding sequence of luciferase. CaMKIV alone activated CRE-BP1 trans-activity 4.7-fold. CRE-BP1 seems to be phosphorylated by CaMKIV on two closely spaced threonine residues (Thr-71 and Thr-73), since replacement with alanine inhibited the trans-activity of CRE-BP1. Moreover, co-transfection of CBP or p300 co-activator increased the transcriptional activity of CRE-BP1 further. These results suggest that the increased intracellular calcium ion concentration elicited by glucose might activate CaMKIV and CRE-BP1, which in turn increase insulin gene expression through their association with CBP and p300 co-activator.

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FUNCTIONAL AND MOLECULAR CHARACTERIZATION OF B-CELL $G\alpha_{olf}$ GENE USING OLIGO ANTISENSE

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We have undertaken molecular characterization and functional studies of $G\alpha_s$ and $G\alpha_{olf}$ in various B cell lines and pancreatic tissues. Antisense (-36-1, upstream of ATG) for in situ hybridization (ISH) were used in combination with specific antibodies raised against the 100-118 sequence for western blot to comprehend the $G\alpha_{olf}$ topology from mRNA to functional protein. The validity of the probes was attested by its specific expression in adult rat striatum. Here, we show that $G\alpha_{olf}$ mRNA was expressed in rat pancreatic B cells by ISH using the digoxigenin-labeled antisense probe. This further extend our previous results demonstrating the presence of the $G\alpha_{olf}$ protein in pancreatic B cell obtained by optical and ultrastructural studies. We next assessed the effectiveness of the antisense using HIT-T15 cells in which $G\alpha_{olf}$ protein was expressed. Fifteen bases of the phosphothioate specific 5' noncoding region covering the ATG translation initiation codon of $G\alpha_{olf}$ as antisense templates were selected. Antisense RNA blocks the expression of the targeted protein by hybridizing $G\alpha_{olf}$ mRNA, preventing translation. Antisense and sense oligonucleotides were transfected into HIT-T15 cells for 72 hours by simple diffusion or lipofection and tested for $G\alpha_{olf}$ expression by immunoblotting. Cells containing antisense $G\alpha_{olf}$ showed a decrease in the expression of $G\alpha_{olf}$ when compared to either the control cells or cells containing sense oligonucleotides. Staining of immunoblot with specific antibody to $G\alpha_s$ demonstrated that this inhibition was specific to $G\alpha_{olf}$ in HIT-T15 cells. Immunofluorescence of $G\alpha_{olf}$ antibody was also shown to be much lower in antisense-transfected cells. Further studies are undertaken to elucidate the role of $G\alpha_{olf}$ protein in the control of the pancreatic B cells development and in the regulation of insulin secretion with the prospect to obtain a B-cell line where the $G\alpha_{olf}$ antisense will be permanently expressed.

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β CELL DYSFUNCTION IN THE MOUSE WITH THE β CELL-SPECIFIC GLUCOKINASE GENE DISRUPTION.

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The role of glucokinase (GK) in the pancreatic β cell was systematically examined in the heterozygous mouse with targeted disruption of the β cell-specific GK gene at the age of 20-40 weeks. The heterozygotes were with significant hyperglycaemia (the mean random sample plasma glucose, 13.3 mM) with normoinsulinaemia and with normal body weight; the microscopic appearance and insulin content of the islets were unchanged. In the heterozygotes' islets, both the first and second phases of insulin release was reduced upon stimulation with 16.7 mM glucose; when the concentration-dependency was examined with 3-40 mM glucose, the EC50 was elevated with the maximum response being unaltered. The K^+ATP channel-independent glucose action was exaggerated, if any, in the heterozygotes' islets, however, the EC50 was elevated in this branch too. Discrimination of α and β glucose was impaired in the heterozygotes' islets. A daily sc insulin injection for 2 weeks resulted in modest but significant lowering of plasma glucose in the heterozygotes, which was accompanied by a dramatic depression of glucose-stimulated insulin release by the islets. The data is compatible with the GK glucose sensor concept inasmuch as glucose sensitivity is reduced in the heterozygotes' β cell. Preservation of K^+ATP channel-independent glucose action and anomeric malaise in the heterozygotes' β cell are likely due to chronic hyperglycaemia.

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EFFECT OF ISLET CATECHOLAMINE RELEASE ON INSULIN SECRETION

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The presence of catecholamines (CAs) as well as the enzymes involved in their synthesis (tyrosine - hydroxylase [TH] and dihydroxyphenyl- alanine [DOPA]-decarboxylase) suggest that CAs can be synthesized by insular cells. Furthermore, we have previously shown that normal adult rats fed for a week only with carbohydrates increased the TH activity and decreased the norepinephrine (NE) content in their pancreatic islets, together with a significant reduction in the release of insulin in response to glucose. The aim of this work was to study the role of endogenous CAs on the control of insulin secretion. Thus, we studied the release of DOPA, dopamine (D), and NE (measured by HPLC) as well as of insulin (measured by radioimmunoassay) in response to glucose (3 or 16 mM) in islets isolated from normal rats. Insulin secretion was also studied in islets incubated with 3 or 16 mM glucose in the absence or presence of α_2 (yohimbine [Y], 0.01 to 1 μM), and α_1 (Prazocin [P], 0.01 to 0.5 μM) adrenergic receptor antagonists. Medium DOPA concentration was lower in islets incubated at a high glucose concentration (313 vs 1.0 pg/ml), whereas D and NE concentrations were higher at 16 mM glucose than in those islets incubated at a low glucose concentration (D: 70.4 vs. 133.2 pg/ml; EN: 28.6 vs. 75.0 pg/ml). Islets incubated in the presence of Y significantly enhanced the insulin response to high glucose (Y = 0.1 μM: 14.31 ± 2.71; 1 μM: 21.32 ± 2.19 vs. control : 7.85 ± 1.21 ng/islet/h, $p < 0.05$);conversely, the addition of P in the incubation media significantly diminished insulin secretion in the presence of 16 mM glucose (P= 0.1 μM: 3.98 ± 0.63; 0.5 μM:3.41 ± 0.34 vs. control: 7.37 ± 0.87 ng/islet/h, $p<0.025$).These results suggest that endogenous CAs can be synthesized and released by insular cells in response to glucose, contributing to the paracrine regulation of insulin secretion.

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EXPRESSION OF GLUCOKINASE IN GLUCOSE-UNRESPONSIVE HUMAN FETAL ISLET-LIKE CELL CLUSTERS

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Glucokinase (GK) is the glucose sensor in the adult β cell resulting in fuel for insulin synthesis and secretion. Defects in this enzyme in the β cell are responsible for the genetic disorder maturity-onset diabetes of the young (MODY) with the β cell being unable to secrete insulin appropriately when challenged with glucose. The human fetal β cell is also unable to secrete insulin when exposed to glucose but whether GK is present and functional in this developing cell is unknown. To determine the expression of GK in human fetal pancreatic tissue, cytosolic protein was extracted from human fetal islet-like cell clusters (ICCs) at 17-19 week gestation and examined for protein content and enzyme activity. On Western blots a single band corresponding to GK was seen at 52-kDa, and this was similar in amount to that obtained from human adult islets. GK was measured fluorometrically, the Vmax being less in fetal ICCs than adult islets: 8.7 vs 20.7 nmol/mg protein/h; similar Km values were found in both ICCs and islets. No attempt was made to determine which cells in an ICC contained GK. Glucose utilization was determined radiometrically, the Vmax of the high Km component being less in ICCs than islets: 31.3 pmol/ICC/h vs 101.4 pmol/islet/h. Culturing of ICCs for 3-7 days in medium containing 11.2 mmol/l glucose resulted in a 3.7-fold increase in the Vmax of GK and a 1.8-fold increase in glucose utilization. These enhanced activities of glucose phosphorylation and glycolysis, however, did not lead to the β cell being able to secrete insulin when exposed to glucose. In conclusion, glucokinase is present and functional in human fetal ICCs but the inability of the human fetal β cell to secrete insulin in response to an acute glucose challenge is not due to immaturity of this enzyme.

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Effect of Neuroendocrinological and Second Messenger Signals on Insulin Secretion in ßTC Cells.

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Clonal beta cell lines, such as ßTC6-f7 cells, that maintain glucose sensitive insulin secretion allow a greater understanding of the cellular mechanisms that induce glucose potentiated insulin release (GPIR). We have characterized the effect of selective agonists and antagonists at the histamine, serotonin, dopamine, acetylcholine, CCK, GLP-1, ß-adrenergic, and α-adrenergic receptors, in addition to intracellular second messenger activity such as arachidonic acid metabolites on GPIR. Insulin secretion (IS) from cultured βTC6-f7 cells (passage 48-52) was quantified using a scintillation proximity assay (SPA). Conditions that simulate low (0 mM glucose 25 μM IBMX) and high (10 mM glucose and 25 μM IBMX) glucose were used to obtain dose response relationships for each agent and compared in each assay with the IS induced by the imidazoline RX871024 (RX). Some of the most interesting responses were: chlorpheniramine, an H-1 antagonist, induced a GPIR (EC50 = 23 μM, 2.5 fold increase in IS at the maximal dose 100 μM) comparable to RX (EC50 = 21 μM, 2.5 fold increase in IS at the maximal dose 100 μM). Additional H agonists and antagonists will be discussed. 3-PPP, a dopamine agonist, induced a lower maximal GPIR (2 fold) and higher EC50 (40 μM) than RX, 6-7 ADTN, a dopamine agonist inhibited IS. Muscarinic agonist, APE, had a five fold lower EC50 (4 μM) and was glucose sensitive. Atropine blocked this effect and by itself did not have an agonistic or antagonistic effect on IS. Alprenolol, a ß-adrenergic antagonist, induced GPIR similar to RX. GLP-1 has a nM activity (EC50=5 nM) although a small fold induction (1X) of IS in this cell line. Sulfonylureas. CCK. prostaglandins have also been examined and dose response GPIR for these agents will be discussed.

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DISSOCIATED EFFECTS OF 3-O-METHYL-D-GLUCOSE UPON D-GLUCOSE UPTAKE, METABOLISM AND INSULINOTROPIC ACTION IN RAT PANCREATIC ISLETS

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The transport of D-glucose across the B-cell plasma membrane is currently considered not to represent a rate-limiting factor in the metabolism and, hence, insulinotropic action of the hexose. In the present study, however, 80 mM 3-O-methyl-D-glucose (3OMG) was found to inhibit insulin release evoked, over 90 min incubation, by D-glucose. This effect, which was concentration-related in the 30 to 80 mM range, was not reproduced by 80 mM D-fructose or D-galactose. Moreover, 3OMG failed to affect insulin release evoked by 2-ketoisocaproate (10 mM) or the association of L-leucine and L-glutamine (10 mM each). In islets exposed for 90 min to 16.7 mM D-glucose, 3OMG also decreased both D-[5-^{3}H]glucose utilization and D-[U-^{14}C]glucose oxidation. The fractional inhibition of insulin release, relative to that of glucose metabolism, was comparable in islets exposed to either 3OMG or D-mannoheptulose (1 mM). In perifused islets exposed to a sudden rise in D-glucose concentration, 3OMG delayed the early secretory response, which was more markedly affected than the second phase of insulin release. Yet, whether over 5 or 20 min incubation, 3OMG failed to decrease, and even slightly increased, the radioactive intracellular content of islets exposed to 16.7 mM D-[U-^{14}C]glucose. Likewise, D-glucose (16.7 mM) tended to augment the radioactive intracellular content of islets exposed to 80 mM 3-O-[^{14}C-methyl]-D-glucose. The phosphorylation of D-[U-^{14}C]glucose by islet homogenates was slightly but significantly decreased, however, by 3OMG. These findings suggest that, except during the early secretory phase, the inhibition by 3OMG of glucose-stimulated insulin release is not merely attributable to an impaired hexose transport into the B-cell.

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1,25-DIHYDROXYVITAMIN D_3 INFLUENCE ON ISLETS INSULIN RELEASE AND PKC PATHWAY IN VITAMIN D_3 DEFICIENT RATS.

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As previous works with islets from normal (N) rats and 4 week-vitamin D_3 deficient rats (-D) showed a more rapid stimulatory influence of 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] on insulin response to acetylcholine (Ach) than to glucose stimulus, we studied the in vitro insulin release (RIA assay) in similar conditions, taking into account the Ach-induced activation of the PKC pathway. A 45 min induction time of 10^{-8} M 1,25$(OH)_2D_3$ induction was not sufficient to improve the insulin response over basal release to 16.7 mM glucose of 50 perifused islets from -D (+0,69±0,15 vs N rats: +2,88±0,25 ng/min ; n=7; p<0.001), whereas the insulin response to glucose could be restored when same groups of islets were first submitted to both influences of 1,25$(OH)_2D_3$ and 10 µM Ach before glucose stimulation whatever it concentration was: 16.7, 11.1 or 8.3 mM. When -D islets were pre-exposed to 10^{-8} M 1,25$(OH)_2D_3$ and 8.3 mM glucose before a stimulation by Ach, their insulin release increased, especially during the PKC-dependent 2nd phase of Ach stimulation. This potentiating effects could be suppressed by the addition of H_7, a PKC inhibitor (+0,60±0.06 vs +1.44±0.19 ng/min without H_7; n=7; p<0.001). Prior exposure of -D islets to 8.3 mM glucose, 10 µM Ach and 50 µM MOG (a diacylglycerol kinase inhibitor) rendered glucose much more efficient on insulin release from islets preexposed to 10^{-8} M 1,25$(OH)_2D_3$ (+0,38±0.07 ng/min; n=8) versus control islets (+0.13±0.04 ng/min; n=8 ; p<0.01) with 10^{-12} M inactive concentration of the steroid. These results sustained the hypothesis that the beneficial influence on insulin release of prior exposure to 1,25$(OH)_2D_3$ and Ach was mediated via the activation of PKC pathway.

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EVALUATION OF THE ROLE OF GTP IN GLUCOSE STIMULATION OF INSULIN SECRETION

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Glucose metabolism in pancreatic β cells leads to an increase in the ATP/ADP ratio which may participate in the regulation of insulin secretion. We have reported recently that good correlations also exist between guanine nucleotide levels in islets and insulin secretion. However, to determine whether variations in GTP concentration play a specific role in stimulus-secretion coupling, this concentration should be modified selectively. This was attempted by culturing mouse islets overnight in the presence of mycophenolic acid (MPA), an inhibitor of GMP synthesis at the level of IMP-dehydrogenase. The drug (25-50 µg/ml) did not affect the insulin content, but decreased the GTP content of the islets (by 65 %, P< 0.001). During subsequent incubation, glucose (15 mmol/l)-induced insulin secretion was inhibited (by 50 %, P< 0.001), while basal secretion (3 mmol/l glucose) was unaffected. However, MPA also lowered the ATP/ADP ratio in the islets. Addition of guanine to the culture medium (to stimulate the salvage pathway of GTP synthesis) restored normal GTP levels corrected the ATP/ADP ratio, and partially prevented the inhibition of insulin release. In contrast, attempts to stimulate ATP synthesis specifically (by provision of adenine or adenosine) failed to reverse any of the effects of MPA. It is concluded that guanine and adenine nucleotide pools are tightly linked in islet cells and cannot be specifically affected by MPA probably because of the activity of the nucleoside diphosphate kinase and of the role of GTP in several reactions leading to adenine nucleotide generation. MPA is not an adequate tool to support or exclude a specific role of guanine nucleotides in the control of insulin release.

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AUTOANTIBODIES AGAINST CD38 (ADP-RIBOSYL CYCLASE /CYCLIC ADP-RIBOSE HYDROLASE) IN DIABETIC PATIENTS.

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Glucose is the primary stimulus of insulin secretion. We have proposed a model for insulin secretion by glucose via cyclic ADP-ribose (cADPR)-mediated Ca^{2+} mobilization in pancreatic B-cells: In the process of glucose metabolism, millimolar concentrations of ATP are generated, inducing cADPR accumulation by inhibiting the cADPR hydrolase activity of CD38, and cADPR acts as a second messenger for intracellular Ca^{2+} mobilization from the endoplasmic reticulum for insulin secretion. In the present study, we expressed human CD38 (amino acid residues 45-300) in *E. coli* and purified it to homogeneity. Using the purified CD38, we examined the presence of autoantibodies against CD38 in the sera of 466 Japanese diabetic patients and 75 controls by immunoblot. In the diabetes group, 19% of IDDM patients and 15% of NIDDM patients had autoantibodies against CD38, while only 1 in 75 (1.3%) in the control group exhibited a extremely low level of autoantibody. When sera of patients with autoantibodies against CD38 were added at 10% to the enzyme assay, the cADPR metabolizing enzyme activity of CD38 was inhibited (43-84% of control). Some sera with the anti-human CD38 antibodies reacted with recombinant rat CD38 and inhibited glucose-induced insulin secretion from rat isolated islets (44-86% of control). Therefore, it is quite possible that autoantibodies against CD38 in diabetic patients suppress the cADPR metabolizing enzyme activity of CD38 in pancreatic islets and inhibit insulin secretion to develop and exacerbate diabetes.

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MODULATION OF GLUCOSE-INDUCED INSULIN SECRETION BY CYTOSOLIC REDOX STATE IN BRIN-BD11 CELLS

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Stimulation of β-cell metabolism plays a crucial role in glucose-induced insulin secretion, but some of the underlying mechanisms remain unresolved. Previous studies either did not detect measurable effects of glucose on cytosolic NADH/NAD^+ or did detect them, albeit with a discrepant time course in relation to insulin secretion. We have now assessed the role played by cytosolic redox state in glucose-induced insulin secretion, using the novel glucose-sensitive cell line BRIN-BD11. Exposing the cells to glucose (2-16 mM) for 45min caused a dose-dependent increase in insulin release rate and lactate/pyruvate (L/P) ratio (ie. a measure of cytosolic NADH/NAD^+ ratio). Glyceraldehyde (10 mM) mimicked the stimulatory effect of 16 mM glucose on both insulin release and L/P ratio. Pre-incubating the cells with the NAD^+ precurssor niacin (100 μM) for 45 min supressed the glucose-evoked rise in the L/P ratio and inhibited (40%) the concomitant increase in insulin release. Niacin nearly suppressed the rises in cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$, assessed from single cells by fura-2 microfluorometry) evoked by acute (5 min) exposure to 16 mM glucose. Niacin had no effect on tolbutamide (100 μM) - or high K^+ (30 mM)- evoked $[Ca^{2+}]_i$ rises and insulin release. Thus, the drug did not interfere with either voltage-sensitive Ca^{2+}channels, intracellular Ca^{2+} buffering or more distal events in the stimulus-secretion coupling cascade. The data indicate that changes in cytosolic redox state of the pancreatic β-cell account for a significant part of the insulinotropic capacity of glucose, by a mechanism that may involve direct shuttling of reducing equivalents into the mitochondria.

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ANTI-CD38 ANTIBODY MODULATES INSULIN SECRETION THROUGH SOME MECHANISM OTHER THAN K-ATP CHANNEL.

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【Aim】 We made sure that cyclic ADP-ribose (cADPR; cADPR synthetase is nearly equal CD38 antigen), which recently drew the public attention as a newly second messenger of intracellular calcium regulation, had might act against the membrane potential (K-ATP channel) in the early phase of insulin secretion with patch-clamp technique. 【Method】 We practiced a patch-clamp technique (on-cell configuration) to pancreatic beta cells isolated from Wister Rat. In on-cell experiments extracellular solution (PBS: phosphate buffer solution) with or without each loaded substance (D-glucose 20mM, diazoxaide 0.3mM, glibenclamide 0.1 μ M) was applied. Two types of anti-CD38 antibodies, both anti-T10 monoclonal antibody and anti-CD38-fragment (287-297: N-CVKNPEDSSCT-C) polyclonal antibody, were preloaded for 1 hour before patch-clamp experiments. The activity of K-ATP channel was compared each open-provability value (Po value). 【Result】 The Po values were 0.057 ±0.076, 0.020±0.022, 0.070±0.072 and 0.001±0.005 in control group and 0.133±0.109, 0.011±0.017, 0.111±0.062 and 0.001±0.003 in anti-CD38 antibody preloaded group, for PBS, high glucose, diazoxaide and glibenclamide, respectively. The Po value for each loaded substance (D-glucose, diazoxaide, glibenclamide) was not distinguishable between the control group and anti-CD38 antibody preloaded group. 【Conclusion】 The effect of anti-CD38 antibody preloading on insulin secretion is not present directly against K-ATP channel. So, we guess that that effect may be present against another step of insulin secretion, for example, voltage-dependent calcium channel or mobilization of intracellular calcium or the other.

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POSSIBLE MECHANISMS OF THE K-ATP CHANNEL-INDEPENDENT STIMULATION OF INSULIN SECRETION BY GLUCOSE

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Glucose controls insulin secretion by regulating Ca^{2+} influx in B cells through changes in K^+-ATP channel activity and membrane potential, and by modulating the effectiveness of cytoplasmic Ca^{2+} on exocytosis. The second mechanism is still poorly understood and was investigated in the present study. Insulin release from mouse islets stimulated by 30 mmol/l K^+ (and 250 μmol/l diazoxide) was sustained in the presence of high glucose, but only transient in its absence in spite of a sustained rise of $[Ca^{2+}]_i$ in B cells. This progressive decline of the secretory response might suggest that the releasable pool rapidly empties unless it is refilled by glucose. However, stimulation of microtubule polymerization (taxol) or depolymerization (nocodazole) similarly decreased the secretory response to low or high glucose, without changing the pattern. Contrary to previous suggestions, the effect of glucose could not be ascribed to activation of PKC because it was resistant to three inhibitors which antagonized the effect of a phorbol ester. No evidence could be found for the intervention of a recently described ATP-sensitive phospholipase A_2, for a signal originating from the endoplasmic reticulum, or for a role of the nitric oxide pathway. In contrast, the effect of glucose was decreased by okadaic acid and calyculin A, two inhibitors of protein phosphatases which did not influence the ATP/ADP ratio. In conclusion, numerous possible mechanisms of this second effect of glucose have now been ruled out. The best correlations remain with the changes in adenine nucleotide levels. This study further indicates that phosphatases might be involved at a stage that has yet to be identified.

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NITRIC OXIDE MODULATES INSULIN AND GLUCAGON SECRETION INDUCED BY CHOLINERGIC STIMULATION

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Immunohistochemical evidence has been obtained for the occurrence of a constitutive nitric oxide synthase (cNOS) in both endocrine cells and nerves in the pancreatic islets. By a combined in vivo and in vitro approach we have studied the role, if any, played by nitric oxide (NO) in insulin and glucagon secretion induced by cholinergic stimulation. Carbachol-induced insulin release from isolated islets in the presence of basal glucose (7mmol/l) was greatly enhanced by the NOS inhibitors N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-monomethyl-L-arginine (L-NMMA). In contrast, carbachol-induced glucagon release was suppressed by the NOS-inhibitors. A similar effect by L-NAME on insulin release induced by the protein kinase C activator TPA was observed. Glucagon release was not affected. Carbachol-induced hormone release was not influenced by the enantiomer D-NAME, which is devoid of NOS inhibitory properties. Carbachol-induced insulin release from islets depolarized by 30 mmol/l K^+ in the presence of 250 μmol/l diazoxide was still increased by L-NAME, although the response was less pronounced. The intracellular NO donor hydroxylamine suppressed insulin release and enhanced glucagon release. Moreover, in vivo studies showed that the insulin secretory response to an i.v. injection of carbachol was greatly enhanced by pretreatment with L-NAME. In contrast, carbachol-induced glucagon response was suppressed by a previous injection of L-NAME. The data strongly suggest that NO is an important regulator of cholinergically stimulated insulin and glucagon secretion, being a negative modulator of insulin and a positive modulator of glucagon release. The effect on insulin release is, at least partly, independent of membrane depolarization events.

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EFFECTS OF PROTEIN KINASE ON INSULIN SECRETION VIA ATP-SENSITIVE K^+ CHANNEL-INDEPENDENT PATHWAY.

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We examined the possibility that glucose-induced insulin secretion can be elicited by protein kinase activations by a pathway independent of ATP-sensitive K^+ channels (K_{ATP} channels), using 100 μM diazoxide-hyperpolarized islets. No detectable $[Ca^{2+}]_i$ changes were observed by Fura-2 fluorometry under these conditions. Protein kinase C (PKC) activation was found to augment K_{ATP} channel-independent insulin release even at a lower glucose concentration (1.6 mM or 3.3 mM ; $p<0.01$). On the other hand, protein kinase A (PKA) activation augmented K_{ATP} channel-independent insulin release at higher glucose concentrations ($\geq$ 11.1 mM; $p<0.01$). Meanwhile, protein kinase G activation did not involve the mechanism. These facts indicate that the two mechanisms depend on phosphorylation of functionally distinct target proteins. Because mannoheptulose depressed PKC or PKA related insulin release, some intracellular metabolic signals derived from the sugar should be necessary for these pathways. This study clearly shows the existence of a novel regulatory system in pancreatic ß cell: glucose augments insulin release in a dose-dependent manner within a range of physiological concentration through a pathway independent of K_{ATP} channel closure under protein kinase activations without detectable $[Ca^{2+}]_i$ elevation.

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A ROLE FOR PARACRINE NITRIC OXIDE IN THE EARLY PHASE OF GLUCOSE-STIMULATED INSULIN SECRETION

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Nitric oxide (nitrogen monoxide, NO) acts as a signal transducer in a variety of cells. There is substantial evidence to suggest that NO could also be involved in the insulin secretory pathway of pancreatic β-cells. In this study the effect of NO on acute glucose-stimulated insulin secretion from isolated islets of Langerhans and the localization of constitutive NO-synthase were investigated. Newborn rat islets were precultured in RPMI-1640 for 5-6 days and perifused with Krebs-Ringer buffer (KRHB) at a flow rate of 600µl/min in a 300µl perifusion chamber. After an equilibration period of 45min in KRHB + 1.6mM glucose, 0.5mM L-methylarginine (L-NMMA), or 10µM carboxy-PTIO, respectively, was added to the buffer. Glucose concentration in the perifusion medium was kept at 1.6mM for another 10min, was then raised to 16mM for 40min and was finally reduced to 1.6mM for 15min. For light- and electron-microscopic studies semithin and ultrathin sections, respectively, were analyzed with anti-NO-synthase antibody and antibodies against the classical islet hormones. Perifusion in the presence of the NO synthase inhibitor, L-NMMA, resulted in an inhibition of the early phase of glucose-stimulated insulin secretion by 66%: area under the curve (AUC) 12.2±3.2pg/islet x min vs. 35.4±3.8pg/islet x min in control islets ($p<0.001$). The second phase was not influenced by L-NMMA: AUC 34.6±3.8pg/islet x min and 36.1±9.2pg/islet x min in control and L-NMMA-exposed islets, respectively. Addition of 10µM carboxy-PTIO, a NO scavenger, to the perifusion medium resulted in an inhibition by 49% of the first phase of insulin secretion. The AUC was 34.4±7.3pg/islet x min and 17.5±5.3pg/islet x min in controls and carboxy-PTIO-exposed islets, respectively ($p<0.05$). The second phase was not affected, the AUC being 38.7±17.2pg/islet x min and 34.0±15.2pg/islet x min in controls and carboxy-PTIO-exposed islets, respectively. Light- and electron-microscopic studies revealed that pancreatic islets constitutively express NO-synthase in α- and δ-cells, where it is confined to the secretory granules. These data, thus, indicate that NO is important in the signal transduction pathway of the early phase of glucose-stimulated insulin secretion and that NO is likely to act in a paracrine and not in an autocrine manner.

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PARTICIPATION OF CREATINE KINASE IN CONTROL OF INSULIN SECRETION

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We have shown that the rat insulinoma cell line INS-1 contains brain type-creatine kinase (CK) as well as the mitochondrial CK isoform. The aim of this study was to elucidate whether the CK system takes part in the control of energy flow from the mitochondrium to energy-dependent sites of insulin secretion. To this end, INS-1 cells were loaded with the creatine analogue cylcocreatine (cCr) which is readily phosphorylated by CK and can serve as an energy donor at ATP-consuming sites, but at a severely lower rate compared to creatine phosphate. After loading cells for 24 hours with cCr ATP content of perchloric acid extracts of whole cells was measured by the firefly luciferase/luciferin method. Insulin secretion and insulin content were determined in parallel. Results: Incubation of INS-1 cells with cCr leads to dose-dependent intracellular accumulation of cCr. Percentage of phosphorylated cCr was generally greater than 90 %. With 5 mM extracellular cCr, cCr phosphate accumulated to 20 mmol/l cell water. ATP in these cells was 0.9 mmol/l cell water which was not significantly different from non-treated cells. However, insulin secretion in cCr-loaded cells was enhanced 3-fold ($p<0.05$), while insulin content was unchanged. Enhanced insulin secretion and cCr content were almost fully reversible after 24 hours of incubation in cCr-free medium. In conclusion these results suggest that in clonal beta cells CK participates in mitochondrium-plasma membrane energy transfer believed to be necessary for glucose-stimulated insulin secretion. Energy transfer cannot be demonstrated on the sole basis of ATP measurements and may be better explained by kinetic analysis as has been recently shown for adenylate kinase-catalyzed phosphotransfer (JBC 271:16544-52, 1996).

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ISLET ACID GLUCAN-1,4-α-GLUCOSIDASE IN RELATION TO NITRIC OXIDE AND INSULIN SECRETION

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We have previously shown that activation of the islet lysosomal enzyme acid glucan-1,4-α-glucosidase increases, whereas nitric oxide (NO) inhibits, glucose-induced insulin release. Isoforms of α-glucosidehydrolases contain several cystein-residues being possible targets for NO-induced thiol inactivation processes. We therefore investigated, in isolated islets, the influence of NO on insulin release and islet acid α-glucosidehydrolase activities. It was observed that the intracellular NO donor hydroxylamine (300 µmol/l) markedly inhibited glucose-induced (16.7 mmol/l) insulin release. At the same time hydroxylamine strongly suppressed the activities of acid glucan-1,4-α-glucosidase, acid α-glucosidase and N-acetyl-β-D-glucosaminidase. The activities of acid phosphatase as well as neutral α-glucosidase were unaffected by the NO donor. Islet homogenates exposed to the spontaneous NO donor sodium nitroprusside (SNP) lost approximately 20-50% of their acid α-glucosidehydrolase activities during a 60 min incubation period. Acid phosphatase decreased by 25%, whereas N-acetyl-β-D-glucosaminidase was unaffected. When isolated islets were incubated at 16.7 mmol/l glucose in the presence of the specific NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME; 5 mmol/l) the activities of the acid α-glucosidehydrolases were markedly increased, compared with the 16.7 mmol/l controls. Concomitantly there was a large increase in insulin release. Other lysosomal enzyme activities and the neutral α-glucosidase were not influenced. The data suggest that a possible mechanism for NO-induced inhibition of glucose-stimulated insulin release is exerted through an NO mediated inactivation of the acid glucan-1,4-α-glucosidase activity.

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STIMULATION OF INSULIN RELEASE BY MICROMOLAR CONCENTRATIONS OF A NOVEL SUCCINIC ACID ESTER

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Esters of succinic acid are currently under investigation as possible tools for stimulation of insulin release in NIDDM. They are indeed well suited to bypass site specific defects of D-glucose metabolism in diseased B-cells. In recent studies, two potential limitations of this therapeutic approach were already dealt with. Thus, it was first shown that several esters of succinic acid not susceptible to lead to the undesirable generation of methanol by intracellular hydrolysis are, like the methyl esters, potent insulin secretagogues. Second, some esters of succinic acid remain able to stimulate insulin release after enteral administration. The present work deals with two other objections to the use of succinate esters in NIDDM, namely the necessity of administering high amounts of these esters and, hence, their utilization as gluconeogenic precursor. In isolated rat pancreatic islets, 1,2,3-tri(methylsuccinyl)glycerol ester (3SMG) stimulated both biosynthetic and secretory activities. The release of insulin was increased by 3SMG both in the absence and presence of D-glucose. At a close-to-physiological hexose level (7 mM), as little as 10 μM 3SMG significantly augmented insulin output. Likewise, in anaesthetized rats, the plasma insulin concentration was increased by 2.72 ± 0.26 ng/ml (n = 6) two min after the intravenous injection of only 0.07 μmol of 3SMG per g body wt. With such a low amount, the risk of an untoward increase in gluconeogenesis is probably negligible. In conclusion, therefore, the present work provides essential support to the proposal that suitable esters of succinic acid could be used as insulinotropic agents in the treatment of NIDDM.

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CITRULLINE-ARGININOSUCCINATE-ARGININE CYCLE IN β-CELLS COUPLED TO Ca^{2+}-SIGNALING AND INSULIN RELEASE VIA NITRIC OXIDE

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In the presence of stimulatory glucose (8.3 mmol/l), L-citrulline at physiological concentrations (0.1-1 mmol/l) under arginine-deficient conditions increased the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) in rat pancreatic β-cells and HIT cells, an insulinoma cell line, as demonstrated by ratiometric fura-2 microfluorometry. The enzymes involved in citrulline metabolism, argininosuccinate synthetase (ASS) which metabolizes L-citrulline to L-argininosuccinate, and argininosuccinate lyase (ASL) which metabolizes L-argininosuccinate to L-arginine, as well as the cerebellar-type NOS, were immunohistochemically localized in rat islets. The mRNAs of all three enzymes were also expressed in rat islets. Argininosuccinate and arginine at 0.1-1 mmol/l also increased $[Ca^{2+}]_i$ in rat β-cells. An NO-donor increased $[Ca^{2+}]_i$ in rat β-cells. Furthermore, citrulline also increased insulin release from rat islets in 8.3 mmol/l glucose. The citrulline-induced $[Ca^{2+}]_i$ increase and insulin release were inhibited by NMMA, an inhibitor of NOS. We propose that citrulline and arginine at physiological concentrations are recycled through ASS-ASL-NOS circuit, thereby activating a NO-mediated Ca^{2+} signalling pathway, which appears to be linked to potentiation of glucose-induced insulin release.

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FAILURE OF D-FRUCTOSE TO STIMULATE PROTEIN BIOSYNTHESIS IN RAT PANCREATIC ISLETS

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Under suitable experimental conditions, D-fructose displays a modest but sizeable insulinotropic action. For instance, it was recently documented that, even in the absence of any other exogenous nutrient, D-fructose stimulates insulin release from rat pancreatic islets provided that the ketohexose is present in the incubation medium at a 240 mM concentration. Some doubt was expressed, however, on the view that the insulinotropic action of D-fructose would involve the same coupling factors as those currently incriminated in the process of glucose-induced insulin release. For instance, an apparent dissociation was observed between the secretory and metabolic effects of D-fructose in islets prepared from normal rats. These findings led us to investigate, in the present study, whether D-fructose, tested in high concentrations, mimics the stimulant action of D-glucose on biosynthetic activity in rat pancreatic islets. The islets were incubated in the presence of L-[4-^{3}H]phenylalanine (3.7 μM) and, as required, D-glucose (16.7 mM) or D-fructose (80 and 240 mM). After 90 min incubation, the islet content in tritiated TCA-soluble material was not significantly different in islets deprived of exogenous nutrient or exposed to either D-glucose or D-fructose. The amount of tritiated TCA-precipitable material found in the islets after incubation in the presence of 16.7 mM D-glucose was much higher ($P < 0.001$) than the basal value. D-fructose, however, whether tested at a concentration of 80 mM, which is close to the threshold value for the insulinotropic action of the ketohexose, or at 240 mM failed to stimulate the labelling of islet peptides. These findings reinforce the view that the stimulation of insulin release by D-fructose does not entail the same metabolic determinants as those operative in glucose-stimulated islets.

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BIOCHEMICAL AND FUNCTIONAL ASPECTS ON ISLET HAEM OXYGENASE ACTIVITY AND CARBON MONOXIDE FORMATION.

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Nitric oxide has been the molecule of the last decade, and its role as an endocrine modulator in the pancreatic islets is still controversial. The last few years yet another two-atomic gas, carbon monoxide produced from heme under the influence of the heme oxygenase (HO) enzyme, has been postulated also to function as a neuroendocrine modulator. In the present study we have detected and quantified CO production in mouse islet homogenate using a sensitive gas chromatographic method. Islet hormone release studies using the CO precursor hemin and the HO inhibitor zincprotoporphyrin IX indicate that CO is a positive modulator of both insulin and glucagon secretion. Exogenously applied CO also stimulates islet hormone secretion, in contrast to exogenously applied NO, which inhibits insulin secretion. The effects of CO seem mainly to be mediated through an elevation of intracellular cGMP levels. Further, hemin has been shown to potentiate L-arginine stimulated insulin secretion, an observation which could reveal a possible connection between the heme-CO and L-arginine-NO pathways. CO produced from heme might inhibit the nitric oxide enzyme activity and thereby L-arginine derived NO formation. We have previously shown NO to be a negative modulator of insulin release. In conclusion, mouse pancreatic islets contain a constitutive heme oxygenase enzyme producing detectable amounts of CO, which stimulates both insulin and glucagon secretion. There seems to be an important connection between the CO - system and the NO - system in islet tissue.

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DO TOLBUTAMIDE AND DIAZOXIDE INFLUENCE INSULIN SECRETION BY MODULATING Ca^{2+} EFFICACY IN B CELLS ?

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Sulfonylureas trigger insulin secretion by blocking K-ATP channels of the B cell membrane, thereby causing depolarization, Ca^{2+} influx and rise in cytoplasmic Ca^{2+} ($[Ca^{2+}]_i$). It has recently been suggested that they also potentiate the action of Ca^{2+} on exocytosis. We have already challenged this hypothesis, but further tested it here with normal mouse islets cultured overnight in RPMI medium containing 10 mM glucose. Depolarizing islet cells by raising extracellular K from 4.8 to 15, 30 and 60 mM, abolished $[Ca^{2+}]_i$ oscillations induced by 15 mM glucose and progressively raised $[Ca^{2+}]_i$. The effect of 100 µM tolbutamide on $[Ca^{2+}]_i$ was marked in 4.8 mM K, smaller in 15 mM K, minor in 30 mM K and absent in 60 mM K. The effect on insulin secretion paralleled that on $[Ca^{2+}]_i$. Tolbutamide was also ineffective in islets depolarized with 60 mM K in the presence of only 0.5 mM $CaCl_2$ (instead of 2.5 mM), without or with dibutyryl cAMP, or in the presence of low (3 mM) instead of high (15 mM) glucose. Repaglinide is a non-sulfonylurea drug that blocks K-ATP channels but does not potentiate the action of Ca^{2+} on exocytosis. In the presence of meglitinide, a parent compound, tolbutamide did not affect $[Ca^{2+}]_i$ (expected from the common action on K-ATP channels) and did not increase insulin release further (unexpected if tolbutamide also increases the efficacy of Ca^{2+}). In contrast to sulfonylureas, diazoxide has been reported to decrease the effectiveness of Ca^{2+}. However, 250 µM diazoxide was without effect on $[Ca^{2+}]_i$ and insulin release in islets depolarized by 60 mM K in 0.5 or 2.5 mM external $CaCl_2$. In conclusion, no evidence for a modulation of the Ca^{2+} action on exocytosis by tolbutamide or diazoxide could be found in normal mouse B cells.

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PACAP AS AN INTRA-ISLET AMPLIFIER OF GLUCOSE-INDUCED INSULIN SECRETION.

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It has been shown that pituitary adenylate cyclase activating polypeptide (PACAP) is localized in the pancreas and potently augments glucose-induced insulin release by increasing cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in islet β-cells. We examined whether PACAP is involved in the regulation of glucose-induced insulin secretion by islets. High glucose (8.3-20 mM)-stimulated insulin release from isolated rat islets was attenuated by a specific anti-PACAP antiserum. The islet incubation medium with high glucose possessed a capacity, which was neutralized by PACAP antiserum, to increase $[Ca^{2+}]_i$ in rat β-cells, as measured by fura-2 microfluorometry. PACAP-immunoreactivity was localized in the entire region of rat islets. Furthermore, PACAP mRNA expression and PACAP biosynthesis were detected in islets and a β-cell line, MIN6. The (type-I) PACAP receptor was immunohistochemically observed in islets. $[Ca^{2+}]_i$ measurements revealed a substantial population of glucose-unresponsive β-cells, many of which were recruited by 10^{-13} M PACAP into $[Ca^{2+}]_i$ responses. These results indicate that PACAP is a novel islet hormone, which is synthesized and released by islets and then, in an autocrine manner, arouses and potentiates β-cell responses to glucose, thereby amplifying glucose-induced insulin secretion in islets.

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CALCIUM SIGNALLING IS INVOLVED IN THE INSULIN-RELEASING EFFECT OF 4-HYDROXYISOLEUCINE

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The novel amino acid 4-hydroxyisoleucine (4-OH-Ile), extracted from *Trigonella foenum graecum L* seeds, stimulates insulin release from pancreatic islets; it also increases insulin secretion from isolated rat pancreas, in a glucose-dependent manner. This study was aimed at evaluating more precisely the influence of glucose in the insulinotropic effect of 4-OH-Ile, and investigating the role of calcium signalling in this effect. Insulin secretion was studied using the perfused rat isolated pancreas and cytoplasmic Ca^{2+} ($[Ca^{2+}]_i$) was measured in mouse β-cells loaded with the indicator fura-2. In the presence of 5 mmol/l glucose, 4-OH-Ile (200 µmol/l) did not modify insulin secretion. The difference in the area under the curve for insulin secretion compared to controls (Δ $AUC_{10\text{-}min}$) was 0.5 ± 0.4 ng. In the presence of 6.6, 7.4, 8.3 and 10 mmol/l glucose, the Δ $AUC_{10\text{-}min}$ were 5.6 ± 2.4, 57.5 ± 12.1, 77.1 ± 20.0 and 186.1 ± 14.4 ng, respectively. At concentrations of 0.5-1.0 mmol/l, 4-OH-Ile was ineffective on the cytoplasmic Ca^{2+} concentration of β-cells exposed to 3 mmol/l glucose. Elevation of glucose to 11 mmol/l resulted in large amplitude oscillations of $[Ca^{2+}]_i$, which were unaffected by the presence of 0.5 mmol/l 4-OH-Ile. In contrast at 7-8 mmol/l glucose, 0.5 mmol/l 4-OH-Ile induced large amplitude oscillations of $[Ca^{2+}]_i$. Reduction of glucose from 11 to 7 mmol/l inhibited the large amplitude $[Ca^{2+}]_i$ oscillations in most β-cells but they could be restored by 0.5 mmol/l 4-OH-Ile. In conclusion, the results show clear glucose dependence of the insulin-releasing effect of 4-OH-Ile. Moreover, the $[Ca^{2+}]_i$ data indicate that the amino acid potentiates the effect of intermediary glucose concentrations on Ca^{2+} signalling.

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CALCIUM AND SODIUM DEPENDENCY OF THE INSULINOTROPIC EFFECT OF PACAP38 IN HIT-T15 CELLS

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a pancreatic neuropeptide, which stimulates insulin secretion. Although the mechanism of its insulinotropic action is far from clear, the peptide raises cellular cAMP, increases cytosolic Ca^{2+} and induces formation of inositol phosphates (IPs) in insulin producing HIT-T15 cells. Earlier studies have shown that the mechanism of the insulinotropic effect of glucagon-like peptide-1 (GLP-1) not only involves cAMP, but is also Na^+ and Ca^{2+} dependent. Therefore, in this study, we examined the dependency of Na^+ and Ca^{2+} for the increases in insulin, cAMP and IPs after activation by PACAP38 in insulin producing HIT-T15 cells. IPs were measured from cells prelabeled with [2-^{3}H]-myoinositol, and eluted by anion chromatography. Removal of extracellular Ca^{2+} at 10 mM glucose abolished the insulinotropic effect of PACAP38 completely, without affecting the increase in cellular cAMP (44±8.9% in controls vs. 64±20% in the absence of Ca^{2+}, n=5, n.s.). PACAP38 increased IPs formation in the presence of Ca^{2+} (60±25%, n=3), but not in the absence of Ca^{2+} (-1±5.3%, n=3). Furthermore, removal of Na^+ from the medium and replacing it with N-methyl-D-glucamine reduced the insulinotropic effect of PACAP38 (430±48% in controls vs. 150±17% in the absence of Na^+, n=12, $P<0.001$) and the increase of cellular cAMP (100±13% vs. 24±7.9%, n=5, $P<0.01$), but the formation of IPs was not affected (81±8.9% vs. 95±23%, n=3, n.s.). In contrast, the markedly stimulated IPs formation induced by gastrin releasing peptide at 100 nM was diminished in the Na^+ free medium (980±150% vs. 213±39%, n=3, $P<0.01$). We conclude that the insulinotropic effect of PACAP38 in HIT-T15 cells, like that of GLP-1, is both Ca^{2+} and Na^+ dependent, whereas the PACAP38 induced formation of cAMP is dependent of Na^+ but is independent of Ca^{2+}. The results therefore suggest that after stimulation by PACAP38, Na^+ is required for the formation of cAMP whereas Ca^{2+} is required for the distal events of exocytosis in insulin producing HIT-T15 cells. Finally, we also suggest that formation of IPs is not of mechanistic importance for the insulinotropic action of PACAP38 in these cells.

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INSULIN RELEASE DEPENDS ON GLUCOSE CONCENTRATION, INCUBATION TIME, TEMPERATURE, AND PH TESTED IN MOUSE AND RAT ISLETS

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The purpose of the present study was to investigate the differences in insulin response of isolated mouse and rat islets under different conditions.The islets of both species were isolated and tested under the same conditions.Insulin was measured by means of RIA.The statistic was made by means of SIGMASTAT package. The numbers of experiments were between 16 and 24 for each point of different series. Insulin secretion was strongly dependent on glucose concentration for both species:3.3mM glucose did not induce insulin release (about 1ng/ml/islet) whereas 16.7mM glucose induced the same insulin release as 30mM glucose secreted insulin after 2 hours was 2-fold higher, $p<0.05$ in isolated mouse islets (about 6ng/ml/islet) than in rat islets (3ng/ml/islet).Insulin release was also dependent on incubation time:a longer incubation time elicited insulin secretion constantly, after 6h at 16.7mM glucose the insulin secretion of mouse islets was nearly 4-fold higher (30 ng/ml/islet), $p<0.05$ than of rat islets (8ng/ml/islet).Insulin release was enhanced in isolated pancreatic mouse and rat islets at higher temperatures. At 40°C the insulin release was 1.5-fold, $p<0.05$ increased compared to the insulin response at 37°C. The insulin content released by mouse islets was about 10 ng/ml/islet compared to 6 ng/ml/islets, $p<0.05$ whereas the rat islets released 7 ng/ml/islet at 40°C and 4 ng/ml/islet. $p<0.05$ at 37°C. We also demonstrated that alkaline pH reduced insulin secretion. In the range of pH7.2 to pH7.6 insulin release was almost the same for mouse and rat islets.At a pH of 8.0 there was a dramatic increase of insulin release in mouse islets (6.1 to 11.9 ng/ml/islet,$p<0.05$) whereas the rat islets (3.7 to 4.1 ng/ml/islet,$p<0.05$) did not release as much insulin as mouse islets. In conclusion, both mouse and rat islets released insulin in a time- and glucose concentration-dependent manner, insulin secretion was enhanced at higher temperatures and alkaline pH, but the released insulin content was much higher in mouse islets than in rat islets. The present data have demonstrated that the mouse islets seem to be more sensitive compared to rat islets under different conditions.

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SPECIFICITY OF ADENYLYL CYCLASE ISOFORMS EXPRESSION IN PANCREATIC ISLETS AND ACINI

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Eight adenylyl cyclase (AC) isoforms were described specifically in mammalian tissues, from broadly distributed types II and IV, to brain specific types I and VIII, but not in pancreas. In the present study we have investigated the presence and the distribution of six different isoforms, AC-I, II, III, IV and V/VI in neonate and adult mouse, rat, and hamster pancreas using antibodies raised against the C-terminal h817-836, rh1071-1090, r1125-1144, r1045-1064, hm1149-1165 sequences, respectively. Localization of AC in pancreatic endocrine cells, was performed using double labeling with either insulin or glucagon. The specific AC protein band was evaluated by western blotting in RINm5F cell line. AC-II was: (1) the most prominant protein expressed in rat and mouse pancreas, (2) strong expression was restricted to A and B cells of rat and mouse and (3) exclusively seen in A cells of adult hamster pancreas. In adult rat, ultrastructural study revealed the abundancy of AC-II isoform with a more pronounced labeling in the secretory granules and the plasma membrane of A and B cells. A marked fluorescence was observed with AC-III in A and B cells of 3-day-old mouse pancreas, with a lower intensity in 3-day-old rats and not detected in adult rats. Isoforms IV and V/VI were also expressed in B and A cells of 3-day-old mouse and with a lower intensity in 3-day and adult rats. Furthermore, scattered labelings were detected in exocrine area. AC-I was faintly, if any, expressed both in rat and mouse islets. This isoform as well as AC-III and IV was not detected in adult hamster. Western blots show 120, 100, 155, 110-135 and 105-140 kD bands corresponding to the AC-I, II, III, IV and V/VI isoforms, respectively. Coexistence of AC-III with G_{olf}, and of AC-II with G_s, G_i, Gq in the B cells suggest a possible association of these molecules into complexes required for the transduction of signal in B cells.

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ROLE OF THE CYSTEINE STRING PROTEIN CSP IN INSULIN SECRETION FROM PANCREATIC β-CELLS

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We investigated the putative function of CSP in insulin sceretion as this protein has previously been implicated in the regulation of neurotransmission. In addition, two splice variants have been cloned recently from chromaffin cells, localized to exocytotic vesicles and proposed to function via their DnaJ-domains as chaperones in the folding of proteins required to induce secretion by membrane fusion of secretory granules with the plasma membrane. Western blot analysis demonstrates the presence of a 36 kDa CSP in HIT-T15, INS-1, RINm5F and MIN6 cells. Subcellular fractionation and immuno-cytochemistry using confocal microscopy localizes CSP mainly on secretory granules and on synaptic-like microvesicles in INS-1 and primary β-cells. Despite its known high degree of palmitoylation in its cysteine string domain CSP was removed from membranes only by detergent (Triton-X100) but not by ethanolamine and, therefore, behaves as an integral membrane protein. Upon transient coexpression of human insulin gene and myc-tagged CSP splice variants, both CSP forms are localized to vesicular structures in hamster HIT-T15 cells. Furthermore both splice variants inhibit insulin release evoked by Ca^{2+}-influx as measured by the release of human C-peptide used as a reporter gene. In contrast, a point mutation in CSP (G91V) abolished its inhibitory effect although its subcellular distribution was comparable to the wild-type. Transient overexpression of antisense CSP in HIT-T15 cells largely reduced stimulated insulin release. We are currently investigating the effect of highly conserved residues in the DnaJ-domain by the use of different point mutations. In conclusion, CSP is a secretory granule protein in insulin-secreting cells and the functional observations are compatible with a chaperone function of CSP on the secretory machinery required for insulin secretion.

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GLUCOSE DEPENDENT INSULIN SECRETION BY THE ADENYLATE CYCLASE (AC) / cAMP-SYSTEM IN THE INSULINOMA CELL LINE ßTC6,F7.

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It is well known, that compounds that enhance intracellular cyclic adenosinmonophosphate (cAMP) are able to stimulate insulin secretion of beta cells only in the presence of high glucose concentrations (glucose dependent). However the mechanisms underlying this effect are remain controversal. The aim of the present study was to investigate the glucose dependent insulin secretion by the AC/cAMP-system on the recently developed insulinoma cell line ßTC6,F7 (passages 54-85). All experiments were carried out by static incubations of the cells in the absence and in the presence of 10 mM glucose. In contrast to glucose, that had no effect on insulin secretion itself, the phosphodiesterase inhibitor IBMX (25 µM) stimulated insulin secretion in the presence of glucose by 1.5-fold ($p<0.001$). The AC-stimulator forskolin enhanced insulin release in the presence of glucose dose dependently with an EC_{50} of 1 µM and its maximal stimulation at 3 µM (2.2-fold, $p<0.001$). In the absence of glucose, IBMX and forskolin had only a slight effect on insulin release. GLP-1 (7-36) as a physiological insulin secretagogue was also able to potentiate insulin release only in the presence of glucose and IBMX (1.5-fold, $p<0.001$). In contrast GLP-1 had no effect on insulin release with or without IBMX in the abscence of glucose. 8-bromo-cAMP the stable analogue of cAMP stimulated insulin release in the presence of glucose at higher concentrations by 1.5-fold (100µM, $p<0.001$). Surprisingly, all glucose dependent insulin secretions by the above-mentioned secretagouges were completly blocked by 200 µM Diazoxid. 9-(tetrahydro-2-furyl)adenin (SQ22536) an AC-inhibitor was able to inhibit the forskolin induced but not the IBMX induced insulin release. The glucose dependent insulin releasing effects of forskolin and IBMX were not inhibited by 100 nM of the proteinkinase A inhibitor H89. We conclude that at first the glucose dependent insulin secreting AC/cAMP system in ßTC6,F7 insulinoma cells is functional and secondly cAMP exerts its effect on insulin secretion in a glucose dependent mechanism at the beta cell membrane that does not include proteinkinase A.

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THE 55 kDa CA^{2+}/CALMODULIN KINASE II δ_2 (CaMkin II) COMIGRATES WITH INSULIN SECRETION GRANULES IN INS-1 CELLS

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CaMkin II phosphorylates insulin secretion granule proteins including synapsin in ß-cells and is activated by glucose. The mRNAs of CaMkin II δ_2, δ_6, $ß_3$ and γ were found in insulinoma cells. We investigated the intracellular distribution of CaMkin II to assess its role in insulin secretion. **Methods:** Antibodies were generated in rabbits against the bacterially expressed C-terminal 200 amino acids of CaMkin II δ_2 which crossreact with bacterially expressed CaMkin γ. INS-1 cells were fractionated on 5 to 20 % sucrose gradients or on metrizamide step gradients. Insulin, synaptophysin (synaptic-like vesicles), Na/K-ATPase, 78 kDa heat shock protein (ER), arylsulfatase (lysosomes) were determined as marker enzymes. **Results:** A 55 kDa CaMkin II immunoreactivity corresponding in size to the recombinant δ_2 variant was present predominantly in the detergent insoluble compartment and fainter in cytosol. Fractionation of postnuclear supernatant on sucrose gradients showed close comigration with insulin granules and no comigration with synaptic-like vesicles, plasma membrane, ER or lysosomal markers. On metrizamide step gradients the 55 kDa CaMkin II also apperard in the insulin granule fraction. **Conclusion:** The comigration of CaMkin II δ2 with insulin secretion granules suggests a role in insulin secretion. The poor detergent solubility may indicate cytoskeletal components which may localize CaMkin II in granule recruitment. Other isoforms of CaMkin II should be larger (γ, $ß_3$) or smaller (δ_6) but were not detectable with our antibody. Remarkably, CaMkin II δ_2 was not present on synaptic-like vesicles containing GABA in contrast to its association with synaptic vesicles in brain.

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B-CELL INSENSITIVITY LINKS TO Ca^{2+}-INDEPENDENT DECREASE IN cAMP GENERATION AND RIGHT-SHIFT IN cAMP-INDUCED INSULIN RELEASE

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Diazoxide (D) blocks glucose-induced insulin secretion and thereby protects against excessive stimulation, which is a major cause of hyperglycemia-induced B-cell insensitivity. D was used to explore the role of the adenylatecyclase-cAMP system for insensitivity. Rat pancreatic islets were cultured for 22 h in RPMI with 27 mM glucose (G) and with or without 325 µM D. In the presence of 3.3 mM G previous D increased forskolin-induced islet cAMP from 18.3±2.2 to 23.0±2.1, from 46.2±10.6 to 68.1±15.5 and from 163±21 to 231±37 fmol/islet for forskolin concentrations 0.1, 1 and 25 µM respectively, $p<0.05$ or less. These effects were not due to D per se since co-culture with D and 6 mM G failed to influence cAMP levels. In islets cultured at 27 mM G, co-culture with D enhanced insulin secretion only at 0.1 µM and not at higher concentrations of forskolin. Omission of Ca^{2+} to preincubation (30 min) and final incubation media did not diminish the cAMP response to 25 µM forskolin and did not reduce the enhancing effect of previous D. Insulin responses to the cAMP analogue (Sp)5,6-dichloro-1-β-D-ribofuranosyl-benzimidazole-3',5-cyclic monophosphorothioate (BIMPS) were tested in 60 min final incubations together with 6 mM G. At concentrations 0.01, 0.025 and 0.05 mM previous D increased insulin responses by 73, 217 and 69% respectively, $p<0.025$ or less. However, previous D failed to affect insulin responses to 0.1 mM BIMPS. Omission of calcium reduced the insulin response to 0.05 mM BIMPS comparatively more after previous D than in islets cultured without D. Conclusions: Averting excessive stimulation by D moderately increases forskolin-induced cAMP responses. This effect is not secondary to perturbations in calcium inflow. Furthermore, previous D left-shifts insulin release in dose-response to elevations of cAMP but does not alter maximal responses.

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Effect Of Protein Kinase Cβ Selective Inhibitors On Insulin Secretion and Beta cell activty.

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Phorbol esters such as 4-β-phorbol-12-myristate-13-acetate (PMA) have been shown to stimulate insulin secretion under high glucose conditions from pancreatic islets presumably by activation of protein kinase C (PKC). Staurosporine has been shown to inhibit high glucose/PMA induced insulin secretion in pancreatic islets. Likewise, we have found potentiated insulin secretion to occur under high glucose conditions on phorbol ester, 4-β-phorbol-12,13-dibutyrate (PDBu), challenge (ED50=10 nM) in transformed beta cells (BTC6-f7). Staurosporine was able to inhibit insulin secretion on PDBu challenge in BTC6-f7 cells (IC50=30 nM). However staurosporine is a more potent inhibitor of both calcium calmodulin (IC50=4 nM) and src tyrosine kinase (IC50=1 nM) than most PKC isozymes (PKCβII IC50=19 nM). We tested the effect of several of the PKCβ isozyme selective antagonists we have developed, such as LY333531, on insulin secretion under basal, high glucose, and on phorbol challenge in BTC6-f7 cells. Cells were treated with PDBu, staurosporine, and various PKC inhibitors for one hour. Activity of the compound was assessed by measuring insulin secretion via insulin RIA. Cell viability was assessed by testing the compound in low glucose. LY333531 as well as other analogues from the series inhibit PKC activity but were unable to inhibit PDBu stimulated insulin secretion. In the absence of PDBu these PKCβ selective inhibitors stimulated insulin secretion under high glucose concentration in βTC6-f7 cells. The above results indicate that a PKCβ selective antagonist does not inhibit insulin secretion and is capable of stimulating insulin secretion only under high glucose conditions, from transformed βTC6-f7 cells.

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ABNORMAL cAMP CONTENT AND INSULIN RELEASE IN ISLETS OF SPONTANEOUSLY DIABETIC GK RATS.

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We have tested the possibility that a defect in the islet cAMP production could be involved in the failure of the glucose-induced insulin release in the diabetic GK rats, a recognized model of NIDDM. Exposure to increasing glucose concentrations induced a rise of the cAMP content in the control islets. In the GK islets, cAMP content was 1.6-fold higher as compared to controls at 2.8mM glucose and increasing glucose concentrations elicited no further increase. This coincided with a very low glucose-induced insulin release by the same GK islets. Forskolin markedly enhanced both cAMP and insulin release at 16.7mM glucose in GK islets and to a lesser extent in control islets. IBMX increased the cAMP content to the same extent in both type of islets. Nevertheless, insulin release was significantly higher in the GK islets as compared to control islets. GLP-1 amplified the cAMP content and insulin release to the same extent in both type of islets. Somatostatin decreased cAMP content and insulin release more efficiently in GK islets that in control islets. Kinetics of insulin release in vitro by islets were tested using perifusion procedure. Following exposure to 16.7mM glucose a biphasic pattern of insulin release was observed in the control islets whereas it induced only a modest increase in insulin output in GK islets. Accordingly, the incremental insulin response to 16.7mM glucose was 4-fold decreased in GK as compared to control rats. IBMX and GLP-1 enhanced the secretory response to 16.7mM glucose respectively by 5-fold and 4-fold in control rats and by 12-fold and 5-fold in GK rats. Under these conditions a clear biphasic pattern of the insulin release was regained in GK islets. In summary, ß-cell of diabetic GK rats showed 1) an increased cAMP content already at 2.8mM glucose 2) a defective glucose-stimulated cAMP generation. However it is possible to regain competence of the GK ß-cells to glucose with agents that artificially rise their intracellular cAMP.

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INDUCIBLE OVEREXPRESSION OF CA^{2+}/CALMODULIN KINASE II δ_2 IN INSULINOMA CELLS BY MEANS OF RETROVIRAL INFECTION

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Ca^{2+}/calmodulin-dependent kinase II (CaM kinase II) participates in stimulated insulin secretion. A promising approach to elucidate its exact role is overexpression in insulin secreting cells. Retroviral vectors allow clean and stable insertion of single copies of foreign genes. Inducible gene expression permits to investigate effects of inserted genes without variations introduced by clonal differences. **Methods:** Retroviral producer cell lines of a replication deficient retroviral vector with an estrogen responsive 5' LTR driving a neomycin resistance and the CaM kinase II δ_2 cDNA were generated and used to infect the RINm5F insulinoma which was previously infected with estrogen receptors (ER). CaM kinase expression levels were quantified by immunoblotting with antibodies generated in rabbits and kinase activity was measured by ^{32}P-transfer. **Results:** After infection and selection of RIN cells expressing ERs, the E_2 response was tested by transient transfection with an E_2 responsive luciferase reporter plasmid yielding an up to 20-fold increase in luciferase counts, while no stimulation was detected in normal RIN cells. ER positive cells were subsequently infected with the E_2 responsive CaM kinase II δ_2 vector and selected. CaM kinase II immunoreactivity in transduced but unstimulated cells was almost twice that of untransduced RIN cells. Stimulation with 10 nM E_2 led to a 5- to 6-fold increase. CaM kinase phosphotransfer activity increased 6-fold upon stimulating with E_2 over normal RIN cells. **Conclusion:** Stable insulinoma cell lines with E_2 inducible overexpression of CaM kinase II δ_2 can be generated using a novel E_2 inducible retroviral vector. This inducible overexpression will allow detailed studies on the physiological role of CaM kinase II in insulin secretion. Supported by DFG.

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POTENTIATION BY FORSKOLIN OF GLIQUIDONE INSULINOTROPIC ACTION IN THE PERFUSED PANCREAS OF NORMAL AND GK RATS

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The insulinotropic action of hypoglycemic sulfonylureas is modulated by the metabolism of nutrients in the B-cell, and could thus be altered in NIDDM because of an impaired sensitivity of the B-cell to glucose. The present study deals with a possible modality for potentiation of the B-cell response to the hypoglycemic agents in NIDDM. It indeed explores the influence of forskolin upon gliquidone-induced insulin release in the perfused pancreas of normal and GK rats. The effect of gliquidone (1 μM) upon insulin and glucagon output was examined, at a low concentration of glucose (2.8 mM), in the absence or presence of forskolin (1.3 μM). In normal rats, the diterpene exerted little effect upon basal insulin release but markedly augmented gliquidone insulinotropic action. Such a potentiation was observed whether the response to the sulfonylurea was examined first in the absence and then in the presence of forskolin, or in the reverse sequence. In GK rats, forskolin dramatically augmented both basal and gliquidone-stimulated insulin output. In relative terms, forskolin increased to a much greater extent the response to gliquidone in GK rats than in control animals. Forskolin enhanced basal glucagon output, especially in normal rats, and unmasked the glucagonotropic potential of gliquidone in GK rats. These findings suggest a dual mechanism -metabolic and energy-independent- for the response of islet cells to the forskolin-induced generation of cyclic AMP. They also draw attention to the improvement by endogenous cyclic AMP of the B-cell secretory response to hypoglycemic sulfonylureas.

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INSULIN SECRETION FROM PANCREATIC β-CELLS: CONTRADICTORY EFFECTS OF PROTEIN KINASE C INHIBITORS

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Previous studies that have used chemical inhibitors of protein kinase C (PKC) in β-cells have yielded conflicting evidence as to the importance of PKC in nutrient-stimulated insulin release (IR). There are many types of chemical PKC inhibitors available, which interact with various regions within the enzyme structure. We have investigated the effect of three PKC inhibitors on IR from intact rat islets. Two of the inhibitors target the ATP-binding site of PKC: Ro 31-8220 (Ro), which is selective for PKC but not for individual isoforms; and Gö 6976 (Gö), which preferentially inhibits Ca^{2+}-dependent PKCs. The third inhibitor safingol (S) competitively interacts with the phorbol ester binding site of PKC. Ro (10 μM) inhibited phorbol ester (PMA)-induced IR (20 mM G, 100±6.5%; 20 mM G + 500 nM PMA, 142.5±5.7%; + Ro, 101.4±3.6%, $p<0.001$, $n=9$) and partially inhibited glucose (G)-stimulated IR by 38.5±2.2% ($p<0.001$, $n=9$). Similarly, Gö also inhibited PMA-induced IR (10 mM G + 500 nM PMA, 292.1±21.9% of G-stimulated response; + 1 μM Gö, 130.7±21.4%; $p<0.01$, $n=7$) but unlike Ro, did not compromise G-induced IR (10 mM G, 2.12±0.36 ng/islet/h; + 1 μM Gö, 2.21±0.38; $p>0.2$, $n=8$). In some experiments, S (1-100 μM) inhibited PMA- and G-induced IR in a concentration-dependent manner (10 mM G + 500 nM PMA, 183.7±26.3% of G-induced response, + 100 μM S, 94.1±5.4%, $p<0.01$, $n=7$; 10 mM G, 100±13.4%, + 100 μM S, 25.5±4.6%, $p<0.001$, $n=6$). However, unlike Ro and Gö, the effects of S tended to be irreproducible between experiments and on some occasions these concentrations of S were without effect on either PMA- or G-induced IR. These apparently contradictory results using different PKC inhibitors presumably reflect non-selectivity of some or all of the inhibitors and reinforce the conclusion that caution should be applied when using chemical inhibitors of PKC in pancreatic β-cells.

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DISSOCIATION BETWEEN CYTOSOLIC Ca^{2+} AND INSULIN RESPONSES IN GLUCOSE-STIMULATED ISLETS FROM DIABETIC GK RATS

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Goto-Kakizaki (GK) rats have been extensively used as an animal model to study defective insulin release in NIDDM. Since impaired β-cell metabolism appears to be a major contributing factor to the GK diabetic syndrome, it has often been assumed that intracellular Ca^{2+} signalling might also be compromised in β-cells from GK rats. We have assessed this hypothesis by undertaking a comparative study of cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$, *via* fura-2 microfluorometry) and insulin secretion in glucose-stimulated isolated islets from normal Wistar and GK rats. GK rats from the Coimbra colony (11-14 weeks) were overtly hyperglycemic in the fasting state and displayed pronounced glucose intolerance. Raising glucose concentration from 2 to 11 mM evoked a biphasic insulin response from normal islets (10-fold stimulation over basal at 20 min) and a feeble monophasic response from GK islets (0.2- and 2-fold stimulation over basal at 20 and 50 min, respectively). In contrast, the depolarizing aminoacid arginine (20 mM) and the K_{ATP} channel blocker tolbutamide (500 μM) stimulated insulin release and $[Ca^{2+}]_i$ to about the same extent in normal and GK islets. Glucose raised the $[Ca^{2+}]_i$ to the same final extent in normal and GK rats, albeit with a different time course (notably slower for GK islets). In contrast to normal rat islets, GK islets often displayed fast $[Ca^{2+}]_i$ oscillations. We conclude that long term stimulation of glucose metabolism in β-cells from diabetic GK rats is sufficient to elicit an appropriate Ca^{2+} signal while being insufficient to provide full support to the Ca^{2+}-dependent reactions leading to exocytosis (for example, in the form of adequate cytoplasmic ATP levels).

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Effects of PD098059, an inhibitor of the MAPK cascade, on insulin secretion from pancreatic β-cells.

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We have investigated the expression of members of the MAPK cascade in a glucose-responsive pancreatic β-cell line (MIN6) and examined the effects of modulation of signalling through the cascade on insulin secretion in response to nutrients and an activator of protein kinase C (PKC). Western blotting studies using specific antisera demonstrated that MIN6 cells express MAPK (42 and 44kDa isoforms), MEK (45 and 46kDa isoforms), the activator of MAPK, as well as raf-1 (~70kDa) and MEK kinase (~76kDa), which activate MEK in the cascade. The MAPK cascade is blocked by PD 98059 (PD), a highly selective inhibitor of the phosphorylation and activation of MEK. MIN6 cells showed a dose-dependent increase in insulin secretion in response to glucose (2mM: 2.2±0.2ng/5000 cells/h; 8mM: 2.9±0.2; 14mM: 5.0±0.5; 20mM: 5.7±0.7; n=9; P<0.001 by ANOVA) and also responded significantly to 10mM ketoisocaproate (KIC; 162±7% basal; n=9; P<0.001). Pre-treatment of MIN6 cells with 50μM PD for 1-2 hours did not significantly affect insulin secretion in response to 20mM glucose(G) (20mM G: 5.2±0.31ng/15,000 cells/h; 20mM G+PD: 6.1±0.39; n=7; P>0.1) nor to 10mM KIC (10mM KIC: 5.78±0.39ng/15,000 cells/h; 10mM KIC+PD: 5.7±0.22; n=6; P>0.8). Similarly, pre-exposure of islets to a range of PD concentrations (0.16-50μM) had no effect on glucose-stimulated insulin secretion from normal rat islets (2mM G: 0.44±0.10ng/islet/h; 20mM G: 2.21±0.30; +1.6μM PD: 1.96±0.10; +50μM PD: 2.54±0.32; n=8-9; P>0.7 by ANOVA). Activation of PKC with 500nM PMA potentiated glucose-stimulated insulin secretion from both MIN6 β-cells and islets, and this was not significantly affected by inhibition of the MAPK cascade with 50μM PD (MIN6: 20mM G+PMA: 7.85±0.62ng/15,000 cells/h; 20mM G+PMA+PD: 6.66±0.77; n=8, P>0.1; Islets: 20mM G+PMA: 3.27±0.45ng/islet/h; 20mM G+PMA+PD: 2.61±0.50; n=8, P>0.3). These results suggest that β-cells express components of the MAPK cascade, but that signalling through this pathway is not essential for secretion in response to nutrients, nor to activators of PKC.

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GLUCOSE TOXICITY ON PANCREATIC β-CELLS IS NOT DUE TO INSULIN ACTION OR HYPEROSMOLARITY AND IS COUNTERACTED BY PACAP.

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Whether the glucose toxicity on pancreatic β-cells is produced directly by hyperglycemia or via high levels of insulin or hyperosmolarity induced secondary by hyperglycemia was examined. Isolated single β-cells from Wistar rats were cultured in a normal condition (5.6 mM glucose) and in conditions with high glucose (22-33 mM), insulin (10^{-5} M) or mannitol (27.4 mM). After culture for 2-3 days, the β-cell response to glucose (8.3 mM), tolbutamide (300 μM) and arginine (10 mM) was assessed by measurement of the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) by fura-2 microfluorometry. Percentage of β-cells responding to glucose with an increase in $[Ca^{2+}]_i$ was significantly lower (40%) in the high glucose culture group than in control group (70%), whereas no difference was observed between the insulin, mannitol and control groups. The responsiveness to tolbutamide and arginine was not different between all the control and test groups. When pituitary adenylate cyclase activating polypeptide (PACAP) was added in high glucose culture, the responsiveness to glucose was partially restored (50%) and $[Ca^{2+}]_i$ oscillations were frequently superimposed on the $[Ca^{2+}]_i$ increase in response to glucose. In conclusion, the glucose toxicity is not due to the action of high levels of insulin or hyperosmolarity but primarily to the hyperglycemia itself. PACAP has an ability to protect β-cells against hyperglycemia.

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REGULATABLE CARBOXYL METHYLATION OF THE CATALYTIC SUBUNIT OF PROTEIN PHOSPHATASE 2A IN THE PANCREATIC β CELL

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Recently, we identified that the 36 kDa catalytic subunit of protein phosphatase 2A (PP2Ac) undergoes carboxyl methylation (CM) in normal rat islets and INS-1 cells, an effect accompanied by increased PP2A activity. Recent studies have described adenine and guanine nucleotides, or glycolytic intermediates, as modulators of PP2A activity in the pancreatic β cell. Therefore, we examined whether these molecules regulate the CM of PP2Ac in β cells. ATP (-82%), ADP (-81%), GTP (-83%), or GDP (-88%) all inhibited PP2Ac CM. Moreover, the latter was also inhibited by mM concentrations of glucose-6-P (-64%), glucose-1,6 P_2 (-44%), fructose-6-P (-18%) or fructose-1,6-P_2 (-53%), compatible with described inhibitory effects of these modulators on the phosphatase activity. Additionally, however, the CM of PP2Ac was significantly stimulated by divalent metal ions (Mn^{2+} >Mg^{2+} > Ca^{2+} > control); furthermore, the nucleotide phosphate-mediated inhibition of CM of PP2Ac was completely reversed by Mn^{2+} or Mg^{2+}, suggesting that the effects of phosphorylated molecules are generalized and non-specific. In most cells, acetyl CoA carboxylase (ACC) is regulated by phosphorylation (inactive)-dephosphorylation (active) and indeed, recent studies have implicated PP2A in the regulation of hepatic ACC activity by dicarboxylic acids and Mg^{2+}. Therefore, we investigated whether dicarboxylic acids and metal ions regulate the CM of PP2Ac (and its activity) and ACC in β cells. Glutamate or succinate stimulated (+ 42-84%) the CM of PP2Ac and increased ACC activity (+ 150%) in β cell homogenates. Okadaic acid (50 nM), a specific inhibitor of PP2A, abrogated the CM of PP2Ac as well as Mg^{2+}- or glutamate-stimulated ACC activity in β cell homogenates. These data imply that CM -derived changes in PP2A activity may contribute to regulation of key enzymes (e.g., ACC) of β cell intermediary metabolism.

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GOTO-KAKIZAKI RAT, A MODEL OF GLUCOSE PRIMING RATHER THAN GLUCOSE TOXICITY?

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This study was designed to delineate the nature of β cell dysfunction in a model of genetically determined non-obese diabetes, the Goto-Kakizaki (GK) rat. Pancreatic β cell function was analyzed immediately after weaning and 5 weeks thereafter, comparing animals with or without insulin treatment during the interval. In 3.5 week-old GK rats, fasting plasma glucose was significantly elevated with normoinsulinaemia, and the islet insulin content was reduced by 33%. When incubated with 3-30 mM glucose *in vitro*, the islets from 3.5 week-old GK rats showed reduced glucose sensitivity, i.e., the EC50 values were 19.5 and 15.9 mM, and the Hill constants for the positive cooperativity 2.1 and 4.2, in the islets of GK and the control rats, respectively. On the other hand, the maximum response to glucose was not attenuated when reduced islet insulin content was considered. In 8.5 week-old GK rats, hyperglycaemia worsened and glucose-stimulated insulin release by the islets more severely impaired. A daily sc insulin (Novolin U®) injection from the age of 3.5 to 8.5 weeks significantly prevented worsening of hyperglycaemia, which was accompanied by a near-total obliteration of glucose (6-30 mM) -stimulated insulin release by the islets: An acceleration of β cell dysfunction by insulin treatment in the GK rat implies the β cell dysfunction is not due to glucose toxicity in this model. On the contrary, the rat may well be a model of β cell glucose priming.

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G-PROTEIN-mRNA REGULATION: A MECHANISM FOR HETEROLOGOUS DESENSITIZATION OF INSULIN SECRETION IN INS-1 CELLS

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Prolonged exposure of cells to an agonist usually results in an attenuation of the cellular response. We investigated the regulation of mRNA levels of G-protein α_S- and α_{i2}-subunits in an insulin-secreting cell line (INS-1) and its influence on insulin secretion by northern blot analysis using specific DIG-labeled DNA probes and by RIA. Exposure to epinephrine (50 µM) for 8 h decreases α_S- and α_{i2}-mRNA levels to 58% and 72%, resp. After 24 h exposure α_{i2}-mRNA levels are increased to 150%, whereas α_S-mRNA levels reach control levels. Isoprenaline (10 µM) does not influence either mRNA level. Treatment with the specific α_2-agonist UK 14.304 (1 µM) (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline) shows the same effects as epinephrine indicating that epinephrine actions are mediated via α_2-adrenoceptors. Exposure to either incretin hormone glucagon-like peptide I (GLP-1) (10 nM) and gastric inhibitory polypeptide (GIP) (10 nM) increases α_S- and α_{i2}-mRNA levels (GLP-1: 129% and 137%; GIP: 131% and 182%). The Ca^{2+}-channel blocker verapamil causes a reduction of α_{i2}-mRNA (85%). Incubation with either GIP or GLP-1 together with verapamil decreases α_{i2}-mRNA levels (54% and 77%) showing that the increase of α_{i2}-mRNA depends on Ca^{2+}-influx into the cell. This regulation of mRNA levels is accompanied by reciprocal changes in insulin secretion. Looking at heterologous desensitization an 8 h preincubation with UK 14.304 leads to an increased insulin secretion due to stimulation with GLP-1 (227%) or 8.3 mM glucose (137%) compared to non-pretreated cells (143%, 100%).The GIP effect on insulin release is decreased by pretreatment with GLP-1 (239% to 123%), the UK 14.304 effect from 37% to 27% and the glucose effect from 100% to 47%. Similar results were obtained for GIP-preincubation. The modulation of insulin secretion parallels the regulation of α_{i2}-mRNA levels. We, therefore, conclude that α_{i2}-mRNA regulation plays an important role in heterologous desensitization of insulin secretion.

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B-CELL GLUCOTOXICITY AND HEXOSAMINE BIOSYNTHESIS PATHWAY

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We tested the hypothesis that glucosamine, a putative activator of glucotoxicity in vitro through acceleration of the hexosamine biosynthesis pathway, may also play a role in glucose-induced impairment of B-cell function. Pancreatic islets were isolated by collagenase digestion from 220-280g male Wistar rats. After 48h islets culture with DMEM containing 5.5 or 22mM glucose, 16.7mM glucose-induced insulin secretion from the islets was estimated by static incubation. 16.7 mM glucose-induced insulin secretion of islets cultured with 22mM glucose was significantly decreased compaired with that of islets cultured with 5.5mM glucose(50.8±10.8 vs. 89.5±7.9, µU/islet/h, $p<0.01$). This impairment of insulin secretion by high glucose culture was partially improved by co-culture with 10µM azaserine, a specific inhibitor of the rate-limiting enzyme(glutamine:fructose 6-phoshate amidotransferase) of hexosamine pathway(68.2±6.7 vs. 50.8±10.8, $p<0.05$). 16.7mM glucose-induced insulin secretion of islets cultured with 5.5mM glucose was significantly inhibited by co-culture with 5 or 20mM glucosamine in a dose-dependent manner(106.8 ±9.6 vs. 63.7±8.8, 33.4±5.6, $p<0.05$), whereas 20mM arginine-induced insulin secretion was unaffected by co-culture with glucosamine. On the other hand, 5mM mannosamine or 5mM galactosamine, added to the same protocol, had no significant effects on 16.7mM glucose-induced insulin secretion. These results indicate that an increase of glucosamine in B-cell hexosamine biosynthesis may play an important role in B-cell glucotoxicity.

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ACTIVATION BY GLUCOSE OF THE CARBOXYL METHYLATION OF γ SUBUNITS OF TRIMERIC G-PROTEINS IN PANCREATIC β CELLS

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Like most low molecular mass G-proteins, γ subunits of trimeric G-proteins undergo post-translational modifications (e.g., carboxyl methylation; CM) at their C-terminii. Herein, we examined whether CM of Gγ is regulatable in isolated β (HIT-T15) cells. Using selective antisera, we localized γ_1,γ_2,γ_5, and γ_7 isoforms in β cells. Of these, GTP[S] significantly stimulated the CM of γ_2 and γ_5 in broken cells. Acetyl farnesyl cysteine (100 µM), a competitive inhibitor of prenyl cysteine methyl transferases, completely inhibited the CM of these proteins. Exposure of intact HIT cells to either glucose (11.1 mM) or KCl (40 mM) resulted in a rapid (within 30 sec) and sustained (at least up to 60 min) stimulation of γ subunit CM. Immunoprecipitation studies indicated that glucose and KCl differentially modulated the carboxyl methylation of each of the four isoforms. Either removal of extracellular calcium (via EGTA) or depletion of intracellular GTP (via the synthesis inhibitor, mycophenolic acid) markedly attenuated the ability of glucose or KCl to stimulate the CM of these proteins. Pretreatment of intact HIT cells with pertussis toxin (PTx; 100 ng/ml overnight), which selectively inactivates G_i and G_o, markedly attenuated the stimulatory effects of glucose or potassium without altering the concomitant rises in $[Ca^{2+}]_i$. Glucose-induced CM of isoforms γ_2 and γ_5 was abrogated by coprovision of (2 µM) indomethacin. Conversely, PGE_2 (10 µM), which is known to activate G_i and G_o in HIT cells and thereby would dissociate α from βγ, stimulated the CM of isoforms γ_2 and γ_5 in intact and broken HIT cells. These data indicate that the CM of γ subunits of trimeric G-proteins in insulin-secreting cells may be facilitated by dissociation of the trimer into α and βγ. Regulation of such a cascade by glucose, an effect dependent on calcium influx and the consequent activation of phospholipases releasing arachidonic acid, implies a role for the CM of γ subunits in β cell function.

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DIFFERENTIAL IMPACT OF PALMITATE AND GLUCOSE ON INSULIN RELEASE FROM INS-1 CELLS.

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Studies in β-cell lines and islets have demonstrated that elevated glucose concentrations have a detrimental effect on the ability of the β-cell to secrete insulin. In vivo and in vitro studies have furthermore revealed that elevated levels of free fatty acids induce impaired β-cell function corresponding to the abnormalities being present in NIDDM. Since increased glucose and lipid levels often operate concomitantly in diabetes, it is important to clarify how the combination of elevated glucose and fatty acids influence insulin secretion from β-cells. The aim of the present study was to elucidate how the exposure to high palmitate and glucose in combination influences the insulin release from INS-1 cells. Firstly, we focussed on insulin output after culturing for 4 days at either 3.3, 6.6, 11.0, or 16.7 mM glucose with concomitant addition of 0, 0.05, 0.1 or 0.2 mM palmitate respectively. Subsequently, we tested the responsivness of the INS-1 cells to 3.3 mM (basal secretion) and 16.7 mM glucose (stimulated secretion). Cells were cultured in a modified RPMI 1640 medium containing different glucose and palmitate concentrations. Insulin secretion was measured by RIA. Mann-Whitneys nonparametric test was used for comparisons. At all glucose concentrations applied (3.3-16.7 mM) the addition of 0.05 mM palmitate for 4 days caused an increase in insulin output ($p<0.05$), whereas the exposure to higher palmitate concentrations (0.2 mM) resulted in decreased insulin output ($p<0.05$) compaired to culturing without palmitate. After 4 days culture in the prescence of 3.3 mM glucose and 0.1 mM palmitate basal insulin secretion was increased compaired to culturing without palmitate ($p<0.05$), whereas the addition of 0.2 mM palmitate had no influence on basal insulin secretion. In contrast in the prescence of higher glucose levels (6.6-16.7 mM) palmitate (0.1-0.2 mM) caused an inhibition of basal insulin release ($p<0.05$). When culturing the INS-1 cells in increasing glucose levels (from 3.3 to 16.7 mM) the glucose stimulated insulin secretion declined. The simultanous addition of an increasing amount of palmitate resulted in a more pronounced suppression of glucose stimulated insulin secretion. **In conclusion** We have demonstrated, that the inter-relationship between glucose and palmitate plays a crucial role for the INS-1 cell function.

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Beta-Cell Growth and Differentiation and Islet and Pancreas Transplantation

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ROLE OF MAP KINASE IN THE FORMATION OF INSULIN-PRODUCING CELLS FROM PANCREATIC AR42J CELLS

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Rat pancreatic AR42J cells are derived from an acinar cell tumor and possess properties characteristic of both exocrine and neuroendocrine cells. When AR42J-B13 cells, a subclone of AR42J, are exposed to a combination of activin A and hepatocyte growth factor (HGF), they stop growing and convert into insulin-secreting cells within three days. The present study was conducted to identify the signal transduction pathway involved in the differentiation of AR42J cells induced by activin A and HGF. AR42J cells were incubated for three days with activin A and HGF in the presence or absence of wortmannin, rapamycin or PD098059. Then insulin-producing cells were determined by immunocytochemistry. Wortmannin, an inhibitor of phosphatidylinositol 3-kinase did not affect the formation of insulin-positive cells induced by activin A and HGF. Likewise, rapamycin, an inhibitor of activation of p70 S6 kinase, did not affect the effect of activin A and HGF. In cotrast, PD098059, an inhibitor of activation of MAP kinase kinase, inhibited the formation of insulin-positive cells in a concentration-dependent manner. In AR42J-B13 cells, HGF but not activin A induced a sustained activation of MAP kinase, which was blocked by PD098059. These results suggest that the MAP kinase pathway is involved in the formation of insulin-secreting cells from amylase-secreting AR42J cells.

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REDUCED INSULIN RELEASE FROM MICROENCAPSULATED PANCREATIC ISLETS AFTER TRANSPLANTATION.

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The application of a semipermeable membrane as a mechanical barrier is an attractive approach to the protection of transplanted tissue from the host's immune system. The present study was undertaken to assess the function of islets microencapsulated in alginate-poly-L-lysine-alginate (APA) membranes after their transplantation into streptozotocin-diabetic and normal, non-diabetic recipients. Isolated rat pancreatic islets were microencapsulated in APA-membranes and transplanted intraperitoneally into recipients. Islet containing microcapsules were recovered by peritoneal lavage 14 days after transplantation into diabetic mice and after 1, 7 and 14 days after implantation to normal recipients. Recovered microencapsulated islets were subjected to determination of the in vitro insulin release during incubation.

Microencapsulated islet transplantation restored normoglycemia in diabetic recipients. In contrast, the freshly-prepared free islet grafts were rapidly rejected.

The microencapsulated islet implants removed from diabetic recipients showed a significant response to 20 mM glucose plus 1 μM forskolin challenge during incubation in vitro, although the amount of insulin released to the incubation media was significantly reduced as compared to freshly-microencapsulated islets.

Reduction of insulin secretion was also found during incubation of microencapsulated islets retrieved from normal recipients 1, 7 and 14 days after transplantation. The insulin secretion from islet explants from diabetic mice did not differ from that of normal controls (0.68±0.2 vs. 0.93±0.2 ng/45 min/islet; $p>0.05$).

Our results suggest that islet transplantation into peritoneal cavity may impair islet function. This finding may explain why the relatively large number of islets is required to achieve normal or near-normal blood glucose control in diabetic recipients.

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Properties of Human Pancreatic β-Cell-Lines Developed Using Retroviral Vectors Expressing SV40 T-Antigen and H-ras^{val12}.

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Our goal is to develop cell lines from human β-cells for diabetes therapy and for research on pancreatic endocrine cell biology. We have created human pancreatic endocrine cell lines, HAP-5, TRM-1, and TRM-6, from 18 week ICCs and 24-week fetal islets, respectively. They were isolated using a retroviral vector expressing SV40 T-antigen and H-ras^{val12}. This vector, LoTPRRNLo, contains a *lac* repressor binding site in the LTR for inducible oncogene expression. The cell lines express markers consistent with their origin from endocrine cells and endocrine cell precursors, including low levels of insulin in early passages. However, insulin expression was lost over time. Interestingly, later passages of the adult β-cell line HAP-5 expresses glucagon and somatostatin, but not insulin, by both RT-PCR and radioimmunoassay. The fact that cell lines from mature endocrine cells produce only low levels of pancreatic hormones suggests that oncogene expression is causing dedifferentiation. We have found that primary pancreatic endocrine cells undergo similar changes in differentiation state when grown in monolayer culture on extracellular matrix in the presence of hepatocyte growth factor, suggesting that the signal transduction pathways that are being stimulated in the primary cells and in the cell lines are overlapping. Expression of the lac repressor gene in the cell lines results in growth arrest due to oncogene downregulation. Current efforts are focused on inducing differentiation in the cell lines as well as developing new cell lines using oncogenes that may permit retention of differentiated function. In summary, we have developed an efficient system for creating human pancreatic β-cell lines, but more work needs to be done on maintaining differentiated characteristics.

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SUCCESSFUL ISLET TRANSPLANTATION USING PLANAR TYPE DIFFUSION MEMBRANE DEVICES

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Immunobarrier devices may protect transplanted islets from immune destruction in diabetic recipients, but there are questions about the number of islets that be supported within these devices. Alginate (1%) solution was used to form a gel to prevent clumping of islets in the devices (two laminated PTFE membranes held together by titanium rings, Baxter Healthcare). Varying numbers of syngeneic mouse islets (1000, 750, 500, 250) were loaded into each device, which were implanted into epididymal fat pads of streptozocin diabetic B6AF1 mice. Some mice received two devices containing 500 islels each. After transplantation random blood glucose levels and body weight were measured for 4 weeks. The percentage of mice achieving glucose levels <200mg/dl were 34.2%(1000), 29.6%(750), 43.8%(500), 18.8%(250) and 59.3%(500×2). The mean insulin content of these devices for these groups ranged from 2.5 to 12.9 μg, with there being no correlation with the initial number of islets. Many mice maintained euglycemia for 12 weeks. These results provide new information about optimal packing density for these devices. With this approach 500 islets appears to be the optimal number with no benefit being obtained from adding larger numbers. Limitation of oxygen supply is likely to be responsible for this limitation in packing density. These results provide information about the sizes (surface area) of diffusion devices that are required for successful transplantation.

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Ex vivo expansion of human pancreatic endocrine cells on HTB-9 matrix.

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Cell transplantation, as a therapy for diabetes, will be facilitated by ex vivo expansion of cells without loss of differentiative characteristics. The aim of this study was to determine the optimal conditions for in vitro growth of functional human pancreatic endocrine tissue. We examined the mitogenicity of matrices from a variety of cell lines. Proliferation was more pronounced in cells growing on matrices from bladder carcinoma cell lines and significantly higher in monolayers grown on matrix from the human cell line HTB-9 ($p>0.05$). After 14 days there was a >100 fold proliferative increase, augmented to >200 fold when hepatocyte growth factor/ scatter factor was added. However, hepatocyte growth factor/ scatter factor induced a rapid decrease in insulin content. Without the growth factor, fetal cell monolayers increased 4 fold without insulin loss and 12 fold with insulin levels still at 40% of unexpanded cells. Adult islet cells increased 3 fold without insulin loss and 5 fold with insulin levels still at 75%, while retaining a normal response to secretagogues. Together, these results show that HTB-9 matrix provides the best stimulatory effects on replication of human endocrine cells with little loss of in vitro function.

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A BIO-ARTIFICIAL ENDOCRINE PANCREAS (BIO-AEP) FOR THE TREATMENT OF DIABETES.

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Immunoisolation is a potentially important approach to transplanting islets without any immunosuppressive therapy. Immunoisolation systems have been conceived in which the transplanted tissue is separated from the immune system of the host by an artificial barrier

A diffusion chamber for a bioartificial endocreine pancreas (Bio-AEP) was constructed by placing pancreatic B-cells trapped in a mixed matrix containing agarose and collagen-gel, supplemented with nicotinamide, in the center of a ring holder sandwiched between nuclepore membranes , which were shielded by silicone.

In vivo experiment, we studied nucleopore membranes of different pore size (0.6, 0.4, 0.2, 0.1 and 0.05um) employed as the semipermeable membrane providing a mechanical barrier between the endocrine pancreatic graft and the host's immune system. In a twenty-week experiment, a return to normoglycemia was observed in STZ-diabetic rats implanted with Bio-AEPs fitted with membranes of pore size 0.1 um. However, in other rats implanted with Bio-AEPs with membranes of differeant pore sizes, normoglycemia continued for only a very short period.

The experiment was evaluated a suitable pore size for the nuclepore membrane for immunoisolation during xenoimplantation of the Bio-AEP. The protective effect of the Bio-AEP from humoral immunity was determined in vitro, using sensitized sheep erythrocytes (EAs). A compliment protein could not destroy the cell membranes of EAs in the diffusion chamber with 0.1um pore size.

The results indicated that the Bio-AEP with a 0.1 um pore size membrane should be useful for the implantation of xenograft pancreatic endocrine cells in diabetic animals and may open a new field in the therapy of human IDDM.

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LONG-TERM INCUBATION OF HUMAN FETAL ISLETS WITH GROWTH HORMONE: A DOSE DEPENDENT EFFECT ON INSULIN SECRETION

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In our previous studies we have shown that a long-term in vitro incubation with growth hormone (GH) preserves the insulin secretion capacity of isolated human fetal islets in the pretransplantation period, but the dose dependency of this effect has not yet been clarified. The aim of this study was to analyse the effect of a long-term (15 days) incubation with 250 μg/l (protocol A) , 500 μg/l (protocol B), 1000 μg/l (protocol C) and 2000 μg/l GH (Genotropin, Kabi Pharmacia) (protocol D) on glucose+theophyllin-stimulated insulin response of isolated human fetal islets. The islets were obtained from pancreata originating from 16 - 24 old fetuses by collagenase digestion, and cultured in the media containing 10% fetal calf serum at 37^0C, 5%CO2. The insulin secretion capacity was evaluated by determining insulin levels in the culture media after one hour incubation with 1.67 and 16.7 mmol/l glucose+5mmol/l theophyllin sequentially and expressed as the percentage of the increase in insulin levels after stimulation. We found that in protocols A and B the glucose+theophyllin-stimulated insulin response declined significantly, showing similar patterns of the decline in the islets incubated with GH and in those without GH (A: GH+: day 0: 736.5+/-61.6%, day 15:478.9+/-39.4%, $p<0.05$; GH-: day 0: 342.6+/-31.6%, day 15: 235.7+/-29.7%, $p<0.05$; B: GH+: day 0: 747.5+/-72.6%, day 15: 513.8+/-45.8%, $p<0.05$; GH-: day 0: 331.2+/-29.5%, day 15: 219.8+/-34.6%, $p<0.05$). However, in protocols C and D, the insulin response did not decline in the GH-incubated islets (C:day 0: 731.7+/-69.5%, day 15: 735.5+/-64.8%; D: day 0: 737.8+/-67.8%, day 15: 734.+/-72.8%; p=NS respectively). Simultaneously, the secretory response remarkably declined in the control islets incubated without GH (C: day 0: 357.6+/-37.9%, day 15: 232.8+/-27.8%; D: day 0: 332.6+/-39.3%, day 15: 253.2+/-28.4%, $p<0.05$ respectively). Our results have demonstrated that the long-term incubation with GH was efficient in preventing the decline of the in vitro insulin secretory capacity of isolated human fetal islets, but this beneficial effect was dose dependent and it could not be achieved with very low GH concentrations.

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A COMPARATIVE STUDY OF ALLOXAN TOXICITY IN ISLET GRAFTS PREPARED FROM DIFFERENT SPECIES

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Alloxan has for long been known as a diabetogenic substance in experimental animals, but we recently showed that human islets are resistant to alloxan. There are, at present, no systematic comparative studies with regard to the alloxan sensitivity of β-cells in different species. Therefore, we compared the alloxan sensitivity of grafts prepared from adult human, mouse, rat, dog, guinea pig and fetal pig islets.

For this purpose the islets were transplanted under the kidney capsule of nude mice. Two weeks later (eight weeks in case of fetal pig islets) alloxan (85 mg/kg) or saline (controls) was injected i.v. One week thereafter the mice were killed and the islet grafts and pancreata fixed for immunohistochemistry. The number of β-cells was then evaluated with a semi quantitative method in sections immunostained for insulin. As expected the endogenous pancreata showed total β-cell destruction in all alloxan injected recipients. The morfology of the human islet grafts did not differ from that seen in the saline controls. The same hold true for the β-cells of the guinea pig and pig islet grafts. Quite in contrast, the β-cells of the islet grafts from mouse ($p<0.001$ vs control, non-parametric Mann-Whitney test), rat ($p<0.001$) and dog ($p<0.05$) were almost totally destroyed. Preliminary data suggest that in alloxan-exposed guinea pig islets there was a high proliferative activity evidenced by auto-radiographical examinations.

This study shows that there are distinct species differences with regard to the β-cell sensitivity to the classical diabetogenic substance alloxan. The mechanisms involved have so far not been possible to identify, but it is obvious that the present experimental protocol lends itself very suitable for such studies. The finding that, in relation to man, both close (pig) and more distant (guinea pig) species have alloxan-resistant β-cells might implicate that different mechanisms, evolved separately, underly this resistance.

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DISTRIBUTION AND DEVELOPMENTAL CHANGE OF ANNEXIN V IN RAT PANCREATIC ISLETS

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Annexins are a family of structurally related proteins which bind to phospholipids in a Ca^{2+}-dependent manner. Annexin V is a member of the annexin family and it has recently been reported to form Ca^{2+}-channels and may act as a regulator of protein kinase C. Thus, annexin V may play a role for hormone secretion and differentiation in endocrine tissues. In this study, we investigated the expression patterns and the dynamic changes of annexin V in the rat pancreatic islets during postnatal development by both Western blot analysis and immunohistochemistry using polyclonal anti-annexin V antibody. We performed the isolation of pancreatic islets from 3 weeks-old (w.o.) to 15 w.o. of male Sprague-Dawley rats for Western blot analysis. Also rat pancreas from 1 w.o.to 15 w.o. were fixed with 4% paraformaldehide in PBS and embedded in paraffin for immunohistochemistry. Pancreatic islets were exclusively shown to express annexin V, while exocrine tissues were not. Capillary endotherial cells in pancreas were also stained. Annexin V was present in both α and β cells in islets. Strong staining was detected on the cell membrane and in peri-membranous area of islet cells. The expression level of annexin V in pancreatic islets gradually increased with postnatal development, but it became constant after 12 weeks-old. The present study suggested that annexin V may be related to the maturation of the function of pancreatic islets.

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NORMAn PANREATIC ISLETS TRANSPLANTATION IMPROVES DIABETIC PROGRESSION OF NIDDM MODEL(OLETF) RATS

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To investigate the effect of normal islets transplantation on NIDDM, we employed Otsuka Long Evans Tokushima Fatty (OLETF) rats which manifest obesity, degeneration of pancreatic islets, proliferation of mesangial matrix and nodular lesion of renal glomeruli as a NIDDM model rat. Normal islets from Wister King A rats were implanted into the portal vein of OLETF rats at their age of 12 weeks before onset of diabetes (n=7). Normal 1500 islets were cultured at 24℃ for 7 days and transplanted into the liver. Fasting plasma glucose and insulin, body weight, and histopathological changes in the pancreas and kidneys were evaluated 33 weeks after transplantation. Body weight gain in the transplanted group rats was started to be suppressed 6 weeks after transplantation ($p<0.05$). Islets transplantation lowered fasting plasma glucose level 20 weeks after grafting ($p<0.05$), but leaving fasting insulin levels unaffected. Hyperplastic and fibrotic changes in pancreatic islets of the transplanted group were suppressed 33 weeks after grafting. The proliferation of the mesangial matrix in gromeruli of the transplanted group was suppressed in comparison to those of controls (normal gromeruli 10.9% vs 24.6%, proliferation of the mesangial matrix: mild 44.2% vs 52.7%, moderate 24.6% vs 14.5%, diffuse 20.2% vs 6.9%; control vs transplanted group, respectively). These results indicate that transplantation of normal pancreatic islets into the portal vein of OLETF rats improves progression of the NIDDM model.

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β-CELL MASS DYNAMICS DURING GLUCOSE INFUSION

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Bonner-Weir and colleagues previously demonstrated that infusion of 50% dextrose for 4 days resulted in a 60% increase in β-cell mass, a 5 fold increase in β-cell replication rate and a 60% increase in individual cell size. Although no morphologic evidence of apoptosis was found, calculation of cell numbers after 4 days of glucose or saline infusion suggests that the increase in mass was not due to an increase in the number of cells and that the 5 fold increase in replication must be balanced by a 5 fold increase in cell death. Presently, we have re-examined this paradigm in an effort to elucidate the dynamic changes in β-cell mass. 50% dextrose was infused at 2 ml/min. Cohorts of glucose infused rats were killed on days 0 (n=3), 1 (n=4), 2 (n=3), 3 (n=4), 4 (n=4), 5 (n=2) and 6 (n=2) and compared to similar cohorts of control animals. During glucose infusion, tail vein plasma glucose levels rose from 6.9 ± 0.1 mM to 25.4 ± 0.8 mM after 1 day. Plasma glucose remained elevated after 2 days of infusion (24.4 ± 1.7 mM) but was reduced by day 3 (11.6 ± 1.1 mM) and persisted at this level for the remainder of the infusion period. There was no significant effect of glucose infusion on plasma free fatty acid levels ($P > 0.05$). β-cell mass was increased in response to the glucose infusion ($P < 0.05$). β-cell mass dynamics were complex, giving the best fit to a 3rd order polynomial of β-cell mass versus time. There was an initial increase in β-cell mass in the first day (6.1 ± 0.8 versus 4.2 ± 1.0 mg), a plateau between days 2 and 5, and a further increase to 9.9 ± 0.4 mg on day 6. These data suggest that β-cell mass dynamics during glucose infusion do not mirror changes in plasma glucose. The presence of hyperglycemia may restrain the growth in the β-cell mass until sufficient adaptation has occurred. (Supported by Medical Research Council of Canada).

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Inefficacy in prolonging pancreatic islets allograft survival after multiple oral administrations of low doses of highly purified islets.

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Oral antigen administration has been shown to prolong allograft survival. The aim of this study was to investigate the implications of the effects which repeated administrations of purified antigenic material in inducing immunologic tolerance of islets transplantation. We used genetically and antigenically distant strains of inbred rats such as Lewis and PVGs. Streptozotocin-induced diabetic PVG rats received daily an intragastric administration of purified islets from donors Lewis rats. These injections were performed from 7 days before TX until rejection occurred. No immunosuppressive agents were administred. Rats were injected with 3000 fresh Lewis islets into an affluent of their superior mesenteric vein..We did not find any significant difference between islet survival rates in the tolerized and control groups: mean graft survival was 3 days (range 0-4) for the tolerized group and 2 days (range 0-7) for the control group. Histological analysis from the animals with the shortest survival of the graft shows the presence of endothelitis in association with large infiltration of monocytes in the liver. On the other hand, animals with a longer survival of the graft did not have the endothelites. These results suggest that multiple oral administrations of low doses of highly purified islets is not as effective in achieving islets survival rate as those reported for single dose administration of low purified pancreatic tissue. The results obtained make us believe that at this moment in time this experiment is not apt to induce allograft immunologic tolerance.

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LIMITED REPLICATION OF PANCREATIC ß-CELLS CHRONICALLY EXPOSED TO HIGH GLUCOSE.

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To study the effects of chronic exposure to high glucose on pancreatic ß-cell replication, rat islets were cultured at 11.1 and 22.2 mM glucose for 7 or 14 days with or without human growth hormone (hGH) (1µg/ml). BrdU was added for the final 24 hours of culture; ß-cell replication was expressed as percentage of BrdU positive ß-cells. Insulin secretion was measured by RIA. ß-cell replication was increased after 7 days of culture at 22.2 mM glucose (11.1 mM: 1.01±0.19%; 22.2 mM: 1.64±0.19%, $p<0.05$), but not after 14 days (11.1 mM: 0.21±0.04%; 22.2 mM: 0.33±0.09%). However, islets cultured at 22.2 mM glucose for 14 days maintained a higher insulin secretion (11.1mM: 1.98±0.18 ng/h/islet; 22.2mM: 3.03±0.41 ng/h/islet, $p<0.05$). hGH increased ß-cell replication both in islets cultured at 11.1 mM glucose (7 days: 4.24±0.48%, $p<0.001$; 14 days: 1.99±0.18%, $p<0.001$) and 22.2 mM glucose (7 days: 2.64±0.23%, $p<0.01$; 14 days: 1.89±0.10%, $p<0.001$) compared to islets cultured with no hGH. In summary, when ß-cells were chronically exposed to high glucose the capacity to increase replication in response to glucose became limited, but islets were able to respond to other mitogenic factors, suggesting that the limited ß-cell replication was specific for glucose.

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EFFECT OF VASOACTIVE INTESTINAL POLYPEPTIDE ON INSULIN SECRETION FROM ISLETS AFTER TRANSPLANTATION OR PROLONGED CULTURE

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Transplanted mouse islets loose their responses to cholinergic and adrenergic stimulation. To test whether the peptidergic response is also modified by prolonged denervation, we studied the effect of vasoactive intestinal polypeptide (VIP) on glucose-induced insulin release from islets kept in culture or transplanted under the kidney capsule of syngeneic BALB/c mice. Insulin secretion induced by 16.7 mmol/l D-glucose was potentiated by VIP (10 nmol/l) by 56±10% (mean±SEM; $p<0.05$) in fresh islets hand-picked directly after collagenase digestion. When islets were purified by dextran density centrifugation (dextran islets) and cultured overnight, the VIP potentiating effect was lost (12±9%). When dextran islets were transplanted to the kidney for 4 days, removed, and perifused in vitro, the VIP potentiating effect was partially recovered (31±9%, $p<0.05$). The VIP effect was eventually lost 21-day after transplantation (-2±10%). When islets were hand-picked without dextran (hand-picked islets) the potentiating effect of VIP was not affected by overnight culture (56±16%, $p<0.02$). Four days of culture abolished the potentiating effect of VIP in both dextran and hand-picked islets (-19±22%, and -7±16%). When VIP (10 nmol/l) was added to culture medium, the VIP potentiating effect was diminished in both overnight cultured dextran and hand-picked islets (-14±11%, and -13±24%). However, a marked potentiating effect of VIP was observed in 4-day cultured dextran or hand-picked islets (440±170%, and 249±49%). The content of VIP extracted from 4-day islet transplants (423±123 pg/mg wet) was larger than that from 12-week transplants (2.3±0.1) or fresh islets (64±16). In conclusion, the effect of VIP on glucose-induced insulin secretion from islets was diminished by transplantation, dextran and prolonged-in-vitro-culture. The improved response to VIP in 4-day transplants might be related to the high VIP content in the grafts. This concept is supported by the fact that an enhanced VIP response was achieved in 4-day cultured islets by exogenous VIP. We suggest that local neurocrine release of VIP is important for the secretory function of transplanted islets.

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LATE FETAL AND EARLY POST-NATAL MALNUTRITION IMPAIRS RAT ß-CELL DEVELOPMENT

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A rat model of perinatal malnutrition was developped to study the role of nutrition on the development of the endocrine pancreas. Severe caloric restriction (50% of control intake) beginning on day 15 of pregnancy, was applied to female rats. Three offspring groups were studied: control group (C), R group (maternal restriction until the end of lactation) and R/C group (maternal restriction until birth and nursing by control mothers). At day 1, 7 and 21, pups (5-7) were studied for their insulin content, ß-cell volume fraction and proliferative capacity (analysed by quantitative morphometry and incorporation of BrdU *in vivo*).
Maternal caloric restriction led to small-for-gestational-age newborns and severely growth-retarded weanlings (R pups). Rapid catch-up growth was observed in R/C pups. At all time points, significant reduction in pancreatic insulin content, ß-cell volume fraction and total ß-cell mass were observed:

	Ins content,µg/gland		ßcell mass,mg/gland		%labeled nuclei	
	d1	d21	d1	d21	d1	d21
C	8.4±1.2	48.5±2.9	1.07±0.06	2.78±0.42	3.0±0.1	1.2±0.2
R	5.2±0.9	17.9±4.3	0.70±0.06	0.87±0.23	3.0±0.3	1.3±0.2
R/C	-	38.5±4.5	-	1.58±0.18	-	1.0±0.1

At d21, total ß-cell mass and number were reduced by 70% in R pups and 45% in R/C pups, suggesting long-lasting impairment of this cell population. Beta-cell BrdU labeling index at each time point was not different between groups, suggesting malnutrition did not affect the ability of ß-cells to proliferate. At 3 weeks of life, the calculated mean islet number was reduced by 50% in R pups and 37% in R/C pups, accounting largely for the decrease in ß-cell mass.

In conclusion, perinatal caloric restriction impairs ß-cell development; impaired islet neogenesis can be suggested in this rat model.

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TYROSINE HYDROXYLASE IN RELATION TO SOMATOSTATIN IN THE PANCREAS AND ISLET GRAFTS

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During prenatal development, islet progenitor cells coexpress tyrosine hydroxylase (TH) and somatostatin (SOM). To study the co-localization of cellular TH-like immunoreactivity (LI) with somatostatin in differentiated cells under changed environmental conditions, islets from C57BL/6 mice were transplanted to the kidney of syngeneic hosts. For comparison, freshly isolated islets, cultured islets and islets in adult pancreas were also examined. TH-LI cells were demonstrated in all groups. The cells were about the same size as islet endocrine cells and were randomly scattered in the islet tissue. In the grafts TH-LI cells decreased after 3 days, whereas at all the other time points, up to 52 weeks, the number of TH-LI cells appeared to be the same as in adult pancreas. In the transplanted islets some of the TH-LI cells exhibited TH-LI long smooth nerve fiber like extensions. Restaining revealed co-localization of TH-LI and SOM-LI in islet cells. In normal islets *in situ*, 5 ± 2 % (mean ± SEM, 4 mice) of the cells coexpressed TH-LI and SOM-LI, while in 52-week old grafts the corresponding figure was increased ($p < 0.05$) to 29 ± 7 (5 mice). None of the TH-LI cells with nerve fiber like extensions exhibited SOM-LI. The results indicate that there are two populations of somatostatin cells in the islets, and that transplantation induces an increased expression of TH-LI in somatostatin cells.

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Alteration of ß and α cells development in the pancreas of GK rats. A quantitative study.

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In the GK rat, a genetic model of NIDDM, available histological informations concerning the development of the endocrine pancreas are conflicting.We have previously reported that total pancreatic insulin store in GK adult rats from our colony was depleted by 62% and that ß cell mass in GK pancreases was decreased to a similar extent (51%, p<0.05).The aim of this work was to extensively study the development of the endocrine pancreas in GK rat from late fetal life until adulthood.We have determined the relative volume and the mass of ß and α cells by immunohistochemistry and morphometry in 21.5 day-old fetuses, 4, 7 and 14 day-old newborns and 4 month-old adult rats in both GK and Wistar groups. Our results demonstrate that: 1) there is a very early alteration of ß cells development in the pancreas of GK rat: the total ß cell mass in fetal GK pancreas represents only 30% of control value. In newborn GK rats, the total ß cell mass was decreased by 60% compared to that of control newborns. In adult GK rats, the reduced ß cell mass was associated with a noticeable alteration in the architecture of large islets which displayed fibrosis, together with signs of disorganization of the mantle-core relationship. Concerning α cells, the alteration of their development is a late phenomenon compared to the ß cells alteration. A significant decrease of pancreatic concentration of α cells is detectable in 14-day-old GK rats and persists at adult age. In conclusion, these data indicate that the reduction of ß cell mass in GK rats is a n early event and not a consequence of diabetes (hyperglycaemia) and should be considered as a primary feature in the pathological sequences leading to diabetes in GK rats. It is now important to determine the reasons for the delayed fetal ß-cell growth in the GK model and to identify the respective involvement of ß-cell neogenesis and ß-cell replication in the determinism of this alteration.

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DONOR AGE DOES NOT INFLUENCE THE OUTCOME OF ISLET TRANSPLANTATION.

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To know the influence of donor age on the outcome of transplantation, we isolated islets from C57BL/6 mice, aged 32 weeks (Gr. A) or 8 weeks (Gr. B). The islet number isolated from Gr. A was higher than that from Gr. B (219±22 vs. 97±4 islets/mouse, P=0.0001). In addition, Gr. A had larger size (0.129±0.001 vs. 0.117±0.001 mm^2, P=0.0001) and contained more insulin (0.92±0.07 vs. 0.47±0.09 µg/150 islets, P<0.005) as compared with Gr. B. Three hundred islets isolated from Gr. A or Gr. B were syngeneically transplanted under left kidney capsule of streptozotocin-diabetic mice. Both groups had similar patterns of the decrease of blood glucose levels and the increase of body weights after transplantation. Normoglycemia was achieved at 4 weeks in both groups and maintained lifelong (43±4 and 42±4 weeks, respectively). In conclusion, the islets isolated from donors with different ages have different characteristics but both of them are effective on transplantation.

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OFFSPRINGS FROM STREPTOZOTOCIN DIABETIC RATS.LANGERHANS'S ISLETS AFTER BIRTH.

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Female Wistar rats,were injected with streptozotocin i.p;one week later they were divided in 3 groups:A)severely diabetic blood sugar level above 16.5mM/l,B)mildly diabetic between 6.5 and 16.5mM/l,and C)nondiabetic controls.All were mated with normal males.The areas of pup's Langerhans islets were measured 1 and 5 days after parturition.On the 1st day,in pups from mildly and severely diabetic mothers they were smaller than those from the nondiabetic controls(P<0.001);the areas in neonates from severely diabetic mothers showed a more intense decrease than those from mildly diabetic rats(P<0.001).On the 5th day,the areas of Langerhans islets in offsprings from normal mothers decrease and those in pups from diabetic mothers tends to normalize(P<0.01)particulary those from the severely sick group(P<0.01).35% of 65 offsprings from diabetic mothers had fasting hyperglycemia at that time.Inmunohistochemically determination of C-peptide in pancreas shows a significative reduction in pups 1, 5 and 15 days old compared with controls.In summary diabetes during pregnancy markedly reduced the development of the islets of Langerhans in fetus,with a tendency to normalize after birth.

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OPTIMAL INSULIN TREATMENT IN ISLET TRANSPLANTATION

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We have previously shown that insulin treatment reduces the beta cell mass needed to achieve normoglycemia in islet transplantation (Tx). To identify the optimal insulin treatment protocol, 6 groups of streptozocin-diabetic C57Bl/6 mice Tx with 100 syngenic islets, an insufficient beta cell mass to restore normoglycemia, were treated with insulin as follows: group 1 (n=9): day 10 before Tx to day 14 after Tx (-10 to 14); group 2 (n=11): day 6 before Tx to Tx day (-6 to 0); group 3 (n=11): Tx day to day 6 (0 to 6); group 4 (n=7): Tx day to day 14 (0 to 14); group 5 (n=8): day 10 to day 24 after Tx (10 to 24); group 6 (n=18): Tx but not treated with insulin. Grafts were harvested 60 days after Tx, and beta cell mass was determined. On day 60, normoglycemia had been achieved in 100% mice in groups 1, 4 and 5, in 73% in group 2, and in only 45% and 33% in groups 3 and 6 respectively (p<0.05 vs the other groups). Intraperitoneal glucose tolerance, determined only in normoglycemic mice, was similar in groups 1, 2, 4 and normal controls; groups 3, 5 and 6 showed high glucose values after glucose injection (p<0.05 vs normal controls). Beta cell mass was similar in grafts of normoglycemic mice. In summary, the beneficial effect of insulin was maximal when insulin treatment was initiated before Tx day and maintained 2 weeks after Tx. Transient severe hyperglycemia after Tx (groups 3, 5 and 6) had a deletereous effect in beta cell function that was not fully corrected when normoglycemia was achieved.

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TRANSPLANTED FETAL SHEEP PANCREAS: A USEFUL MODEL TO STUDY ENDOCRINE CELL DEVELOPMENT

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The potential of the fetal sheep pancreas as a transplantable source of insulin-producing cells to reverse diabetes after it is grafted, for example, into athymic mice, has been investigated and found to be wanting. This is because ß cells do not differentiate well after they are transplanted. Glucotoxicity is a potential explanation for this because the blood glucose level of recipient mice is higher than that of fetal sheep (7 vs. 1.5 mM). To test the effect of approximating these fetal conditions blood glucose levels of recipient athymic mice were lowered for 4 weeks from 7.3 ± 1.6 mM to a nadir of 3.7 ± 1.7 mM by administration of ultralente beef insulin pellets. This resulted in a 2.7-fold increase in ß cells as a percentage of epithelial-like cells in the graft (controls 17 ± 5, treated 46 ± 14%; P = 0.05) and a 5.9-fold increase in the number of glucagon-containing α cells (controls 7 ± 5, treated 42 ± 14%; P = 0.03). The increase in endocrine cells was probably due to improved formation from undifferentiated cells, but greater proliferation of the mature cells is also a possibility. The effect was transient with endocrine cell numbers diminishing once the effect of insulin administration ceased: ß cells - controls 12 ± 5, treated 10 ± 3; α cells - controls 26 ± 9, trated 11 ± 7%; P = not significant. The predominance of the α cell has been noted previously in transplanted fetal sheep pancreas and is a feature of the adult sheep islet. It is concluded that while transplanted fetal sheep pancreas may not be suitable for reversal of diabetes, it is a useful model for studying how pancreatic endocrine cells develop.

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AN EFFICIENT AUTOMATED METHOD FOR ISOLATION OF PANCREATIC ISLETS

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The first step in transplantation of islands of Langerhans from healthy donors to diabetic individuals requires an efficient automated method for isolation of the pancreatic islets from donors. Such a mechanism has been developed by Ricordi et al. This automated method has two main chambers, one for isolation and another for recirculation and collection. Recently, we have repeated this work building such an apparatus from glass. We observed that there was considerable loss of pancreatic islets due to path traveled from the isolation chamber to the collecting flask. This was experimentally checked by simply increasing the length of tubing. The shortest path between these chambers is obviously zero! We have therefore reconfigured the apparatus of Ricordi et al so that collection of the islets can take place at the isolation chamber. The isolation chamber remains the same as in Ricordi et al and it is kept at 37° C by immersion in a water bath kept at this temperature which in turn is located on top of a shaker. When tests from a stopcock on the isolation chamber reveal that islets are detected, the whole chamber is lifted from the water bath at 37° C and immersed in ice. There is the added advantage that in this way the number of filters required to isolate the islets reduces to one. The yield of islets obtained through this simplification of Ricordi et al increases even though the digestion chamber is not kept at 37° C continuously, provided extra care is taken in the isolation chamber in ice. The optimum time for stopping the digestion must be determined experimentally. As a conclusion, at this new method, the recovery for count of intact isolated pancreatic islets is found to be higher ($p<0.01$) than the classical reference method.

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MECHANISM OF DESTRUCTION OF FETAL PIG PROISLET XENOGRAFTS IN DIABETIC NOD RECIPIENT MICE

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Analyses of the cytokine mRNA expressed specifically during acute rejection of pig proislet xenografts in CBA/H mice have previously revealed a Th2-type cytokine profile. Autoimmune destruction of islet isografts in diabetic NOD mice has been characterised by others as a Th1-type response. The present study examines the susceptibility of pig proislet tissue to disease recurrence in diabetic NOD mice by comparing the intragraft cytokine mRNA profiles expressed during the destruction of NOD-scid islet isografts, SLA^{dd} pig proislet xenografts and SLA^{dd} fetal pig skin xenografts in diabetic NOD hosts. Isografts of NOD-scid skin were analysed in parallel as controls for the non-specific inflammatory response associated with the surgical procedure. Enhanced cytokine mRNA expression was defined as detection of transcripts at least 5 PCR cycles before corresponding NOD-scid skin isografts. NOD-scid islet isografts at days 3 - 8 post transplant showed enhanced expression of cytokine mRNA for IL-2, IL-3, IFN-γ, IL-4 and IL-5; by day 10 only IFN-γ and IL-2 transcripts were specifically enhanced (i.e. TH1-type profile). Histological analyses revealed the presence of damaged islet tissue from day 3. In comparison, pig proislet xenografts showed a marked delay in both graft damage (from day 8) and the enhanced expression of cytokine transcripts i.e. days 5-10 (IL-3) and days 6-10 (IL-2, IFN-γ, IL-4, IL-5, IL-10)i.e. Th0 -type profile. Absence of significant cytokine mRNA expression in NOD-scid skin isografts together with their histological integrity confirmed lack of disease susceptibility. Significantly, pig skin xenografts showed the same kinetics of enhanced cytokine transcript expression as pig proislet xenografts, suggesting that the cytokine profile in the latter grafts reflected xenograft rejection. This study suggests that pig proislet xenografts may be disease-resistant and that their destruction in diabetic NOD hosts is probably due to xenograft rejection.

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A CONTROLLED STUDY OF SIMULTANEOUS ISLET-KIDNEY (SIK) TRANSPLANTATIONS IN EIGHT IDDM PATIENTS.

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Simultaneous islet kidney transplantation is aimed at optimizing metabolic control, prevention of diabetic secondary complications, and improvement of quality of life. We report a controlled study on our last 8 consecutive simultaneous islet-kidney - transplanted IDDM patients and as a control group 8 IDDM patients with kidney transplants alone (KTA). A special immunosuppressive protocol was used and the study was carried out with a mean follow-up of approx. one year. Both patient groups were matched for sex, mean age, body mass index, mean time on dialysis and mean duration of diabetes. Islets were implanted by embolization into the liver via percutaneous-transhepatic catheterization of the portal vein system under local anesthesia. One year survival rate was 100% for patients in both groups, for kidney grafts 8/8 (KTA) and 8/8 (SIK), respectively, and for islet grafts 7/8 (86%). So far, 2 of 8 islet graft recipients (25%) achieved insulin independence, 10 and 12 months after islet implantation, respectively. Kidney rejection episodes were observed in 1/8 and 0/8 recipients, respectively. Mean HbA1c levels were 6.4% in SIK patients and 7.9% in KTA cases, respectively. Mean daily insulin requirement was 10 units in SIK, but 48 units in KTA cases. The number of hypoglycemic episodes per year in kidney transplant alone cases did not change significantly (> 2 pre and post tx) whereas none of the SIK recipients experienced hypoglycemic episodes thereafter (2.6 episodes pre tx). To summarize, ongoing islet allograft function has been documented for an average period of one year after islet grafting in 86% of our SIK recipients with insulin independence achieved in two cases. In contrast to kidney transplanted alone controls, no SIK transplanted patient experienced hypoglycemic episodes during one year follow-up and these patients demonstrated much better metabolic control.

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DEOXYSPERGUALIN REVERSES XENOGRAFT REJECTION OF ENCAPSULATED ISLETS

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To study whether 15-deoxyspergualin (DSG) reverses xenograft rejection of alginate-polylysine-alginate (A-P-A) microencapsulated rat islets, we subcutaneously injected DSG of 0.625 mg/kg body weight daily for 14 days to graft-failed diabetic BALB/c mice that had been intraperitoneally transplanted A-P-A microencapsulated rat islets. Significant decrease of blood glucose (BG) level was found at the 5th day of DSG injection when compared with BG before DSG rescue (11.96±1.31 vs. 14.92±0.79 mmol/L, n=12, p< 0.05). The mean BG level at 14th day of DSG injection was 9.52±1.75 mmol/L. Five days after ceasing DSG injection, BG raised to level similar to that of before DSG injection. The mean body weight of these mice before and after two weeks of DSG injection was 23.5±1.1 and 23.2±1.2 gm, respectively. In conclusion, DSG reverses xenograft rejection of A-P-A encapsulated islets even when overt hyperglycemia has developed.

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LONG-TERM RESULTS OF ISLET-AFTER-KIDNEY (IAK) TRANSPLANTATIONS IN IDDM PATIENTS.

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Islet transplantation in IDDM patients with established kidney grafts is aimed at optimizing metabolic control in order to prevent recurrence of diabetic glomerulosclerosis in the kidney transplant, to halt the progression of secondary complications and to improve quality of life. We report long-term results of 14 consecutive IAK-transplants in IDDM patients. Mean age (yrs) 38.7 ± 2.0, sex (m/f) 10 m, 4 f; body weight (kg) 64.9 ± 2.0, body mass index 23.1 ± 0.4; diabetes duration (yrs) 26.7 ± 1.7; years post kidney tx 5.2 ± 0.7. Number of islet equivalents (IEQ) transplanted per recipient per kg bodyweight 8,230 ± 934 yielded from a mean of 1.5 ± 0.3 donor pancreata. Islets were embolized into the liver (in one case into the spleen) after percutaneous-transhepatic catheter injection via the portal vein in local anesthesia. Primary islet function in terms of basal C-peptide ≥ 0.5 ng/ml after 2 weeks of islet implantation was observed in 14/14 cases. Six patients experienced islet graft failure after day 16, 17, 62, 120, 164, and 301. One patient died of recurrent silent myocardial infarction. Ongoing graft function was observed in the remaining 7 cases with basal C-peptide levels of 0.5-3.2 ng/ml and good metabolic control (HbA1c 6.3-8.2%). In 2 cases insulin-independence was achieved, 230 and 400 days post islet tx, respectively, In the other cases daily insulin requirement is so far reduced by 29-73%. No patient experienced hypoglycemic episodes. To summarize, ongoing islet allograft function has been documented for an average period of 2 years after islet implantation in 50% of our IAK recipients and insulin independence was achieved in two cases. These are, in our experience, remarkable results that strongly support the notion that islet replacement, even if not complete, is associated with very beneficial effects and the absence of hypoglycemia requiring assistance.

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AN IMBALANCE BETWEEN BETA-CELL BIRTH AND BETA-CELL DEATH CONTRIBUTE TO FAILURE OF ENCAPSULATED ISLET GRAFTS

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Alginate-polylysine microencapsulation allows for successful transplantation of allo- and xenogeneic islets in the absence of immunosuppressive therapy. However, the survival of microencapsulated islet grafts is limited, even if capsular overgrowth is restricted to a small percentage of the capsules. In search of other processes than overgrowth contributing to graft failure we have studied the islets in non-overgrown capsules at several time points after allotransplantation in the rat. All recipients of islet allografts became normoglycemic. Grafts were retrieved at four and eight weeks after implantation, and at 15.3 ± 2.3 weeks postimplant *i.e.* two weeks after the mean time period at which graft failure occurred. Overgrowth of capsules was complete within four weeks postimplant. It was usually restricted to less than 10% of the capsules. During the first four weeks of implantation, 40% of the initial number of islets were lost. Thereafter, we observed a decrease in function rather than in numbers of islets, as illustrated by a decline in the *ex vivo* glucose induced insulin response. At four and eight weeks post-implant, Beta-cell replication was ten-fold higher in encapsulated islets than in islets in the normal pancreas. But, these high replication rates were insufficient to prevent a progressive increase in the percentage of necrotic tissue in the islets. This necrosis mainly occurred in the center of the islets, which indicates insufficient nutrition as a major causative factor. Our study demonstrates that not only capsular overgrowth but also an imbalance between Beta-cell birth and Beta-cell death contribute to the failure of encapsulated islet grafts. Our observations indicate that we should focus on finding or creating a transplantation site which, more than the unmodified peritoneal cavity, permits for close contact between the blood and the encapsulated islet tissue.

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EFFECT OF ISLET TRANSPLANTATION ON HYPOGLYCEMIA AWARENESS AND COUNTERREGULATION IN IDDM PATIENTS.

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Iatrogenic hypoglycemia is the most frequent acute complication in patients with insulin-dependent diabetes (IDDM). The major risk factors include the clinical syndromes of defective glucose counterregulation, hypoglycemia unawareness and elevated glycemic thresholds. Therefore, intraportal islet transplantation alone (ITA) was performed in three non-uremic, non-kidney transplanted IDDM patients prone to severe hypoglycemia in whom defective glucose counterregulation and hypoglycemia unawareness have been demonstrated. We used the stepped hypoglycemic clamp technique to determine both the magnitude and the glycemic threshold of the secretory response of the major counter-regulatory hormones and of autonomic and neuroglycopenic symptoms during hypoglycemia and post ITx. Control data were obtained from 10 matched non-diabetic volunteers. Prior to ITx, the secretory response of glucagon and of epinephrine was either absent or severely impaired while the secretory response of cortisol was markedly delayed in all patients. No patient noticed any significant autonomic warning symptoms during hypoglycemia. When the same test was repeated one month after successful ITx (exogenous insulin treatment reduced by about 50%), no improvement of the secretory response of glucagon was observed. However, in two patients, both the magnitude (793 vs 75 and 691 vs 58 pg/ml) and the glycemic threshold (64 and 59 mg/dl vs unmeasurable) of the secretory response of epinephrine were even normalized. In one patient only the magnitude increased (241 vs 179 pg/ml) but without any change in the glycemic threshold (54 vs 55 mg/dl). Moreover, the glycemic threshold of the secretory response of cortisol was reduced in all patients (51 vs 45, 55 vs 49, 52 vs 44 mg/dl). All patients noticed autonomic warning symptoms well before severe neuroglycopenia occurred at a glycemic threshold of 48, 52 and 56 mg/dl, respectively. We conclude that intraportal islet transplantation seems to improve the sympatho-adrenal regulatory mechanism and hypoglycemia awareness even in patients with long-standing IDDM by a yet unknown mechanism.

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DECREASED IMMUNOGENICITY OF NEONATAL COMPARED TO ADULT PIG ISLET ANTIGENS IN NEWLY DIAGNOSED IDDM PATIENTS

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Porcine islets may substitute human sources for the purpose of islet transplantation to diabetic patients. The aim of our study was to determine whether pig islets derived from neonates differ in their immunogenicity from adult islets through evaluation of T-cell proliferation in newly diagnosed IDDM patients and healthy controls. In addition, we studied the correlation between T-cell reactivity to pig islet antigens and human insulin and the titer of various islet autoantibodies such as ICA, IAA, GAD, and ICA512. T-cells and sera of 16 IDDM patients and 15 healthy controls were studied. Adult pig islets were isolated using the collagenase method while a non-enzymatic procedure was used for the neonatal tissue. Purified islets underwent sonication and adjustment to an appropriate protein concentration. A stimulation index >2 was considered as a positive T-cell response. The percentage of positive responses in the two groups is presented in the table below:

	Insulin		Adult pig islets		Neonatal pig islets	
	0.5µg/ml	5µg/ml	1µg/ml	10µg/ml	1µg/ml	10µg/ml
Controls	0%	0%	20%	29%	0%	14%
IDDM	38%	40%	50%	60%	6%	27%

No association was found between a positive proliferative T-cell response and the titer of the various islet antibodies tested. In conclusion, our results suggest that neonatal pig islets induce a significantly lower T-cell proliferative response as compared to adult islets, indicating that the neonatal source may be immunologically more appropriate for the purpose of future islet xenotransplantation.

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POSSIBLE INVOLVEMENT OF VON WILLEBRAND FACTOR IN PANCREATICGRAFT THROMBOSIS IN SIMULTANEOUS KIDNEY-PANCREAS TRANSPLANTATION

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Early post operative graft thrombosis remains the second main cause of failure in pancreas transplantation. Thus, the aim of this study was to compare retrospectively coagulation and fibrinolysis in groups of simultaneous kidney and pancreas transplanted type I diabetic patients having, or not, experienced thrombosis of their pancreatic vein. From December 1990 to January 1996, 26 simultaneous kidney-pancreas transplantations with bladder drainage were performed in 26 uremic type I diabetic patients. Acute thrombosis of the pancreatic vein was observed in 4 patients (group A) whereas 22 patients did not develop thrombosis (group B). The control groups, composed of 15 patients each, were : kidney transplanted (group C) or haemodialysed (group D) non diabetic patients and type I diabetics with HbA1C < 8% (group E) or ≥ 8% (group F) who were not in end stage renal failure. Begining at least 6 months after transplantation, we analysed haemostatic factors (fibrinogen, thrombin and prothrombin times), coagulation inhibitors (C and S proteins), fibrinolysis (plasminogen inhibitor) and endothelial cell abnormalities (Von Willebrand factor: VWf). Micro- and macro-vascular complications were evaluated using a score ranging from 0 to 12. Haemostatic factors, coagulation inhibitors and fibinolysis were similar in both groups A and B. In contrast, VWf differed significantly in group A: 3.49±0.93 IU/ml versus group B: 2.04±0.92 IU/ml ($p<0.05$). VWf in group A was also significantly higher than that of control groups C, D, E and F. The score of vascular complications increased in both groups A and B but was significantly higher in group A: 9±0.81 versus 6.07±1.75 ($p<0.01$). A correlation ($R=0.812$, $p<0.05$) between the severity of vascular complications and VWf was observed. These results suggested the possible involvement of VWf in the pathogenesis of pancreatic graft thrombosis in kidney-pancreas transplantation.

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CHEMOTAXIS OF MURINE MACROPHAGES TOWARDS ENCAPSULATED PANCREATIC ISLETS : *IN VITRO* AND *IN VIVO* STUDIES.

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The aim of the present work was to study, *in vivo* and *in vitro*, the involvement of pancreatic islets in the cellular reaction observed at the surface of biocompatible artificial membrane after transplantation of encapsulated islets. Two hundred pancreatic rat islets encapsulated with the AN69 membrane (Hospal, France) by means of PTFE rings were cultivated at 37°C in RPMI medium or implanted for 2 and 7 days in the peritoneal cavity of diabetic mice (streptozotocine induced). Empty devices and free islets served as controls. *In vivo*, the cellular reaction observed on removed devices (empty or filled) was identified by SEM and phagocytosis tests. *In vitro*, a modified Boyden chamber was used to evaluate the migration of activated peritoneal murine macrophages toward the supernatant of cultivated islets. The chemotactic index was expressed as the ratio : number of cells attracted by the supernatant / number of cells attracted by culture medium. After 2 and 7 days of implantation, only few cells adhered on the empty devices while numerous cells, mainly macrophages (80%), colonized the devices containing the islets. The cellular adhesion did not modify the fiber network of the membrane (SEM). After 2 days of implantation, macrophages covered 45% of the surface of devices filled with islets versus 5% of the empty ones. These values reached respectively 65% and 15 % after 7 days. *In vitro*, after 2 and 7 days of culture, the chemotactic indexes of encapsulated islets were respectively 1.51±0.31 and 1.37±0.39 whereas that of free islets were higher: 2.40±0.42 and 2.63±0.47 respectively. *In vitro*, preliminary studies showed a stronger chemotactic effect of the supernatant of free islets after 1 hour incubation in 4g/l glucose in comparison with the effect observed with 1g/l glucose. Encapsulation of pancreatic islets decreases the chemotactic effect of islets suggesting that the artificial membrane blocked the chemotactic factor of islets. Insulin in high concentration is suspected to be one of these factors.

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GLUCAGON LEVEL AFTER PANCREAS AND KIDNEY TRANSPLANTATION. SEVEN YEARS FOLLOW UP.

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The aim of the study was to assess the level of glucagon in plasma of IDDM patients after simultaneous pancreas and kidney transplantation. The study group comprises 6 individuals (2 males and 4 females) aged 30-40 years. These patients had IDDM for 9-26 years complicated by chronic renal failure. Since the transplantation they are on the immunosuppressive treatment. Every 12 months the fasting level of C-peptide was measured to evaluate beta-cells secretion and the level of glucagon as indicator of alpha-cells function was controlled. The average value of fasting glycaemia was 5.0±0.2 mmol/l (90±3.6 mg/dl). HbA_1c fell from 8.3% in the first year after transplantation to 6% in subsequent years. Serum C-peptide values were in normal range (0.95-3.0 ng/ml).

In all patients more than 2-fold increase of plasma glucagon level was observed immediately after transplantation. High levels plasma glucagon sustained during whole period of observation. Plasma glucagon rose significantly - in average by 239.5% (the lowest 350 pg/ml (124%) - in 4th year and 580 pg/ml (284%) in 7th year (normal range 50-200 pg/ml). This increase of plasma glucagon did not correlate with the serum plasma C-peptide levels. Conclusion: Successful pancreas transplantation in patients with IDDM led to hyperglucagonemia which had no influence on carbohydrate tolerance. The reported high levels of glucagon might result from double quantity of alpha cells.

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ISLET MICROENCAPSULATION USING A PIEZOELECTRIC DROPLET GENERATOR

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For islet transplantation the size of microcapsules may play a crucial role. The diameter should preferably approximate that of the islets for an optimal diffusion of oxygen, nutrients and insulin. In this study we report a new encapsulation technique that produces small barium-alginat capsules. A piezoelectric droplet generator developed in our laboratory was used instead of the earlier air-jet procedure, by which a minimal diameter of about 700µm can be realized due to inevitable turbulence at the outlet of the nozzle. To evaluate the efficacy of the new method, a comparative study was performed. Porcine islets were microencapsulated in barium alginate beads using the new or old droplet generator. The piezoelectric resulted in 350-500µm transparent, spherical droplets. The whole encapsulation time did not exceed five minutes. comparatively, the diameter of microcapsules made by air-jet were 750 µm and the procedure took more than thirty minutes. Ten days later, the functionality of microencapsulated islets was assessed by determination of the glucose-dependent insulin secretion. The stimulatory insulin response did not differ between both groups ($p>0.05$). It was 140.08±38.04% over the basal secretion ($n=38, p<0.01$) with the piezoelectric generator and 96.98±19.72% ($n=41, p<0.001$) with the air-jet procedure. Haematoxylin-Eosin-stained sections of microencapsulated porcine islets confirmed the well-preserved viability. We conclude that the use of new droplet generator allowed the time-saving-production of small microcapsules and did not impair viability and glucose-dependent insulin secretion of porcine islets *in vitro*. Due to the 4-fold more diffusion-surface it might improve survival of encapsulated tissue after transplantation.

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TRANSPLANT THERAPY FOR END-STAGE DIABETIC NEPHROPATHY: A SINGLE CENTRE EXPERIENCE

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The aim of the present study was to compare the patient and graft survival rate, occurence of complications and metabolic control in 4 groups of transplant (Tx) recipients treated for end-stage diabetic nephropathy. Since 1983, isolated primary kidney Tx was performed in 56 Type 1 (group K1) and 26 Type 2 (group K2) recipients. In 38 recipients, combined kidney and segmental duct-occluded pancreas Tx was done (group Ps+K). Other 42 patients were treated by combined kidney and whole pancreas Tx with bladder drainage of pancreatic duct and extraperitoneal placement of the pancreatic graft (group Pw+K). Cummulative 2-year survival rates (%) are shown in the following table:

Group	n	Recipients	Kidney	Pancreas
K1	56	86	72	-
K2	26	74	54	-
Ps+K	38	72	56	35
Pw+K	42	90	80	78

The highest complication rate was found in the Ps+K group. After switching to the whole-organ technique (group Pw+K) the post-operative morbidity remained significant, but the number of serious complications was reduced. One year after Tx glycosylated hemoglobin was found to be in the normal range in Ps+K and in Pw+K groups but not in the K1 and K2 groups (mean ±SE 6.28±10, 4.85±0.09, 9.01±0.12 and 8.95±0.12 %, respectively). Glycosylated hemoglobin was lower and K value higher (intravenous glucose tolerance test) in the Pw+K than in the Ps+K group ($p<0.01$). We conclude that the results of Tx therapy in diabetic recipients have improved considerably in the recent time and that the combined whole-pancreas and kidney Tx is the most favorable treatment option for the majority of Type 2 uremic diabetic patients.

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INSULIN SECRETION AND PERIPHERAL SENSITIVITY ESTIMATED BY CIGMA IN PANCREAS-KIDNEY TRANSPLANT RECIPIENTS

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After successful simultaneous pancreas-kidney transplantation (SPK) normoglycemia is established in spite of the presence of insulin resistance and reduced β cell secretory capacity. To assess insulin sensitivity (IS) and β cell secretory capacity (BC) posttransplant, we introduced Continuous Infusion of Glucose with Model Assessment (CIGMA), as described by Turner (Oxford, UK). We performed CIGMA in 29 bladder-drained SPK patients with systemic insulin delivery (24 male/5 female) >1 year posttransplant and in 32 non-diabetic patients (21 male/11 female), matched for impaired creatinine clearance, with a kidney transplant and immunosuppression (KTx, n=18) or without a transplant (NoTx, n=14). The 3 groups were comparable with respect to mean±SD age (44±9 years), BMI (24±3 kg/m^2), HbA_{1c} (5.6±0.5%), and endogenous creatinine clearance (64±28 ml/min). Mean±SD glucose and insulin values after 1 hour infusion of 5 $mg.min^{-1}.kg\ IBW^{-1}$ glucose stimulation, were 8.6±1.8 mmol/l and 43±20 mU/l in the SPK group and 7.3±1.0 mmol/l with 36±21 mU/l and 8.4±1.6 mmol/l with 28±14 mU/l in KTx and NoTx respectively. Stimulated c-peptide measurements were 2.2±0.9(SPK), 2.9±1.1(KTx), and 2.6±0.9 (NoTx)nmol/l, and were inversely correlated with creatinine clearance ($r=-0.49; p<0.001$). Estimated IS was equal in non-diabetic KTx (69±39 U) and NoTx (71±36 U), indicating immunosuppression did not cause insulin resistance in KTx. However, IS was clearly diminished in the SPK patients (42±15 U). C-peptide values, corrected for creatinine clearance, were used to estimated BC to eliminate the effect of the liver's insulin extraction bypass. CIGMA showed no differences in BC between SPK (131±89 U) and NoTx (135±51). We conclude that, using this simple and accurate test, SPK recipients suffer from more insulin resistance than KTx patients, probably mainly due to the pre-existing diabetic state and hyperinsulinemia per se. The effect of immunosuppressive agents appears to be limited. Pancreas graft function is able to reach non-transplanted levels.

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DIPEPTIDYL PEPTIDASE IV RESISTANT ANALOGUES OF GLUCAGON-LIKE PEPTIDE-1: *IN VITRO* AND *IN VIVO* STUDIES

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The insulinotropic hormone glucagon-like peptide-1 (GLP-1) is a substrate for dipeptidyl peptidase IV (DPP IV), which is responsible for GLP-1 inactivation *in vivo*. The enzyme removes a dipeptide from the N-terminus to form GLP-1 (9-36)amide, which is an antagonist *in vitro*. DPP IV is highly specific and has strict substrate requirements. This study was undertaken to examine whether small modifications of the N-terminus of GLP-1 would confer resistance to degradation by DPP IV. Modified GLP-1 analogues were prepared by solid phase peptide synthesis, substituting the alanine at position 8 of GLP-1 with either threonine, glycine, serine or α-aminoisobutyric acid (Aib). All 4 analogues stimulated cAMP production in BHK cells transfected with the GLP-1 receptor. Incubation of the analogues with porcine plasma *in vitro* at 37°C revealed a prolonged t½ compared to GLP-1 itself (GLP-1, 28.3±1.5; thr^{8}GLP-1, 202±19; gly^{8}GLP-1, 161±21; ser^{8}GLP-1, 173±12 min) whilst degradation of Aib^{8}GLP-1 was undetectable after 6 hr. The analogues were studied in 4 groups of halothane-anaesthetised pigs, in which each group (n=4) received separate 30 min iv infusions of GLP-1 and one analogue, in a cross-over design with 80 min between each infusion. Blood samples were assayed for GLP-1 immunoreactivity using both C- and N-terminally directed antisera, except for Aib^{8}GLP-1 which could be measured only with the C-terminal assay. For all 4 groups, there was no difference between the C-terminal t½ for GLP-1 or the analogue (4.0±0.1 vs 4.2±0.4, thr^8 GLP-1; 4.3±0.5 vs 3.5±0.7, gly^8 GLP-1; 4.1±0.2 vs 4.2±0.2, ser^8 GLP-1; 4.3±0.3 vs 4.4±0.2 min, Aib^{8}GLP-1), but the N-terminal t½ for each analogue was prolonged ($p < 0.01$) compared to GLP-1 (0.7±0.2 vs 3.9±0.2, thr^{8}GLP-1; 0.9±0.003 vs 3.3±0.4, gly^8 GLP-1; 0.9±0.06 vs 3.7±0.4 min, ser^{8}GLP-1). For all 4 groups, the N-terminal t½ was shorter ($p < 0.01$) than the C-terminal t½ for GLP-1, but there was no difference between N- and C-terminal t½ for the analogues. These results indicate that modification of the N-terminus of GLP-1 makes the peptide resistant to DPP IV action. However, the plasma t½ *in vivo* cannot be extended beyond that determined by the C-terminal assay, due to the presence of other non-DPP IV-mediated degradation pathways.

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EFFECTS OF GLP-1 ON REGULARITY, FREQUENCY, AMPLITUDE, AND MASS OF COORDINATE PULSATILE INSULIN SECRETION IN HEALTHY HUMANS.

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GLP-1 is a peptide hormone released from the gut upon luminal stimulation. It is a potent insulin secretagogue, and a potential future drug in the treatment of NIDDM. The majority (~70%) of insulin is secreted as series of punctuated secretory bursts superimposed on a basal insulin secretion. In healthy humans insulin secretion is mainly regulated through perturbation of mass and frequency of the secretory bursts, and the mode of delivery of insulin into the circulation seems important for insulin action. To assess effects of GLP-1 on mass, frequency, amplitude and overall contribution of pulsatile insulin secretion, we applied a recently validated deconvolution model on insulin concentration data before and during infusion of GLP-1 and saline in 8 healthy humans. Following GLP-1 infusion there was an abrupt increase in the peripheral serum concentrations of C-peptide (696±65 vs 1538±165 pM) and insulin (49±8 pM vs 138±21 pM). This increase was mainly due to an increase in secretory burst mass (28.2±4.4 vs 100.1±15.8 pmol/L/pulse, $p<0.001$), and amplitude (4.3±2.2 vs 12.7±7.7 pmol/L/min, $p<0.002$), whereas the secretory burst frequency was not affected by the infusion of GLP-1 (11.5±0.7 vs 12.6±0.6 pulses/hour, $p=0.4$). Consequently the detected contribution of pulsatile to overall insulin secretion was increased from 56±4 to 77±4 percent ($p<0.005$). The regularity of the oscillating insulin secretion was not perturbed by GLP-1 as estimated by ApEn values ($p=0.7$). None of the above parameters changed during saline infusion ($p>0.3$) We conclude that GLP-1 infusion stimulates insulin secretion through amplification of the mass and amplitude of the insulin secretory bursts, amplifying the pulsatile component of insulin secretion.

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THE RECEPTOR MECHANISM INVOLVED IN NEURO-INCRETIN EFFECT OF GLUCAGON-LIKE PEPTIDE-1

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We have recently found that an intraportal appearance of glucagon-like peptide-I (7-36)amide (tGLP-1), but not full-length GLP-1, is specifically recognized by the hepatic vagal nerve, and this recognition further augments the pancreatic vagal efferent activity in a reflex way, suggesting a novel neuroincretin effect of the peptide. To clarify further the receptor mechanism involved in the hepatic vagal recognition of tGLP-1, the effect of exendin (9-39)amide (EX), a specific antagonist of tGLP-1 receptor in the beta cells, on the recognition was examined. A 1-min bolus intraportal injection of tGLP-1, but not the vehicle, increased by ca. 2-fold the impulse discharge rate of the hepatic afferent vagus for >90 min at a physiological (0.2 pmol) or a pharmacological (4.0 pmol) dose in rats anesthetized with urethan and chloralose ($P<0.01$ vs. the preinjection value, n=6 and n=7, respectively). When a 100 times molar dose of EX to tGLP-1 known to inhibit the insulinotropic effect of the tGLP-1 was administered intraportally 5 min before the tGLP-1 injections, the facilitating effect of the tGLP-1s on the vagal afferents was observed without any modification in the amplitude and time-course of the afferent activities (n=5, each). Moreover, when the EXs were administer-ed 10 min after the tGLP-1s, where the afferent activities had already started to increase by tGLP-1, the tGLP-1s again increased the hepatic vagal afferent activities unmodifiedly (n=5, each). The present results shows that the tGLP-1 receptor system involved in the hepatic vagal recognition for tGLP-1 is different from that in the beta cells, implying existence of subtype(s) in the receptor.

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STIMULATION OF PULSATILE SECRETION OF INSULIN AND GLP-1(7-36)AMIDE FOLLOWING BY ORAL GLUCOSE.

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GLP-1(7-36)amide (GLP-1) is secreted from inestinal L-cells of the distal jejunum, ileum and colon within a few min following nutritional stimulation in a pulsatile fashion, as shown previously. As GLP-1 has insulinotropic properties in vivo and in vitro we were interested in the coregulation of GLP-1 and Insulin (I) secretion. This topic was investigated in 5 healthy volunteers (mean age 27.8±3.6 y, BMI 23.4±1.2 kg/m²). A standardized oral glucose load with 100 g glucose (OG; Dextro-O.G.T, Boehringer, FRG) was performed after an overnight fast (app. 10 h) and after 1 h investigation of basal hormone secretion. The changes in plasma concentrations and dynamics of the secretion of GLP-1 and I induced by the glucose challenge were investigated for 1 h. Plasma hormone concentrations were determined radioimmunologicaly, GLP-1 using the C-terminally specific antiserum 89890 and I using an insulin specific ria. Pulsatile elements of GLP-1 and I secretion were determined by deconvolution with the DESADE program. Under fasting conditions we found 5.4±1.95 GLP-1 and 5.20±0.84 I pulses per h and pulse amplitudes for GLP-1 of 8.60±3.76 pmol and for I of 21.7±9.15 pmol. GLP-1 and I plasma levels increased app. +8 min following the OG . the frequency of GLP-1 and I pulses remained unchanged while pulse amplitudes of GLP-1 increased to 19.34±7.42 pmol and of I to 222.2±55.9 pmol ($P<0.05$, ANOVA). The integrated amount of hormone secreted (AUC) was basally 248±17.7 pmol x min for GLP-1 and 1337±50 pmol x min for I. During the 1 h after the OG the AUC of GLP-1 increased to 688±35 and of I to 10485±866 pmol x min. Crosscorrelation analysis revealed no correlation of the basal GLP-1 and I pulses, but a significant crosscorrelation after the OG for a time-lag -2.0±6.3 min; R=0.70±0.28; $P<0.05$). Conclusion: I and GLP-1 secretion is increased in parallel by glucose ingestion. A significant cosecretion of both peptides is found only after nutritional stimulation, but the time-lag between the pulse maxima of GLP-1 and I varies individually. Factors involved in the coregulation of GLP-1 and I might include neuronal or humoral mechanisms.

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IMMUNOCHARACTERIZATION AND FUNCTION OF GLUCAGON- LIKE PEPTIDE -1 ISLET RECEPTOR IN SYRIAN HAMSTER .

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Molecular and functional analyses were performed to show the presence of glucagon-like peptide -1 islet receptor (GLP-1R) in normal syrian hamster (SH). Three bands of 44, 71 and 134 kDA were detected using a polyclonal antibody (S. Mojsov) raised against the extracellular part of the rat GLP-1R detected in isolated SH islets (SHI) and HIT-T15. In cryptic intestinal cells and fibroblasts only one unspecific band of 134 kD was observed. Western blot of rat islets and RINm5F cells revealed a similar pattern except the addition of a band at 95 kD. A GLP-1R mRNA in SHI of 2.7 kb was detected using a human cDNA probe of 1.6 kb pair in low stringency conditions. A cDNA probe of 219 bp coding for the rat transmembrane stretchs IV and V of GLP-1R gave no signal in SH although both probes showed the expected transcripts at 2.7, 3.4 and 3.7 kb in rat islet and RINm5F extracts. In perfusion experiments, after 3.3 mM glucose for 25 min, the response at 8.3 mM (min 27-42) was biphasic. tGLP-1 at 10^{-9} M, for 25 min of exposure, caused a dramatic increase in insulin output reaching 705 ± 28 % of controls at 8.3 mM whereas the effect of glucagon reached only 207±8 % ($P<0.001$). Glucagon values, which significantly decreased at 8.3 mM glucose compared to the basal values (3.3 mM) did not decrease further upon perfusion of tGLP-1. In comparison, a human insulinoma showed a similar response to glucagon and tGLP-1 reaching 3.6 and 3.9 times the basal level i.e. 800 and 825 microU insulin /10^6 cells, respectively. Our data demonstrate the presence and the species specificity of the SHI GLP-1R. A GLP-1R recognizing part similar to that of rat with a 44 and a 71 kD forms corresponding to the immature peptidic and the glycosylated peptidic receptor respectively was observed. For mRNAs the nature of the transcripts differs from that of rat and human. The insulinotropic activity of tGLP-1 through its receptor is five times that of glucagon. Thus, evidence for the presence of a functional GLP-1R in the SHI was provided by our experiments.

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ATROPINE BUT NOT α- OR β-ADRENERGIC BLOCKERS EXERT EFFECTS ON GLP-1(7-36)AMIDE AND INSULIN SECRETION IN HUMANS.

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Within a few min following nutritional stimulation GLP-1(7-36)amide is secreted in man in a pulsatile manner as shown previously. Intestinal delivery of glucose seems not to be major mechanism for the stimulation of GLP-1 secretion. Neuronal e.g. vagal mechanisms are under debate and were investigated in the present study in 5 healthy male volunteers (BMI 23.4±1.2 kg/m², age 27.8±3.6 y) during 60 min under resting conditions and during another 60 min following an oral glucose load (OGL, 100g dextrose/400 ml) with and without blockage of cholinergic and α- or β-adrenergic transmission using atropine, propranolol or phentolamine infused from 30 min before and throughout the OGL. Blood was collected every 2 min for the estimation of plasma glucose (G), GLP-1(7-36)amide (GLP-1) and insulin (I). The plasma concentrations were integrated in segments of 30 min (AUC). Basally no effect of the various blockers was detected comparing segment 1 (-blocker) and 2 (+blocker). Following the OGL the G_{AUC} was reduced to 83/86 % of control (segment 3/4), the I_{AUC} to 55/57 % , the GLP-1_{AUC} to 47/63% ($P<0.005$) by atropine, whereas propranolol or phentolamine had no significant effect. Atropine induced a delay in gastric emptying that was not seen after propranolol or phentolamine. But, the inhibitory effect on GLP-1 and I secretion exceeded the motility effect. From our data we conclude that nutrient dependent GLP-1 and also I secretion is modulated by the parasympathetic nervous system in humans in vivo.

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SOMATOSTATIN IS A MAJOR, PARACRINE REGULATOR OF GLUCAGON-LIKE PEPTIDE-1 (GLP-1) SECRETION.

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GLP-1 secretion from the endocrine cells of the ileum is stimulated by unabsorbed luminal nutrients. Meal responses are rapid, suggesting neural or endocrine control of secretion. We studied GLP-1 secretion from isolated porcine ileum, perfused with synthetic Krebs-Ringer bicarbonate, in response to electrical stimulation (8 Hz, 10 mA, 4 ms imp. duration) of the mixed extrinsic nerves, and infusion of noradrenaline (10 nM) , acetylcholine (1 uM), GRP (gastrin-releasing polypeptide 18-27 (10 nM), intraluminal administration of HCl, and inflation by air (a distension stimulus) with and without alpha-adrenergic blockade (phentolamine , 10 uM). Finally, we infused a high-capacity (final perfusate binding capacity: 6 nmol/ml), high affinity (Kd = 10 pmol/l) monoclonal antibody against somatostatin. Nerve stimulation (n = 6) inhibited GLP-1 secretion to 77 ± 3 % of basal, an effect which was abolished by phentolamine (99 ± 2 %). Noradrenaline (10 nM) inhibited (to 74 ± 6 %) and Ach stimulated secretion (to 136 ± 5 %), abolished by atropine. GRP increased GLP-1 secretion (14-fold). HCL and air distension grossly stimulated motility but weakly increased GLP-1 secretion. Somatostatin antibody infusion increased basal GLP-1 secretion between 5- to 12-fold, but the response to GRP, and nerve stimulation was unchanged. We conclude that GLP-1 secretion is inhibited by sympathetic, noradrenergic nerve activity and tonically inhibited by somatostatin in a paracrine manner. Lifting of sympathetic and somatostatinergic inhibition could provide a powerful stimulus for GLP-1 secretion..

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IMPAIRED SECRETION OF GLUCAGON-LIKE PEPTIDE 1 AFTER ORAL GLUCOSE IN ACROMEGALIC AND GH-DEFICIENT PATIENTS

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Glucagon-like peptide 1 (GLP-1) is an incretin from the lower gut with important glucoregulatory actions. It was the aim of the present study to examine plasma glucose, insulin, and GLP-1 responses after oral glucose in patients with acromegaly (growth hormone excess), GH-deficiency (pituitary disease) and normal subjects. In 7 acromegalic (49 ± 11 y.; BMI 31.3 ± 4.6 kg/m^2), 11 GH-deficient patients (57 ± 11 y.; BMI 29.0 ± 1.4 kg/m^2), and 11 normal controls (53 ± 12 y.; BMI 26.9 ± 3.6 kg/m^2), the glucose (glucose oxidase), insulin (IMx), and GLP-1 (RIA) response to oral glucose (75 g/300 ml) was determined over 4 h. Statistics: repeated-measures analysis of variance. Fasting GLP-1 was 11 ± 2 pmol/l in controls vs. 5 ± 1 pmol/l in both acromegalic and GH-deficient patients ($p = 0.016$). Likewise, the AUC over 6 h was 3961 ± 254 in controls, and 2517 ± 319 in acromegalic, and 2562 ± 300 pmol$\cdot$l$^{-1}\cdot$min in GH-deficient patients ($p = 0.0015$). In a multivariate regression analysis, both basal GLP-1 ($p = 0.034$) and the GLP-1 AUC ($p = 0.025$) were significantly related to cortiocotropic insufficiency (present in 3/7 patients with acromegaly and 10/11 GH-deficient patients). There was no significant difference in glucose or insulin responses. In conclusion, GLP-1 release is impaired in patients with acromegaly and growth hormone deficiency. Their common background is pituitary disease. Deficiencies in the corticotropic axis may be especially responsible for reduced basal and nutrient-stimulated GLP-1 release. This points to a hitherto unknown interaction of pituitary and gut hormones.

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GLUCOSE ABSORPTION AND GLP-1 SECRETION.

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GLP-1 secretion from the intestine is stimulated by glucose ingestion in man. The intraluminal administration (*IL*) of 2DG or sucrose did not affect GLP-1 secretion from the isolated perfused canine ileum, and stimulated GLP-1 secretion by *IL* glucose was decreased with *IL* phlorizin and intraarterial administration of ouabain. Thus, GLP-1 secretion is tightly related with glucose absorption and may be coupled with Na/glucose cotransporter (SGLT-1). However, it has been unknown whether SGLT-1 exists in L cell. In this study, we examined SGLT-1 in L cell by immunohistochemical technique. [Materials & Methods] Rat was fixed by transcardiac perfusion with 10% formalin in phosphate buffer. Small intestine was enbetted in paraffin and cut into 10 μm sections. Anti-SGLT-1 rabbit serum and anti- GLI guinea pig serum were used as double immunostaining on the same section. TRITC conjugated anti- rabbit IgG goat γ-globulin and FITC conjugated anti-guinea pig IgG goat γ-globulin were used as second antibodies. Epithelial cells were observed by a conforcal microscope. [Results] SGLT-1 was observed on the brushborder of the epithelium. The basolateral side was not stained by SGLT-1 antiserum. Although L cell was stained GLI antiserum, the luminal side of L cell was not stained with SGLT-1 antiserum. [Conclusion] L cell could not absorb glucose from the intestinal lumen through SGLT-1, and glucose absorption may indirectly regulate GLP-1 secretion from L cell.

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The ethanol augmentation of glucose-induced insulin secretion is abolished by calcium-antagonism with Nifedipine. No evidence for a role of GLP-1.

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Ethanol in itself does not affect basal insulin secretion in man. However, ethanol given before a glucose challange, augments the early insulin response by an unknown mechanism. Ethanol might affect the β-cell directly, or indirectly modulate insulin secretion through hormonal or neuronal activation. The aim of the present study was to examine if the ethanol augmentation of insulin secretion to an intravenous glucose challange could be modified by use of the calcium blocking agent nifedipine, and if that effect was mediated by GLP-1. Eight healty subjects volunteered for the study and in each subject four experiments were performed (control, ethanol, nifedipine and combination) in random order. Intravenous glucose tolerance test was performed with and without pretreatment with oral ethanol and nifedipine. Ethanol pretretment was followed by increased insulin (ethanol 5113,0 ± 863,7 vs control 3160,6 ± 413,8 pmol/l; $p<0,01$, n=8) and C-peptide (ethanol 29,6 ± 3,5 vs control 22,7 ± 1,5 mmol/l; $p<0.05$, n=8) areas after intravenous glucose (0-20 min.), indicating that ethanol augments insulin secretion. Calcium antagonism with nifedipine abolished the ethanol augmentation of insulin secretion, (insulin area 0-20 min, ethanol 5113,0 ± 863,7 vs combination 3730,6 ± 433,2; $p<0,05$, and C-peptide area 0-20 min, ethanol 29,6 ± 3,5 vs combination 22,1 ± 1,3; $p<0,01$). The GLP-1 response (area 0-90 min, n=6) was not significantly affected by ethanol. In conclusion, ethanol augmentation of insulin secretion to a glucose challange is supressed by nifedipine. Our results do not suggest that ethanol affects insulin secretion by stimulating GLP-1 secretion.

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GLUCAGON-LIKE PEPTIDE 1: EFFECTS ON GASTRIC EMPTYING AND ABSORPTION OF SUCROSE IN CONSCIOUS SWINE.

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Glucagon-like peptide 1 (7-37)OH (GLP-1) may participate in the mechanisms, comprising the ileal brake, which slow gastric emptying in the presence of nutrients in the distal bowel. In order to characterize this property of GLP-1, gastric emptying of a 1g/kg sucrose solution was assessed in conscious swine using the dilution of sequential intra-gastric injections of phenol red during the infusion of (a) 0 (b) 0.5 (c) 1 and (d) 4 nmol/kg of GLP-1 (7-37) over a 4h period. The sucrose was labelled with [^{14}C] glucose and the first appearance of label in the portal or peripheral circulation was used to assess absorption.

Dose nmol/kg /4h	n	% remaining in stomach at time (min) 30	60	120	240	Peak Glucose (mmol/L)	Time (^{14}C glucose >0) (min)	Peaks ^{14}C Glucose dpm/ml	Time Min
0	8	53±7	20±5	7±3	1±1	9.4±0.9	15	465±63	60
0.5	5	50±11	22±8	5±1	0.5±0.2	9.7±0.4	15	640±142	30
1	5	72±12	64±13	30±9	18±8	6.3±0.8	30±8	357±67	120
4	4	78±17	70±17	79±11	59±21	5.8±0.3	104±6	86±39	180

There is a dose-dependent slowing of gastric emptying with a resulting decrease in both the glucose and (normalized) label peak. Both the time of first appearance and peak of the label are delayed with increasing dose. The dose-dependent delay in first appearance of label, not correlated to gastric emptying, suggests a separate effect of GLP-1 on glucose absorption. Thus, both gastric emptying and glucose absorption may be independently delayed by this gut peptide.

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IV GLP-1 LOWERS BLOOD GLUCOSE LEVELS IN NIDDM PATIENTS REGARDLESS OF FASTING GLUCOSE, BMI, AND INSULIN CAPACITY.

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GLP-1 administration has been shown to lower blood glucose in small groups of patients with NIDDM, but it is not known how this effect is related to the degree of obesity, the fasting blood glucose (f-BG) concentrations or the insulin secretory capacity of the patients. In this study we infused GLP-1 (1.2 pmol/kg/min) intravenously for 4 to 6 hours into 50 fasting NIDDM patients (age: 40 - 71 yrs.; BMI: 20 - 45 kg/m^2; fasting plasma glucose: 5.7 - 24.5 mmol/l; HbA_{1C}: 5.7 - 13.3 %). Antidiabetic treatment was discontinued 3 days before the infusion. Blood samples were drawn every 30 min, and plasma were analysed for glucose, insulin (ELISA), C-peptide (ELISA), and glucagon. Groups were categorised on the basis of A) BMI (group1: 20 - 25.0 kg/m^2; group 2: 25 - 30.0 kg/m^2; group 3: 30 - 35.0 kg/m^2; group 4: > 35 kg/m^2), B) fasting blood glucose concentrations (group 1: < 10.0 mmol/l; 2: 10 - 15.0 mmol/l; 3: > 15 mmol/l), C) fasting plasma insulin concentrations (group 1: < 25 pmol/l; group 2: 25 - 50 pmol/l, group 3: > 50 pmol/l) and C-peptide responses. GLP-1 lowered blood glucose in all patients (12.1±0.6 to 7.0±0.4 mmol/l) at rates ranging between 1-2 mmol/h independent of their fasting blood glucose, fasting insulin concentrations, BMI or insulin secretory capacity. However, because of the similar fall rate in all groups the blood glucose decrements obtained after 4 hs of infusion were for fasting blood glucose group B1: 8.0±0.2 to 5.3±0.5 mmol/l; group B2: 12.3±0.2 to 6.6±0.2 mmol/l; group B3: 17.9±0.4 to 10.4±0.5 mmol/l. We conclude that GLP-1 may effectively normalize blood glucose in all NIDDM patients presumably as a result of its glucagonostatic activity.

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LIPOLYTIC AND LIPOGENIC ACTIONS OF GLP-1 IN HUMAN ADIPOSE TISSUE

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Insulin-like effects of GLP-1(7-36)amide (GLP-1) in rat liver, skeletal muscle and adipose tissue, have been documented. Also, a lipolytic effect of GLP-1 in rat adipocytes was reported. In this work, we have studied the lipolytic and lipogenic effect of GLP-1, and the adenylate cyclase activation, in adipocytes isolated from human subcutaneous fat tissue obtained, previous informed consent given, from eight patients undergoing abdominal surgery. For lipolysis, 10^5 cells were incubated during 60 min in 300 μl KRB-HEPES buffer, pH 7.4, containing 3% BSA and 3.3 mM D-glucose, in the absence (control) and presence of GLP-1 or glucagon; the glycerol released was enzymatically determined. For cellular cAMP content, assayed by RIA, adipocytes were incubated as above, in the presence of 0.1 mM IBMX, during 5 or 60 min. For lipogenesis, cells were incubated during 2 h in 1 ml of the medium containing 0.4 μmoles ^{14}C-Na acetate (0.05 μCi) as precursor, in the absence and presence of GLP-1 or insulin; cell associated radioactivity was toluene extracted and ß-counted. GLP-1 exerted a significant dose related stimulation of glycerol release in the 10^{-11}-10^{-9}M range, from 127±11% of control, n=12, to 154±11%, n=14, respectively, similar to that obtained with glucagon. GLP-1 also induced a rise in lipids generation in the 10^{-13}-10^{-9}M range, from 116±5%, n=18, to 130±8%, n=18, respectively, being the latter value not different to that of 10^{-9}M insulin. 10^{-8}M GLP-1 increased the adenylate cyclase activity in adipocyte plasma membranes to the same extent than 10^{-8}M glucagon. Furthermore, GLP-1 exerted a significant dose related stimulation of cAMP content in adipocytes, in the 10^{-12}-10^{-8}M range after either 5 min incubation, from 186±17%, n=3, to 621±30%, n=3, or 60 min incubation, from 130±11%, n=9, to 156±17%, n=17, respectively. These data document in human adipocytes that physiological levels of GLP-1 exert own counteracting effects on lipid metabolism, suggesting that at least the lipolytic action maybe mediated by cAMP.

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GLUCAGON-LIKE PEPTIDE-1 RESTORES ACUTE PHASE INSULIN RELEASE TO AGED RATS.

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Glucose intolerance is a common feature of the aging process and aging *per se* is an etiologic factor for Type II diabetes mellitus. To characterize the beta cell abnormalities that occur with aging, we looked at the serum glucose and insulin levels of six young (3-month) and six old (22-month) Wistar rats at 0, 2, 4, 7,10,15, 20, and 30 minutes after an intravenous glucose load (IVGTT, 0.5g/kg glucose). We found that the fasting glucose and insulin levels were not significantly different between young and old rats. However, peak glucose levels were significantly higher in the old (349 ± 10 mg/dl) compared to the young (250 ± 7 mg/dl) animals. Insulin levels in the young animals peaked at 2 minutes (859 ± 171 pmol/l) with a quick return towards fasting levels by 7 minutes. The old animals had a delayed and blunted insulin response to glucose, achieving lower peak insulin levels (656 ± 164 pmol/l) 7 minutes after the glucose load. As insulin levels are also positively modulated by incretin hormones, we quantitated the fasting insulin responses of young and old animals to 0.05, 0.1, 0.2, 0.4, and 0.5 nmol/kg intravenous glucagon-like peptide-1 (GLP-1), the most potent incretin known. Insulin responses were similar in both age groups with maximum insulin responses seen at 0.4 nmol/kg. GLP-1, in conjunction with the IVGTT, restored the acute insulin response to glucose and increased the clearance of glucose in the old animals. It therefore appears that old animals have an impaired glucose-mediated insulin release but maintain their insulin responsivity to GLP-1. This makes it a potentially useful agent for Type II diabetes in the elderly.

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GLYCOGENIC EFFECT OF GLP-1 ON HUMAN SKELETAL MUSCLE

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A potent glycogenic effect of GLP-1(7-36)amide (GLP-1) in rat skeletal muscle has been documented. In this work we have studied the effect of GLP-1 on glycogen synthesis in normal human skeletal muscle cells and *cremaster* muscle, both obtained, previous informed consent given, from subjects without alterations of carbohydrate metabolism, undergoing surgery. Cell cultures were stablished from satellite cells of dissociated muscle tissue *(vastus lateralis),* grown in SKGM with 2% FBS and 1% antibiotics up to near confluence, and then fused for 4 days in alpha-MEM with 2% FBS and 1% antibiotics. Differentiated myotubes were used in the first pass only. We measured the incorporation of D-[U-^{14}C]glucose into glycogen during 60 min at 37 °C, in myotubes incubated in alpha-MEM containing 5.5 mM D-[U-^{14}C]glucose (0.545μCi/μmol), and in muscle strips (*ca.* 2mg protein) incubated in KRB containing 5mM D-[U-^{14}C]glucose (0.047μCi/μmol) and 1% BSA, in the absence (control) or presence of GLP-1 or insulin. In human myotubes from four subjects, the control glycogen synthesis (9.6±1.2 nmol/mg protein, n=15) was increased ($p<0.01$) by GLP-1 at 10^{-10}M (169±12% of control, n=41) and at 10^{-9}M (128±8%, n=41), with an apparent higher potency than that of insulin (126±7%, n=24, $p<0.01$ and 166±17%, n=28, $p<0.001$, respectively). In muscle strips from three subjects, the control glycogen synthesis (15.6±4.1 nmol/mg protein, n=7) was also increased by 10^{-10}M GLP-1 (129±5% of control, n=7, $p<0.001$), as it was observed with 10^{-9}M insulin (123±9, n=7, $p<0.05$). These results document the insulin-like effect of GLP-1 upon skeletal muscle glucose metabolism in man.

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EFFECTS OF VOGLIBOSE AND ACARBOSE ON GLUCOSE HOMEOSTASIS AND GLUCAGON-LIKE PEPTIDE-1 SECRETION IN NIDDM PATIENTS

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The α-glucosidase inhibitors voglibose (AO-128) and acarbose are effective antihyperglycemic agents and were shown to increase plasma glucagon-like peptide-1 (GLP-1) levels in healthy volunteers. The aim of this double-blind study was to compare their effects in diabetics. 170 hospitalized NIDDM patients were randomly allocated to six parallel groups and received 7 days treatment with either three times daily voglibose (0.5, 1, 2, 5 mg), acarbose (100 mg) or placebo. Blood was saved before and 15, 30, 60, 120, 180, and 240 minutes after a standardized test meal on day -1 and day 7 to analyse serum glucose, insulin, plasma gastric inhibitory polypeptide (GIP) and GLP-1. 157 patients (mean age 54,4 years; 135 male) could be analysed. Maximal postprandial serum glucose rise (mean Cmax = 15.2 mmol/l on day -1) was statistically significantly prevented by all active treatments as compared to placebo on day 7 ($p < 0.005$; unpaired one-sided t-test). The strongest effects were seen under 5 mg voglibose (-4.2 mmol/l ≙ -26%; $p < 0.0001$); even 1 mg voglibose (-2.5 mmol/l ≙ -16%; $p < 0.0001$) was superior to acarbose (-1.7 mmol/l ≙ -12%; $p = 0.0044$). Voglibose exerted its antihyperglycemic effect over the total observation period, in contrast to acarbose which did this only for the first two hours. Postprandial increases of insulin (area under the curve = AUC 0-4h; Cmax) were significantly reduced by 1, 2, and 5 mg voglibose in a dose-dependent manner (-34% and -41% under 5 mg; $p < 0.0001$),whereas acarbose did not achieve statistical significance (-9% and -12%; $p = 0.19$ and 0.06). The endogeneous secretion of GIP was suppressed in all active groups. Voglibose increased GLP-1 mean AUC 0-4h by 29 % (under 0.5 and 1 mg) up to 47 % (under 5 mg); due to the high SD only 0.5 and 2 mg voglibose became statistically significant versus placebo ($p < 0.005$). Acarbose induced a weaker increase of 12 %. It is concluded that voglibose ≥ 1 mg three times daily has stronger influence on postprandial glucose / insulin reduction and GLP-1 secretion than acarbose (100 mg three times daily) in NIDDM patients.

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CHOLECYSTOKININ-INDUCED INSULIN SECRETION MEDIATED BY PLA_2 ACTIVATION IN ISLETS IS CA^{2+}-INDEPENDENT BUT PKC-DEPENDENT

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Cholecystokinin (CCK) induces insulin secretion through stimulation of phospholipase C (PLC), subsequent increase of cytosolic Ca^{2+} and activation of protein kinase C (PKC). Recently, we showed that also activation of phospholipase A_2 (PLA_2) is involved in the insulinotropic effect of CCK. However, the mechanism of CCK-induced PLA_2 activation is not known. Therefore, we examined the importance of Ca^{2+} and PKC in CCK-mediated PLA_2-activation and insulin secretion in isolated rat islets. CCK-8 (100 nM; at 5.6 mM glucose)-mediated arachidonic acid (AA) formation, measured as efflux of radioactivity from islets prelabelled with [^{3}H]AA, was unaffected by the Ca^{2+} ATPase inhibitor thapsigargin (1 μM; n.s.; n=6) as well as by the Ca^{2+} channel antagonist verapamil (100 μM; n.s.; n=6), indicating that intracellular Ca^{2+} stores and influx of extracellular Ca^{2+} are of no major importance in CCK-8-mediated PLA_2 activity. This was in sharp contrast to the AA formation due to the cholinergic agonist carbachol (100 μM), which was reduced by verapamil by 36±6% ($p<0.001$; n=6), implying a mechanistic dissimilarity between CCK-8 and carbachol when activating Ca^{2+}-independent PLA_2. Moreover, PKC down regulation, induced by overnight incubation with 12-O-tetradecanoyl phorbol-13-acetate (TPA; 500 nM), reduced CCK-8-induced AA formation by 17±5% ($p=0.031$; n=6), and insulin secretion by 40±16% ($p=0.021$; n=15-16). Furthermore, the specific PLA_2 inhibitor p-amylcinnamoylantranilic acid (ACA; 50 μM) diminished CCK-8-stimulated AA formation by 40±8% ($p<0.001$; n=6), and insulin secretion by 57±17% ($p=0.002$; n=15-16). No additive action was seen by combined PKC down regulation and PLA_2 inhibition, regarding neither AA formation (n.s.; n=6), nor insulin secretion (n.s.; n=16). In conclusion, our results suggest that CCK-induced activation of PLA_2 is Ca^{2+}-independent, whereas carbachol-stimulated activation of PLA_2 is dependent on Ca^{2+}-influx, that the PLA_2-activating and insulinotropic effects of CCK is partly PKC-dependent, and that CCK-mediated insulin secretion involves at least one additional signalling pathway, which is independent of both PLA_2 and PKC.

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INFLUENCE OF ACARBOSE ON GLP-1 RELEASE AFTER A SUCROSE LOAD IN NIDDM PATIENTS.

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In healthy volunteers, α-glucosidase inhibition leads to delayed digestion and to absorption of disaccharides also in the lower gut. As a consequence, release of glucagon-like peptide-1 (GLP-1) after oral sucrose is augmented. This could contribute to the glucose-lowering action of acarbose especially in hyperglycemic NIDDM patients. It was the aim of the study to evaluate the influence of acarbose on GLP-1 release and its possible consequences on plasma glucose, insulin and glucagon levels in NIDDM patients. In a crossover-design, 100 g sucrose were administered orally with 100 mg acarbose or placebo to 11 fasting NIDDM patients (6m/5f; age 62 ± 9 years, HbA_{1c} 9.6 ± 1.5 %) on different days. Plasma levels of glucose, insulin, GLP-1 and glucagon (RIAs) and H_2-exhalation (breath test) were measured over 6 h. The increment in plasma glucose (basal: 166 ± 10 mg/dl) was reduced from (peak) 277 ± 15 (placebo) to 230 ± 15 mg/dl (acarbose; $p < 0.001$). After 60-90 min, H_2-exhalation increased under acarbose ($p < 0.001$). From that time on, acarbose increased GLP-1 plasma levels (6 h AUC) by about 28% (6786 ± 1021 vs. 5302 ± 738 pmol·l^{-1}· min, $p = 0.04$, significant by ANOVA from 210 to 360 min, $p < 0.05$) without enhancing insulin or lowering glucagon concentrations during this time. In conclusion, acarbose augments GLP-1 responses after oral sucrose also in NIDDM patients.

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GASTROINTESTINAL REGULATORY PEPTIDES IN CHILDREN WITH IDDM: DOES THE GLYCEMIC CONTROL MATTER?

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Gastrointestinal disorders are common in patients with IDDM and hyperglycemia has been accused to be a predisposing factor. Gut regulatory peptides (GRP) are well known to be associated with motility disorders. However, their relationship with the glycemic status in children with IDDM is poorly defined. We therefore measured fasting glucose, motilin, neurotensin, and glucagon levels and HbA1c in 52 children (mean age 15.3 years, 22 males) with IDDM (mean duration 7.1 years). Thirty eight patients (73%) had persistent gastrointestinal (GI) symptoms: recurrent abdominal pain 15; chronic constipation 13; chronic dyspepsia 9; recurrent vomiting 1. Twenty three patients (44%) had fasting serum glucose 4.1-8.3 mmol/L and 29 (56%) >8.3 mmol/L. Fasting motilin levels (mean ± SEM: 62.2 ± 5.2 pmol/L) were significantly lower ($p<0.0001$) in IDDM patients compared with 26 healthy controls (mean ± SEM: 162.7 ± 7.2 pmol/L). However, motilin levels were not different between patients with or without GI symptoms, as so as with or without hyperglycemia. Motilin levels had a significant negative correlation with duration of IDDM ($r=-0.38$; $p=0.005$) but no correlation was found with HbA1c. Neurotensin levels were lower than the previously published lower normal level (<6 pmol/L) in 62% of patients (mean ± SEM: 9.4 ± 2.9 pmol/L); glucagon levels were higher than the upper normal level (>200 pmol/L) in 60% of patients (mean ± SEM: 213.1 ± 13.0 pmol/L/) . No significant correlations were found between glucose levels or HbA1c and neurotensin or glucagon levels. In conclusion, under conditions of euglycemia or hyperglycemia GRP do not directly depend on glycemic status. However, their role in IDDM patients should be further investigated.

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IMPROVED INSULIN SECRETION AND ORAL GLUCOSE TOLERANCE AFTER *IN VIVO* INHIBITION OF DPP-IV IN OBESE ZUCKER RATS.

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Glucagon-like peptide 1 (GLP-1), a potent incretin, plays a pivotal role in prandial insulin secretion. GLP-1 is rapidly ($t_{1/2}$:1-2 min) inactivated by the protease dipeptidyl peptidase IV (DPP-IV). DPP-IV is believed to be the sole inactivating enzyme of GLP-1, though a slower process removes both active and inactive GLP-1 from the circulation. Here, we investigated whether DPP-IV inhibition is a feasible approach to improve glucose homeostasis through an enhancement of GLP-1 action. Firstly, the effects of DPP-IV inhibition on insulin secretion stimulated by exogenous GLP-1 were studied in conscious, cannulated rats after 2 hrs food deprivation. Infusion (*iv*)of GLP-1 (5pmol/kg/min in saline with 2% glucose @ 150μl/kg/min for 30 min.) evoked a profound but transient insulin response (area under the curve $(AUC)_{0\text{-}10mm}$ +375±61μU/ml*10 min) after which plasma insulin concentrations returned to basal. Administration of the specific DPP-IV inhibitor SDZ 272-070 (15μmol/kg, *iv*@15min) immediately inhibited plasma DPP-IV activity by 85% and elicited a second insulin response ($AUC_{15\text{-}25mm}$ 223±80 *vs* 45±63μU/ml*10 min (vehicle), $p<0.05$). Thus, DPP-IV inhibition augments the effects of exogenous GLP-1 on insulin secretion. Secondly, the effects of DPP-IV inhibition on oral glucose tolerance were investigated in cannulated female insulin resistant, glucose intolerant, obese Zucker rats and lean controls (8-9 weeks of age). SDZ 272-070 (100μmol/kg, *po*, @ t=-30min) was given 30 min before glucose (1g/kg, *po*, t=0min). DPP-IV inhibition significantly amplified the early phase of the insulin response in obese fa/fa rats and all but normalized glucose excursions. In contrast, DPP-IV inhibition produced no significant effects in FA/?. It is concluded that DPP-IV inhibition improves insulin secretion and impaired glucose tolerance, probably through augmentation of the effects of endogenous GLP-1. This suggests that DPP-IV inhibition might be a useful tool in the treatment of NIDDM.

AUC= area under the curve	fa/fa n=7	fa/fa +DPP-IV inhibitor n=7	FA/? n=9	FA/? +DPP-IV inhibitor n=8
Insulin AUC (μU/ml*15min)	1322±778	4940±778**	567±141	546±212
Glucose AUC (mg/dl*30min)	2556±272##	1647±205**	1294±146	1018±104

#,##: $p<0.05$, $p<0.01$ vs lean control; *,**: $p<0.05$, $p<0.01$ vs obese control

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GLUCOSE- AND TOLBUTAMIDE-INDUCED INSULIN SECRETION IN ADULT RATS TREATED WITH NICOTINAMIDE AND STREPTOZOTOCIN.

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We have previously shown that graded doses of nicotinamide (NA) can partially protect adult rats against the beta cell toxicity of streptozotocin (STZ). On this basis, the aim of the present study was to select and test the best NA dose suitable to produce a mild hyperglycaemia without severe reduction in pancreatic insulin stores. Among the various doses of NA (in the range 180-260 mg/kg) tested in 3-mo-old Wistar rats, the dose of 230 mg/kg (NA 230) given i.p. 15 min before STZ injection (65 mg/kg i.v.) yielded a maximum of animals with moderate elevation of non fasting plasma glucose levels (166 ± 12 vs 119 ± 3 mg/dl in controls, $p < 0.01$). Pancreatic insulin content was about 35% of that of controls. IVGTTs revealed glucose intolerance and abnormal insulin response which remained stable for at least 9 weeks after diabetes induction and interestingly appeared to be sensitive to tolbutamide administration. In the isolated perfused pancreas of STZ plus NA-treated rats, insulin response to an increase in glucose concentration (5 to 11 mmol/l) was significantly reduced versus controls ($p < 0.05$), whereas the response to 0.19 mmol/l tolbutamide was similar to that observed in normal pancreas. Furthermore, pancreatic β cells exhibited hypersensitivity to arginine infusion (7 mmol/l) in the presence of 2.8 mmol/l glucose (105 ± 20 vs 10 ± 4 ng insulin x 20 min in controls, $p < 0.001$). In conclusion, our results indicate that adult rats treated with STZ plus NA 230 show a relative deficiency in insulin secretion which is corrected by sulphonylureas, and might represent an attractive experimental model of NIDDM.

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PROINSULIN BIOSYNTHESIS AND PROCESSING IN NIDDM HUMAN ISLETS

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It has been shown that one of the conversion intermediates together with intact proinsulin are abnormaly secreted by the beta cells of certain NIDDM patients. The **aim** of our study was to investigate the biosynthesis and conversion of proinsulin on NIDDM human islets. **Results**: Pulse-chase and immunoprecipitation techniques gave an evidence 4-fold decrease of proinsulin biosynthesis on the islets from the diabetic vs. islets from two control pancreases. Morphometry analysis shown that it cannot be explained by the loss of beta cell mass. ß-cell mass/pancreas from NIDDM vs. controls: 1.9±0.2% vs. 2.4±0.7% and 2.8±0.1%. We observed, by northern blot, that this defect seems to be focused at the translation level since islets from the diabetic have same proinsulin mRNA content as controls. No particular defects are observed at the proinsulin conversion ratio and neither proinsulin or proinsulin intermediates are secreted into the medium. This low proinsulin synthesis does not seem to be a glucose toxicity related defect because pulse-chase studies performed with control islets exposed for 8 days at 24.4 mM glucose didn't show any diferences on proinsulin biosynthesis, conversion or secretion when were compared to those exposed at 5.5mM. In **conclusion** i) proinsulin mRNA translation is partially bloked on those NIDDM human islets; ii) chronic hyperglicaemia does not affect proinsulin synthesis or conversion, and neither promote proinsulin secretion.

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EFFECTS OF GLUTAMATE ON GLUCOSE TOLERANCE AND INSULIN SECRETION IN A RAT MODEL OF TYPE II DIABETES.

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We have previously shown that glutamate stimulates insulin release via a glutamatergic receptor and improves glucose tolerance in normal rats. In this study, we investigated the effect of glutamate in a rat model of type II diabetes obtained by an i.v. injection of a low dose (35 mg/kg) of streptozotocin (STZ). Glutamate was tested on glycaemia and/or insulin secretion *in vivo* and *in vitro*. After one week, in the postabsorptive state, STZ rats exhibited a moderate hyperglycaemia and a slight decrease of plasma insulin levels (6.9 ± 0.3 mmol/l and 2.6 ± 0.5 mU/l, respectively) compared to controls (5.5 ± 0.1 mmol/l and 4.6 ± 0.7 mU/l, respectively). In STZ rats, during an i.v. glucose tolerance test (0.5 g/kg), insulin response and glucose tolerance were impaired: the increase in insulinemia for 10 min was +40 ± 10 vs +146 ± 29 mU/l ($p<0.01$) and the glucose increment for 30 min was +235 ± 11 vs +191 ± 8 mmol/l ($p<0.01$) in normal rats. The addition of glutamate (9 mg/kg) to the glucose solution increased the insulin response during the first 10 min ($p<0.05$), reduced the hyperglycaemia (20%; $p<0.05$) and increased the glucose disappearance rate ($p<0.001$). *In vitro*, on the isolated perfused pancreas, the biphasic insulin response to a glucose rise from 4.2 to 16.6 mmol/l was reduced by 55% in STZ rats compared to normal animals. Glutamate (400 µmol/l), added simultaneously with glucose 16.6 mmol/l, provoked a 3.6-fold increase of the first phase (5 min) of insulin response ($p<0.001$). In conclusion, glutamate is able to stimulate insulin release and to improve glucose tolerance in moderately diabetic rats. Thus, glutamate receptors of B cells could be a new target for antidiabetic insulinosecretory agents.

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ACCELERATED PROINSULIN CONVERSION IN HUMAN B-CELLS AFTER OVEREXPRESSION OF PC2 OR PC3 BY RECOMBINANT ADENOVIRUS.

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Proinsulin conversion involves cleavage at the B-chain/C-peptide and C-peptide/A-chain junctions. Cleavage (and trimming of basic residues) at just one junction leads to generation of des-31,32-split proinsulin (des-31,32) and des-64,65-split proinsulin (des-64,65) respectively. In man, des-31,32 is the major intermediate. Two endoproteases, PC2 and PC3 have been implicated in proinsulin conversion. These enzymes display specificity for just one proinsulin junction *in vitro*, but little is known regarding their substrate specificity and relative activity *in vivo* within human B-cells. To study this, human pancreatic islets were trypsinized, the cells cultured 2-3 days and then infected (1h) with increasing multiplicity of infection (moi) of adenovirus expressing PC2 (Ad-PC2), PC3 (Ad-PC3) or βgal as control (Ad-βgal). After 24h, cells were pulse-labelled (10 min label [^{3}H]Leu) and chased. Radioactive products were separated by hlpc. Expressing β-gal did not affect proinsulin conversion. For controls (Adβ-gal, moi of 200) after 60 min chase there was 38.5% insulin, 25.0% des-31,32, 2.8% des-64,65 and 33.7% proinsulin. For AdPC2: insulin = 48.4, 78.0, 86.6%; des-31,32 = 15.7, 2.7, 1.0%; des-64,65 = 4.7, 5.3, 3.7%; proinsulin = 31.1, 14.0, 8.7% at moi of 2, 20 and 200 respectively. For AdPC3: insulin = 43.3, 69.4, 86.8%; des-31,32 = 21.3, 14.8, 3.7%; des-64,65 = 3.1, 0, 0.7%; proinsulin = 31.1, 14.0, 8.7 at moi of 2, 20 and 200 respectively. Whereas each enzyme shows the same substrate specificity previously observed *in vitro*, with PC2 cleaving preferentially at the C-peptide/A-chain junction and PC3 at the B-chain/C-peptide junction, both endoproteases appear to be able to cleave at both proinsulin junctions (at least when expressed at supraphysiologic levels). The data thus show that PC2 and PC3 when overexpressed in human B-cells accelerate to an equal extent the conversion of proinsulin to fully processed insulin.

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REDUCED ISLET INNERVATION IN TYPE 2 DIABETIC HAMSTERS

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It is known that pancreatic islets are innervated and that nerves are involved in islet function. However, whether islet innervation is altered during the development of type 2 diabetes is not known. We therefore examined the adrenergic and cholinergic innervation of the islets in different phases of glucose intolerance in a new subline (CHIG/Han) of the Chinese hamster. The animals were characterized by their glucose tolerance, plasma insulin levels and glucose-stimulated insulin secretion in vitro. Pancreas sections were immunostained for neuropeptide Y (NPY) and tyrosine hydroxylase (TH) as markers for adrenergic nerves, for vasoactive intestinal peptide (VIP) presumably localized in cholinergic nerves, and the main islet hormones. The animals were grouped according to their nonfasting plasma glucose levels into nondiabetic (< 7.2 mmol/L), mildly (7.2-15 mmol/L) and severely diabetic (> 15 mmol/L). Severely diabetic hamsters had marked glucose intolerance and impaired glucose (15 mmol/L)-stimulated insulin secretion (-76%, $p<0.01$) of islets. The group with mild diabetes had a glucose tolerance between these two groups, and glucose-stimulated islet insulin secretion was decreased by 54% ($p< 0.05$) when compared with the nondiabetic group. Plasma insulin was increased in nondiabetic (+81%, $p< 0.05$) and mildly diabetic (+119%, $p< 0.01$) hamsters, while approaching basal levels (354.9±37 pmol/L) in the severely diabetic animals. Immunostaining of the pancreas sections revealed that innervation by both adrenergic and cholinergic nerves was markedly reduced in severely diabetic hamsters, whereas the innervation pattern in the mildly diabetic ones was virtually unaffected. Insulin was reduced in the diabetic animals, whereas glucagon staining was increased. Immunostained somatostatin, pancreatic polypeptide (PP) and peptide YY (PYY) was unchanged as compared with the nondiabetic pancreases. In this animal model, the autonomic nerves (adrenergic as well as nonadrenergic) disappear with the loss of glucose-stimulated insulin secretion. This suggests that altered islet innervation is involved in type 2 diabetes.

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CELL-FREE TRUNCATION OF C-PEPTIDE TO des-(27-31)C-PEPTIDE

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Recent studies have shown secretion of a novel form of C-peptide (Cp), truncated C-peptide (tCp), which lacks its 5 C-terminal residues. Truncation of Cp to tCp occurs in both primary and transformed rat B-cells but to a greater extent in the latter. In order to confirm that truncation is a granular event and to establish a cell-free assay to characterize the truncation enzyme, we have now studied rat Cp truncation in two in vitro systems. Granules from INS (transformed rat B-) cells were purified by subcellular fractionation using a Percoll gradient and disrupted by freeze-thaw. Rat Cp was biosynthetically labelled in INS cells and purified by HPLC before use as a substrate. When labelled Cp was incubated with granule contents at pH 6.0 and 37°C there was production of tCP amounting to 30% of total (tCp + Cp) by 4h. There was, however, also extensive degradation of Cp possibly reflecting contamination of granules with lysosomes. To limit such contamination and in order to determine whether truncation activity in granules was soluble or membrane bound, INS cells were incubated for 1h in Krebs-Ringer bicarbonate in presence (stimulated) or absence (control) of a cocktail of secretagogues previously shown to stimulate release of insulin stores (and thus also of soluble but not membrane anchored granular contents). The conditioned medium was concentrated prior to use. Incubation (pH 6.0, 37°C) of the labelled Cp in stimulated conditioned medium led to production of 12.7±4.2% tCp in 4h but only 6.7±0.7% for control. There was no significant difference between control and stimulated medium at 2h. Taken together the data confirm that B-cell granules contain soluble enzyme(s) able to cleave Cp between residues 26 and 27 and demonstrate that the truncation event can be faithfully reproduced in a cell free system.

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LONG-TERM TOTAL PARENTERAL NUTRITION REDUCES ISLET ACID GLUCAN-1,4-α-GLUCOSIDASE ACTIVITY AND INSULIN RELEASE

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To study the effects of long term elevation of serum lipids on nutrient-stimulated insulin secretion and the islet acid glucan-1,4-α-glucosidase system, normal healthy rats were subjected to total parenteral nutrition (TPN) for 10 days. Control rats received saline and had free access to food and water. The levels of serum FFA, triglycerides and cholesterol were greatly elevated by TPN infusion. Pancreatic islets isolated from TPN infused rats and then incubated in vitro showed a greatly reduced insulin release when stimulated by glucose or KIC. The insulin response to the muscarinic agonist carbachol, however, was of similar magnitude as observed in controls. The inhibitory effect of TPN treatment on glucose-induced insulin release (-55%) was only slighly ameliorated by exposure to the carnitine palmitoyltransferase I inhibitor, etomoxir. Further, isolated isles from TPN infused rats displayed a markedly reduced activity of the lysosomal enzyme acid glucan-1,4-α-glucosidase, a putative key enzyme in nutrient induced insulin release. This depression of acid glucan-1,4-α-glucosidase activity was accompanied by a similar reduction of several other lysosomal enzyme activities e.g. acid phosphatase , N-acetyl-β-D-glucosaminidase, β-glucuronidase and acid α-glucosidase. In contrast, in liver tissue from TPN infused animals all these enzymes displayed markedly increased activities. The impaired insulin response to glucose was not readily reversible and persisted in islets isolated 12 h after TPN treatment. Also the in vivo insulin response to an intravenous glucose load at 12 h after TPN treatment was impaired. The present data show that TPN treatment for 10 days induced a severe impairment of nutrient-induced insulin release being accompanied by a marked suppression of the islet acid-glucan-1,4-α-glucosidase activity and other lysosomal enzymes. In contrast, the lysosomal system/lysosomal enzyme activities in liver tissue was greatly increased. The results strongly suggest that the islet lysosome-acid glucan-1,4-α-glucosidase system is of key importance for nutrient-induced insulin release and that long term elevation of serum lipids greatly impairs this signal system.

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NK-lysin, an endogenous antibacterial peptide, exerts an insulinotropic effect in isolated pancreatic islets.

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We investigated whether a recently isolated 78 residue porcine antibacterial peptide, NK-lysin, could have an insulinotropic effect on pig and rat islets. Initially, batches of either pig or rat islets were tested in static incubations at 37°C for 60 minutes. The KREBS-BSA (K-B) buffer contained a low glucose concentration 2.8mM with and without (control) the presence of NK-lysin. A 2-fold increase in insulin release from pig and rat islets was induced by NK-lysin as from 0.1μM as compared to control values. Also, freshly isolated rat islets were perifused at 37°C in K-B buffer containing either 2.8 or 16.7mM glucose. When 0.1μM NK-lysin was present in 2.8mM glucose perifusion studies, it induced a first phase peak of insulin release. This response corresponded to 46% of that induced by 16.7mM glucose only. When NK-lysin was studied in presence of 16.7mM glucose the biphasic pattern of insulin release was amplified. Under these conditions, NK-lysin enhanced only the second phase by 2.5-fold as compared to the measured secretory response with 16.7mM glucose alone. Further, this enhancement returned to normal basal secretory rates upon removal of NK-lysin and in presence of 2.8 mM glucose. These data suggest that this endogenous antibacterial peptide is a novel insulinotropic agent. The mode of action of NK-lysin in stimulating insulin at low and high glucose concentrations remains to be established.

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ALTERATIONS IN ISLETS' INSULIN SECRETION OF FETUS FROM PREGNANT RATS FED AN ISOCALORIC LOW PROTEIN DIET

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Fractional insulin release (IR) in vitro of fetal islet cells is significantly reduced when mother is fed a low protein diet (8%) (LP) instead of 20% (C). cAMP content in LP islets is also diminished. Neither forskolin nor theophyline did restore the IR of LP islets but both normalised the cAMP content. To determine the site of alterations of IR in fetal LP islets, the latter were cultured during 7 days in RPMI and were challenged thereafter during 120 min with various secretagogues which stimulate IR at different sites and compared with control islets. Acetylcholine (ACh) (100 mM), cytochalazine B (Cytoc) (10 mM), glutamine (Glut) (10 mM), leucine (Leu) (10 mM), Ba^{2+} (4.54 mM) and a high K^+ (50 mM) were tested. In C as well as in the LP group, all secretagogues significantly increased IR (%). However, when IR % of LP fetal islets was compared to C, it was significantly reduced in response to Glut+Leu (15.83 ± 1.96 vs 8.30 ± 1.35 $p<0.01$), KCl (17.14 ± 1.33 vs 10.70 ± 1.38 $p<0.01$), or to ACh (15.92 ± 0.68 vs 4.71 ± 0.88 $p<0.01$). But, IR (%) of fetuses from LP group was restored when their islets were stimulated with Cytoc B (10.16 ± 0.82 vs 9.23 ± 0.99) or with Ba^{2+} (15.55 ± 0.70 vs 12.01 ± 1.78). The results show thus that the lack in the secretory mechanism of LP B cells should be located in the late phase of the insulin secretion. Ca^{2+} movements might be involved in this B cells secretory alteration and are being investigated.

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INSULIN RELEASING EFFECTS OF CITRULLUS COLOCYNTHIS FRUIT EXTRACTS IN ISOLATED RAT PANCREAS

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Infusions of the fruits of *Citrullus colocynthis Schrad.*, cucurbitacceae are traditionally used in Morocco and mediterranean countries as antidiabetic medications. The present study was designed to investigate the possible insulinotropic properties of these fruits. For this purpose, different extracts of the components of *Citrullus colocynthis* fruits were obtained: RN_{II} (crude extract), RN_{VI} (hydro-alcoholic extract) and RN_X (purified extract). The insulin secretory effects of these three different extracts were evaluated *in vitro* in the isolated and perfused rat pancreas in the presence of 8.3 mmol/l glucose. These extracts were perfused for 20 min. RN_{II} at 0.1 mg/ml induced an immediate and significant stimulation of insulin secretion. The increase reached + 90% at min 3 ($P < 0.01$) but was transient (5 min). The area under the curve (AUC) for the first 5 min was 2444 ± 170 ng versus 1941 ± 101 ng in control experiments ($P < 0.05$). The same amounts of RN_{VI} and RN_X (0.1 mg/ml) also elicited an immediate and monophasic increase in insulin output (maximum + 40% and + 60% respectively). AUC for RN_{VI} was 2331 ± 150 ng ($P < 0.05$) and for RN_X 2339 ± 100 ng ($P < 0.05$). Moreover, a significant and persistent increase in pancreatic flow rate appeared during RN_{VI} and RN_X infusions: AUC for the 20 min perfusions were 51.0 ± 1.2 ml and 55.9 ± 0.7 ml respectively versus 46.2 ± 0.5 ml in control experiments ($P < 0.01$). In conclusion, our results show that the three different extracts from *Citrullus colocynthis* fruits display an insulinotropic effect which may, at least in part, account for the antidiabetic activities traditionally described for these fruits.

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THE EFFECT OF POOR FOETAL AND NEONATAL NUTRITION ON ISLET FUNCTION IN NEONATAL RATS.

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It has been suggested that poor foetal-neonatal nutrition may predispose adult animals to impaired glucose tolerance and diabetes. We have previously shown that feeding a low protein (5%) diet to pregnant and lactating rats leads to altered islet function in the adult offspring. To investigate further we have studied islet function in offspring aged 6-7 days from mothers fed a low protein or control diet during pregnancy and lactation. Low protein offspring were smaller than control offspring (10.3±0.3 vs 17.1±0.8 g in controls, n=50, $p<0.001$). Insulin release in response to 10mmol/l glucose was significantly impaired in islets isolated from low protein offspring in both batch incubations (14.1±2 (n=7) vs 35.9±3.4 (n=27) μU/islet/hr in controls, $p<0.001$) and in perifusion (15±12 vs 181±102 μU/40min in controls, n=4, $p<0.001$). This was not due to a decreased secretory capacity, as responses of islets to ketoisocaproate and arginine were not significantly reduced. Also, islet insulin content was not reduced (0.69±0.12 vs 0.93±0.09 mU/islet in controls, n=7, NS). To attempt to identify the underlying defect, islet glucose metabolism was measured. Mitochondrial oxidation of glucose was not impaired in low protein offspring (6.73±0.84 vs 7.32±0.73 pmol glucose oxidised/islet/hr in controls at 10mmol/l glucose, n=12, NS). Glucose utilisation, a measure of glycolytic rate, however was reduced (73.4±11 vs 129±15 pmol glucose utilised/islet/hr in controls at 10mmol/l glucose, n=12, $p<0.01$). These data suggest that poor foetal-neonatal nutrition causes an impairment in glucose stimulated insulin release in islets from neonatal rats, due possibly to a defect in the glycolytic pathway.

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ENDOTHELIN-1 AND ENDOTHELIN-3 STIMULATE INSULIN RELEASE FROM ISOLATED RAT ISLETS OF LANGERHANS

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It has been reported, by different experimental models, that insulin induces Endothelin-1 (ET-1) release. Recently, ET-1 has been shown to increase circulating insulin and lower blood glucose in rats. At present, it has not been demonstrated whether this peptide may act at an insular level or influence insulin concentrations by indirect mechanisms. Aim of the present study has been to clarify whether: 1) ET-1 influences insulin release from isolated rat islets, 2) other isoforms of endothelin, like Endothelin-3 (ET-3) show the same action.

Isolated rat islets of Langerhans were employed. 120 minutes incubations with increasing concentrations (10^{-12} to 10^{-9} M) of ET-1 and ET-3 were performed both in presence and in absence of 5.5 mM glucose. At the end of incubation, insulin concentrations were assayed in the medium. No increases of insulin release were observed when islets were incubated in glucose-free medium. Both ET-1 and ET-3 were able to enhance insulin release in presence of 5.5 mM glucose and the increases were statistically significant at 10^{-10} M concentration for ET-1 ($p<0.05$) and at 10^{-9} M concentration for ET-3 ($p<0.05$).

We conclude that ET-1 is able to stimulate directly, in presence of physiological glucose concentrations, insulin release from isolated rat islets. ET-3 exerts, at the insular level, an action similar to that of ET-1.

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MYELIN BASIC PROTEIN STIMULATES INSULIN SECRETION IN RATS *IN VIVO*

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Myelin basic protein (MBP) stimulates insulin and glucagon release from pancreatic islets *in vitro*. Effects of MBP *in vivo* were studied in anaesthetized rats (n=6-8). A reference sample was withdrawn at -10 min from a. femoralis, MBP (10mg/0.5 ml of 0.9% NaCl) was injected at 0 min via v. femoralis, and blood samples were withdrawn in exp A at 10, 20, 30 and 40 min, and in exp B at 2, 4, 6, 8, 10 and 12 min, and replaced with fresh blood from a donor rat. The control rats (n=6) received 0.9% NaCl with similar protocols. The control levels of insulin and glucose were unchanged in exps A and B except for the 12-min glucose value in B ($p<0.05$: repeated-measures ANOVA/Bonferoni t-test). MBP stimulated insulin release in A by 108±10% at 10 min, 163±17% at 20 min, 192±28% ($p<0.01$) at 30 min and by 164±28% at 40 min (means±SE) over the -10 min level. The changes in individual animals ranged from 133% to 508% stimulation at 30 min. The blood glucose level rised from 6.5±0.3 mM to 8.2±0.3, 8.4±0.6 ($p<0.01$), 8.0±0.8 and 7.2±0.9 mM at the aforementioned times. In exp B, the glucose level rised from 6.5±0.4 mM to 7.0±0.4 mM at 2 min, 8.4±0.4 mM ($p<0.01$) at 4 min, 9.1±0.4 mM ($p<0.01$) at 6 min, 9.8±0.4 mM ($p<0.01$) at 8 min, 10.0±0.6 mM ($p<0.01$) at 10 min and 10.5±0.5 mM ($p<0.01$) at 12 min. Insulin plasma values exhibited oscillatory changes in 7 out of 8 rats, one increasing without oscillations. Three out of the 7 oscillating exhibited maxima at 4 and 8 min, the rest showing one maximum at 4 min and/or additional maxima in reverse phase. The combined data showed stimulation of 123±24%, 243±41% ($p<0.01$), 203±35% ($p<0.05$), 263±54% ($p<0.01$), 204±40% and 236±35% ($p<0.01$) at 2, 4, 6, 8, 10 and 12 min, respectively. Since previous studies have shown that MBP injected into rat circulation enters the liver and accumulates in the pancreas, the changes may result from a combined effect of MBP on these tissues. They could explain idiopathic hyperglycaemia in stroke and head injury patients and disturbances in glucose homeostasis in some multiple sclerosis patients.

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LACK OF EFFECT OF LEPTIN ON INSULIN SECRETION. STUDY IN THE PERFUSED RAT PANCREAS.

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In human obesity, as well as in some models of rodent obesity, elevated plasma concentrations of both leptin and insulin have been found. Insulin regulates leptin mRNA and increases plasma leptin concentration. Recently, it has been reported that leptin receptor mRNA is expressed in primary rat pancreatic islets and in the insulinoma cell line ßTC-3. These observations raise the possibility of an adipose-insular B cell axis modulating insulin release. Thus, we have investigated the effect of rat leptin on insulin secretion in the perfused rat pancreas. Perfusate consisted of Krebs-Henseleit buffer supplemented with albumin (0.5%), dextran T-70 (4%) and glucose (5.5 mmol/l). Insulin was analysed by RIA. Synthetic rat leptin (a gift from Amylin Pharmaceuticals, Inc., San Diego, CA, USA), at 10 nmol/l - a concentration close to the upper plasma levels found in obese subjects - failed to modify the insulin response to an increase in perfusate glucose level from 5.5 mmol/l to 9 mmol/l (incremental area: 58±13, Mean±SEM, ng/20 min, N=5 vs. 49.4±11 ng/20 min, N=10 in control experiments; p=0.7). At a supraphysiological concentration (100 nmol/l), leptin was without effect on insulin release in pancreases perfused at 5.5 mmol/l glucose ($F_{20,140}=0.63$) or stimulated by increasing glucose levels from 5.5 mmol/l to 9 mmol/l (Incremental area: 71±5 ng/20 min, N=3, vs. 72±17 ng/20 min, N=7 in control experiments; p=0.96). Our results would rule out an obvious effect of leptin on insulin secretion in the normal rat pancreas, thus challenging the concept of leptin as a direct signal from the adipocyte to the pancreatic B-cell.

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CONSTRUCTION AND EXPRESSION OF RECOMBINANT INSULIN FROM HUMAN INSULIN GENE AND MAMMALIAN EXPRESSION VECTOR PRC/CMV

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To develop a model somatic gene therapy system for diabetes, we constructed a human insulin expression vector in non β cells. As the first step, a insulin cDNA fragment, generated by a complete digestion of PBCA with EcoR I and BamH I, was inserted into the EcoR I/BamH I site of plasmid PBS·SK by ligation of cohesive-ended DNA, which constructs a transition plasmid PBS·INS. Then the plasmid PBS·INS was completely digested by Hind III and Xbal I. A small DNA fragment containing insulin cDNA gene, was subcloned into the expression plasmid PRC/CMV to form recombinant PRC/CMV·INS. Cultured mouse fibroblasts of the Ltk^- cell line were transfected with human insulin expression plasmid PRC/CMV·INS, which contains a gene conferring resistance to the antibiotic G418. Finally, 12 clone of the Ltk cells named Ltk^-·PRI-12, was selective because of its higher insulin-releasing activity compared with other clones. Release of human insulin was assessed through a specific human c-peptide assay(RIA). After culturing for 24 h in the RPMI 1640 medium containing 10mM glucose, Ltk^-·PRI-12 cells release c peptide at about 98 pmol/10^6 cells per 24 h. The study offers a potential somatic gene therapy approach to insulin delivery in diabetes mellitus.

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ISLET AMYLOID POLYPEPTIDE KNOCK OUT MICE DEVELOP A MORE SEVERE FORM OF ALLOXAN-INDUCED DIABETES

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Several studies have shown that islet amyloid polypeptide (IAPP; amylin) is over-expressed in experimental forms of diabetes. This may be harmful in diabetes, because of IAPP's propensity to form amyloid in islets and its negative effects on insulin release and sensitivity. Alternatively, IAPP over-expression could arise as a protective mechanism in diabetes. We recently generated mice with a targeted disruption of the IAPP gene (knock out; KO); this allowed us to examine experimentally induced diabetes in the absence of IAPP. Thus, male IAPP KO (n=10) and wild type (WT) mice (n=10) were given alloxan (70 mg/kg iv). Whereas both KO and WT mice became hyperglycaemic, a more pronounced diabetes gradually evolved in KO mice. At day 7, plasma glucose was 31±3 and 27±4 mM in KO and WT mice (P=0.4), respectively, whereas at day 35 it was 33±3 and 23±4 mM (P<0.05). Furthermore, at day 35, weight loss was greater in diabetic KO than in WT mice (9.0±2 vs 1.9±2 g; P<0.001). IVGTT (1g/kg) was performed at day 7 and 35; glucose and insulin levels were not different in the two diabetic groups. In contrast, control KO mice displayed lower glucose levels than control WT mice at 10-60 min (P<0.05-001), which indicates an enhanced glucose elimination in KO mice. Quantitative *in situ* hybridization revealed that insulin mRNA levels at day 35 were 14±9 and 27±11% of control in diabetic KO and WT mice, respectively (P<0.001 for both); the reduction of insulin gene expression was greater in diabetic KO mice (P<0.001). In conclusion, lack of IAPP in mice is associated with a more severe form of alloxan-induced diabetes. The results suggest that the perturbation of islet function by alloxan is aggravated, because insulin gene expression was lower in diabetic KO mice and the enhanced glucose elimination seen in control KO mice was lost in diabetic KO mice. To speculate, the previously shown ability of IAPP to enhance microcirculation by vasodilatation, which is lacking in IAPP KO mice, may promote islet survival/ regeneration after a diabetic insult, such as alloxan.

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MOUSE PANCREATIC ISLETS CULTURED ON ECM IS A GOOD PREPARATION FOR MICROFLUOROMETRY

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We have examined the use of extracellular matrix (ECM) as a substratum for NMRI mouse islet cultures with special reference to microfluorometry. The techniques for obtaining and maintaining ECM-producing cell cultures and subsequent coating of glass is straightforward. Islets seeded out onto ECM attached readily, firmly and with time spread out. Single islet cultures (1/7/14 days, RPMI 1640) were monitored for glucose-induced changes in $[Ca^{2+}]_i$ (Fura-2), NAD(P)H and FAD fluorescence, mitochondrial membrane potential (Vmit, Rhodamine 123) and insulin release. After an increase in glucose from 3 to 9-10 mM, the well-established initial changes in $[Ca^{2+}]_i$, as well as oscillations upon prolonged exposure, were observed. Measurements of Vmit showed hyperpolarisation under similar conditions. Cultured islets remained responsive to the sugar over at least two weeks, but the tendency to display $[Ca^{2+}]_i$ oscillations at 10 mM glucose was decreased at 2 weeks. Simultaneous measurements of NAD(P)H and Flavine Adenine Dinucleotide (FAD) fluorescence changes in response to glucose stimulation showed rapid increases in NAD(P)H fluorescence and decreases in FAD fluorescence. Stable and dose-dependent NAD(P)H/FAD fluorescence ratio levels were observed after prolonged incubation, similar at 7 and 14 days. The islets responded to glucose challenges from 0-3 mM to 10 and 20 mM glucose with increases in insulin release of about 10- and 30-60 fold, respectively ($p<0.05$). In conclusion, we find this preparation excellent for microfluorometry applications. The fact that the islets are still glucose responsive when they become more flat gives an opportunity to single cell imaging with conventional microscopy techniques. In addition, we suggest that the NAD(P)H/FAD ratio may be a useful parameter when monitoring metabolic activation of pancreatic islets.

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Hypoglycaemic and insulinotropic effects of stevioside in the non-insulin dependent GK rat.

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Stevioside, is a glycoside contained in the leaves of *Stevia rebaudiana* Bertoni that has been used in the traditional treatment of diabetes in Brazil. Recently we found that stevioside potently stimulates insulin secretion from mouse islets. The aim of this study was to investigate the impact of stevioside on blood glucose and insulin levels *in vivo* in the non-insulin dependent GK rat. Students unpaired t-test has been applied for statistical analysis. During an i.v. infusion of glucose (2.0 g/kg body weight) over 1 min without or with stevioside (0.2 g/kg body weight) we observed a pronounced increase in the insulin response to stevioside (51116 ± 10967 vs 21548 ± 3101 µU*120 min; $p<0.05$) and a concomitant suppresion of the blood glucose responses (648 ± 50 vs 958 ± 85 mM*120 min; $p<0.05$) in the diabetic GK rat. Also in the normal Wistar rat stevioside potently stimulated the insulin response (79913 ± 3101 vs 17347 ± 2882 µU*120 min; $p<0.01$) whereas no change in the blood glucose response was found (416 ± 43 vs 417 ± 47 mM*120 min). In the GK rat insulin levels were steadily increasing during the entire 120 min observation period. Despite the prominent insulin response to stevioside no increment in the liver glycogen content could be detected in either the normal or diabetic rat. In conclusion the glycoside, stevioside, possess hypoglycaemic and insulinotropic effects in the non-insulin dependent GK rat and may have a potential as a hypoglycaemic drug.

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THE POTENT INSULINOTROPIC EFFECT OF ENDOTHELIN-1 IS NOT MEDIATED VIA DIRECT ACTION ON THE ISLET β-CELLS: EVIDENCE FOR AN IMPORTANT ROLE FOR α-CELLS.

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The potential involvement of the endothelium derived peptide endothelin-1 (ET-1) in the patophysiology of diabetes has been further strengthened by our recent finding that ET-1 has potent insulinotropic effects on mouse islets. However, the mechanism of action has not yet been clarified. We aimed at exploring if ET-1 interacts directly with the β-cells. For this purpose the action of ET-1 on insulin secretion and insulin mRNA in β-cell lines and purified islet cells was studied. First, we investigated the possible effect of ET-1 (10^{-6} M to 10^{-12} M) on β-cell lines INS-1, βTC-3 and MIN6. At all concentrations studied no significant effects of ET-1 on insulin secretion were found. Corresponding to this, there was no change in insulin mRNA in INS-1 cells. To further strengthen this finding we performed ligand-binding studies on βTC-3 cells with ^{125}I-ET-1 and ^{125}I-glucagon as control. No specific binding of ^{125}I-ET-1 was found on the β-cells at the concentrations studied. This was in contrast to the displaceable binding of ^{125}I-glucagon. Thus we found no evidence of specific ET-1 receptors on the β-cells. In an attempt to study whether these quite unexpected "missing" effects on the β-cells might be due to the use of insulinoma cell lines we used FACS-purified β- and non-β-cells from normal rats. Using the purified β-cells no action of ET-1 on insulin secretion was found. Interestingly, however, when purified β- and non-β-cells (preferentially α-cells) were mixed ET-1 (100 nM) did increase glucose-stimulated insulin secretion. The α-cells therefore seems important for the effects of ET-1 on insulin secretion. In conclusion, the results indicate that the potent insulinotropic effect of ET-1 is not mediated through a direct action of ET-1 on the β-cells but is elicited indirectly via stimulation of the α-cells.

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DELAYED ONSET OF DIABETES AND PRESERVED INSULIN SECRETION IN TROGLITAZONE-TREATED OLETF RATS.

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Troglitazone is thought to improve insulin-resistance. We analyzed whether long-term treatment of troglitazone to insulin-resistant OLETF (Otsuka Long-Evans Tokushima Fatty) rats prevented the development of diabetes or not, and preserved glucose-induced insulin secretion or not. Male OLETF and non-diabetic LETO (Long-Evans Tokushima Otsuka) rats were treated with troglitazone as a 0.2% food admixture from 7 to 60 (65) weeks of age (OLETF+ and LETO+). Others were fed with standard chow (OLETF- and LETO-). Blood samples were taken every 3-4 weeks. At 60-65 weeks of age, rat pancreas was isolated and perfused with 20 mM of D-glucose or 10 mM of L-arginine for 15 min. Post-prandial insulin levels were 606 ± 159 pM (OLETF-) and 260 ± 111 pM (OLETF+) at 30 weeks of age, and 35 ± 4 pM (OLETF-) and 213 ± 151 pM (OLETF+) at 50 weeks of age. Post-prandial plasma glucose levels were gradually increased with age, and reached to 26.3 ± 10.9 mM (OLETF-) and 12.3 ± 1.9 mM (OLETF+) at 50 weeks of age. At 60-65 weeks of age, however, fasting plasma glucose were 12.8 ± 3.0 mM (OLETF-) and 11.0 ± 5.1 mM (OLETF+) (statistically not different). In perfusion study, arginine-induced insulin secretion was preserved in both groups of OLETF rats but glucose-induced insulin secretion was significantly decreased in OLETF- than in OLETF+ rats. In histological examination, loss of islets was less significant in OLETF+ than in OLETF- rats. Troglitazone delayed the onset of diabetes and preserved insulin secretion in OLETF rats. Long-term treatment of troglitazone to OLETF rats appears to attenuate the exhaustion of pancreas caused by insulin-resistance.

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DIVERSITY OF THE TRANSDUCTION SYSTEM EXPRESSION IN PANCREATIC A CELLS

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High glucagon levels unaffected by glucose were observed in diabetes, leading us to investigate the molecules involved in the signal transduction in glucagon-secreting pancreatic islet A cells of adult rats. Immunodetection was performed by light and electron microscopy using antibodies (abs) raised against PACAP receptors (R) C-terminal which takes into account the different subtypes of PACAPR which in preliminary study showed three bands around 42, 50 and 60 kD on western blot of pancreatic extracts of newborn mouse. Other abs used, were raised against the 13-29, 13-29, 291-302, 385-394, 100-119 and 100-118 sequences of α_q, α_{11}, α_{o2}, α_s/α_{olf}, α_s and α_{olf}, respectively. PACAPR was seen in A cells and in pancreatic islet B cells, by light and electron microscopy. The labeling intensity appeared specific of each antibody used, 93093-2 (mainly A cells) or 93094-2 (mainly B cells). $G\alpha_{11}$ and $G\alpha_{o2}$ were highly expressed in A cells, and in vascular endothelial system but not in insuline, and somatostatin or in pancreatic exocrine cells. $G\alpha_{11}$ and $G\alpha_{o2}$ were mainly present in the core and the periphery of glucagon granules. The subunits are likely to be coupled to the m1 and m2 acetylcholine receptors, respectively. $G\alpha_q$, probably linked to the m1 cholinergic receptor, was not found in A cells but was detected in B cells, being expressed mostly in the cytoplasm at the vicinity of the ribosomes. A slight labeling in A cells was found with both $\alpha s/\alpha olf$, αs and α_{olf} specific antibodies. Among the adenylate cyclase, AC-II alone was expressed in A cells. These findings indicate that $G\alpha$ subunits are differentially expressed in A and B cells. Colabeling of AC-II with $G\alpha_s$ and perhaps $G\alpha_{11}$ in the A cells suggests a possible association of these molecules required for the signaling of A cells. PACAPR might interfer with both of these pathways through a transduction system involving $G\alpha_s$ or $G\alpha_{11}$.

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HISTOLOGICAL CHANGES OF CGRP-IMMUNOREACTIVITY IN OLETF RAT PANCREATIC ISLET.

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Calcitonin gene-related peptide (CGRP) is a 37 amino acids neuropeptide translated from the calcitonin gene. In the present study, we demonstrate sequential changes of CGRP immunoreactivity in the pancreatic islets of the non-insulin dependent diabetes mellitus (NIDDM) model rat. [Materials and Methods] Otsuka Long-Evans Tokushima Fatty (OLETF) rat were used as NIDDM model rat and Long-Evans Tokushima Ohtsuka (LETO) rat as control. Histopathological changes in the OLETF rat pancreas were classified into three stage, the early stage (6-20 weeks of age), the hyperplastic stage (20-40 weeks of age) and the final stage (over 40 weeks of age) (Kawano et al. 1992). Animals were killed at 7, 16, 24, 32, 50, 65 weeks of age, and were fixed by transcardiac perfusion with 10% formalin in phosphate buffer. Specific CGRP antiserum which does not crossreact with rat amylin1-37 and islet amyloid polypeptide was used. [Results] Diabetes mellitus in OLETF rat was obvious after 24 weeks of age. CGRP immunoreactivity in the pancreatic islets of OLETF rat and LETO rat was localized in the pancreatic D cell and nerve fibers at all three stages. CGRP positive nerve fibers of OLETF rat were more abundant than those of LETO rat in the early and hyperplastic stages. In the final stage, the pancreatic islets of OLETF rat became atrophic due to the decrease of B cells, and CGRP positive cells of OLETF rat were relatively increased in number. [Conclusion] It is suggested that CGRP is one of factors controlling pancreatic islets function.

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IMMUNOHISTOCHEMISTRY OF THE PANCREAS IN FIBROCALCULOUS PANCREATIC DIABETES

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Nine patients with Fibrocalculous Pancreatic Diabetes (FCPD) had pancreatic biopsies done at time of surgery for abdominal pain. Routine histopathology of all cases and immunohistochemistry in six cases using antibodies to insulin, glucagon, pancreatic polypeptide and somatostatin were carried out. Histology revealed severe atrophy of the entire lobule with replacement by extensive fibrocollagenous tissue. Two cases had evidence of nesidioblastosis. Total number of islets were significantly reduced compared to the normal controls. On immunohistochemistry, the percent of cells positive for insulin, glucagon and somatostatin in each islet was not significantly different from normal, but the overall positivity for these hormones was reduced due to decrease in total number of islets. Pancreatic polypeptide was markedly reduced in all the six cases. In conclusion, in FCPD the islets are destroyed secondary to the inflammatory process. However those that are not destroyed tend to maintain their normal physiological functions.

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EFFECTS OF DEHYDROEPIANDROSTERONE IN RATS INJECTED WITH STREPTOZOTOCIN DURING THE NEONATAL PERIOD.

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Dehydroepiandrosterone (DHEA), an adrenal steroid precursor of both androgens and estrogens, could reportedly bring a fountain of youth in advancing age. Anti-diabetic and anti-obesity effects of DHEA have been documented. Interestingly, this steroid has been shown to induce in the liver the thermogenic enzymes mitochondrial FAD-linked glycerophosphate dehydrogenase (m-GDH) and cytosolic NADP-malate dehydrogenase (malic enzyme). A deficiency of the former enzyme in the insulin-producing B-cell has been identified in several animal models of NIDDM, including that found in adult rats that were injected with streptozotocin during the neonatal period (STZ rats). Therefore, it was investigated whether DHEA may also affect the activity of the above-mentioned enzymes in pancreatic islets of STZ rats and, hence, improve their metabolic and secretory responsiveness to D-glucose. Control and STZ rats were either maintained on a standard diet or given access to food supplemented with DHEA (0.2 %) for 11 days before sacrifice. In both control and diabetic animals, DHEA feeding augmented the activity of the m-GDH and malic enzyme in liver, but not so in either the parotid gland or pancreatic islets. DHEA lowered, also in both control and diabetic rats, the ratio between D-glucose oxidation and utilization and the rate of insulin release in pancreatic islets exposed to a high concentration of D-glucose, as well as the insulin concentration and insulin/glucose ratio in plasma. These findings support the view that, in diabetes, DHEA doesn't improve the metabolic and secretory responses of islet B-cells to D-glucose but, by increasing extra-pancreatic sensitivity to insulin, may allow insulin-producing cells to avoid the otherwise unfavourable consequences of chronic hyperactivity.

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SER 994 IN THE β-SUBUNIT OF THE HUMAN INSULIN RECEPTOR (HIR) IS INVOLVED IN RECEPTOR TYROSINE KINASE REGULATION.

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Human insulin receptor (HIR) is known to be phosphorylated on tyrosine, threonine and serine residues. The functional significance of serine phosphorylation is not understood in detail. We prepared serine to alanine mutations at various candidate positions of HIR to study whether any of these serine residues has an influence on the level of the receptor autophosphorylation or substrate phosphorylation by HIR. The tyrosine phosphorylation pattern in 293 cells overexpressing HIR wild-type (HIR-wt) or the respective HIR mutants were determined by immunoblots using phosphotyrosine antibodies. HIR serine to alanine at position 994 (HIR-994) caused a 2-fold increase both in the receptor autophosphorylation and substrate tyrosine phosphorylation. Our data suggest that serine 994 is a potential inhibitory serine phosphorylation site. In order to evaluate whether this serine is required for the inhibitory effect of high glucose we incubated 293 cells overxpressing HIR-994 with 25 mM 2-deoxyglucose (2-DOG). 2-DOG was able to induce an inhibition of the insulin receptor to the same extent as it was seen with HIR-wt. This suggests that serine 994 is not directly involved in the inhibitory effect of hyperglycemia.

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INSULIN-INDUCED ACTIVATION OF PHOSPHOLIPASE D IN RAT ADIPOCYTES.

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It has been reported that activation of phospholipase D (PLD) is thought to be an important role for agonist-induced signal transduction. It is still controversial whether insulin-induced diacylglycerol (DG) production is associated with phosphatidylcholine (PC) hydrolysis by PLD and PLC. DG activates protein kinase C (PKC), which regulates insulin action. In the present study, we investigate whether insulin and phorbol ester (TPA) provoke PLD activity in rat adipocytes.

PLD activity was measured by the formation of phosphatidylethanol (PEt) in the presence of ethanol or the formation of choline during stimulation with insulin. Adipocytes were stimulated by 10nM insulin or 1 μM TPA after prelabeled with [^{3}H] palmitic acid or [^{3}H] choline, and then [^{3}H] PEt and [^{3}H] choline formation was analyzed by thin layer chromatography. Insulin-stimulated PLD activity was increased by approximately 2-fold for 5 min, and reached maximum by 3-fold for 10 min and continued for 20 min. On the other hand, TPA-induced PLD activity was increased by 2-fold for 5 min, and continued for 20 min. These results suggest that insulin provokes PLD activation and subsequent PKC activation which synergistically stimulates PLD activity. Finally, insulin-induced PLD activation contributes to continued DG formation and chronic PKC activation in rat adipocytes.

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SERINE RESIDUES IN THE C-TERMINAL TAIL OF THE HUMAN INSULIN RECEPTOR (HIR) DETERMINE THE INTERACTION WITH TYROSINE PHOSPHATASE 1D.

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HIR is phosphorylated on tyrosine, threonine and serine residues. The functional significance of serine residues is not understood in detail. We prepared serine to alanine mutations at various candidate positions of HIR. To study whether any of these serine residues is important for substrate phosphorylation by the HIR the tyrosine phosphorylation pattern of 293 cells overexpressing HIR or the respective HIR mutants were determined by immunoblots using anti phosphotyrosine antibodies. HIR serine to alanine at position 1293/94, 1308/1309 and the C-terminal deleted HIRΔCT caused an altered pattern of tyrosine phosphorylated proteins. Increased tyrosine phosphorylation was seen for several bands between 60 and 80 kDa. Using specific antibodies one of the bands at 70 kDa was identified as tyrosine phosphatase 1D (PTP1D). Following the idea that the C-terminal serine residues might be important for the interaction of HIR and PTP1D we cotransfected 293 cells with HIR1293/94, HIR 1308/09, HIRΔCT and PTP1D. We found that expression of HIR1293/94 and 1308/1309 with PTP1D caused a strong tyrosine phosphorylation of PTP1D which is very weak with HIR wildtype and all other $HIR_{ser \rightarrow ala}$ mutants. The data suggest that the C-terminal serine phosphorylation sites are important for the interaction of HIR with PTP1D. An insufficient activation of PTP1D with these receptor constructs might explain the tyrosine phorphorylation of other proteins which is normally supressed by PTP1D.

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EFFECT OF PROTEIN KINASE C ON INSULIN RECEPTOR SUBSTRATE-1 (IRS-1) AND PHOSPHATIDYLINOSITOL 3-KINASE (PI 3-KINASE) ACTIVATIONS IN RAT ADIPOCYTES

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Insulin stimulates tyrosine phosphorylation of IRS-1 and PI 3-kinase via activation of receptor tyrosine kinase. On the other hand, insulin also stimulates protein kinase C(PKC) by synthesis of diacylglycerol (DG) via activation of phospholipase C and D which are coupled by GTP binding protein to receptor. To clarify the relationship between IRS-1-PI 3-kinase and DG-PKC signaling, we studied the effect of protein kinase C (PKC) on PI 3-kinase activity and IRS-1 tyrosine phosphorylation in rat adipocytes. Phorbol ester (TPA) provoked the increase in PI 3-kinase activity using thin layer chromatography and p85 subunit of PI 3-kinase immunoreactivity after anti-phosphotyrosine antibody immunoprecipitation like insulin in time- and dose-dependent manner. TPA also increased IRS-1 immunoreactivity after phosphotyrosine immunoprecipitation like insulin. Incubation of purified conventional PKC containing PKCα, ß and γ with IRS-1 immunoprecipitates using anti-IRS-1 antibody resulted in the phosphorylation of 180 and 165 kDa proteins in the presence of 0.5 mM Ca^{+} +/40 µg phosphatidylserine, and 0.4 µg/ml diolein. These results suggest that conventional PKC phosphorylates IRS-1 and increases tyrosine phosphorylation, followed by PI 3-kinase activation. Finally, activation of PKC and PI 3-kinase by insulin simultaneously induces the translocation of glucose transporter in rat adipocytes.

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THE INSULIN RECEPTOR RK MOTIF CONTROLS RECEPTOR ASSOCIATION/ACTIVATION OF MULTIPLE PKC ISOFORMS.

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We have previously shown that insulin receptor (IR) phosphorylation by protein kinase C (PKC) is critically dependent on a short consensus (Arg_{1152} - Gln_{1153}; RK motif) in the receptor regulatory domain. Neutral for basic amino acid substitutions in the RK motif lead to constitutive activation of the IR kinase. In the present study, we have used RK mutant IRs to investigate the mechanisms of PKC activation by the IR. After exposure to 10^{-7} M insulin for 30 min., NIH-3T3 cells expressing human wild-type IRs (WT) showed a 2-fold increase in plasma membrane-associated PKC activity ($P<0.001$). In contrast, insulin treatment of cells expressing a similar number of any of three RK mutant IRs (Arg_{1152}->Ala; Lys_{1153}->Ala; Arg_{1152}/Lys_{1153}->Ala) decreased membrane PKC activity by >50% ($P<0.01$). IR immunopreciptates from insulin-stimulated WT cells showed a 2-fold increased recovery of PKC activity compared to those from basal cells ($P<0.001$). In the mutant cells, IR-associated PKC activity was 40% higher than in WT fibroblasts ($P<0.01$), but decreased by 60% upon exposure to insulin ($P<0.01$). Western blotting of IR immunoprecipitates from WT cells followed by probing with isoform-specific PKC antibodies revealed receptor association with PKC α, δ, and ζ. IR association with these three isoforms increased by 3-, 1.5-, and 2.5-fold, respectively, upon insulin exposure of the cells ($P<0.001$). Association of PKC α, δ, and ζ with the RK mutant receptors was 40% higher compared to WT ($P<0.01$) decreasing by 2-fold in insulin-stimulated cells ($P<0.001$). Thus, in NIH-3T3 cells, insulin elicited association of its receptor with multiple PKC isoforms. IR-PKC association is controlled by the RK motif and correlates with insulin-induced PKC activation.

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HYPERGLYCAEMIA MEDIATED COMPLEX FORMATION BETWEEN PKC AND THE INSULIN RECEPTOR DEPENDS ON PKC ACTIVATION

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High levels of glucose inhibit insulin stimulated insulin receptor autophosphorylation. This effect can be observed in skeletal muscle as well as in a number of cell culture models. The exact mechanism of this inhibition is still unclear, however protein kinase C (PKC) seems to play a crucial role. We have shown previously that hyperglycaemia leads to the formation of a stable complex between the insulin receptor (IR) and PKC α and ζ. Using A293 cells which were simultaneously transfected with the IR and PKC we have now examined all known PKC isoforms with respect to coimmunoprecipitation with the insulin receptor. Using a monoclonal antibody against the IR we detect a basal level of PKC coprecipitation for all isotypes. In response to high levels of glucose (25mM 2-Deoxyglucose) we see in addition to what we observed earlier increased IR/PKC complex formation also for PKC β1, β2, ε and θ. Next we were interested to understand whether complex formation with the IR in itself is sufficient to mediate PKC's effect on IR kinase inhibition, or whether it requires activation of the enzyme. To address this question we constructed kinase negative versions of PKC by introducing a point mutation in the ATP binding site. In all cases, this mutation totally abolished the glucose induced complex formation with the IR. In summary, we show that hyperglycaemia leads to increased formation of complexes between the IR and PKC α, β1, β2, ε, θ and ζ. This association is dependent on an intact kinase activity of PKC. We speculate that PKC mediates its effect on IR kinase by covalent modification of the IR β-subunit. This might contribute to the normal termination of the insulin signal. Prolonged or increased activation of this complex formation might result in insulin resistance.

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INSULIN ACTIVATION OF INSULIN RECEPTOR KINASE IN INTACT BLOOD CELLS IS NOT IMPAIRED IN NIDDM AND IS NOT INFLUENCED BY HYPERGLYCEMIA.

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Recent studies suggest that hyperglycemia impairs the insulin effect on insulin receptor kinase(IRK)-activation in certain tissues. The present study explored whether IRK-activation is impaired in blood cells of NIDDM patients and whether a reduction of hyperglycemia in these patients leads to improved IRK-function. Blood was collected from 21 nondiabetic control subjects and 22 patients with NIDDM. Moreover, in 11 NIDDM patients with poor metabolic control blood was collected before (day 1) and after (day 8.6±0.4) therapeutic reduction of hyperglycemia. During this period, mean daily preprandial blood glucose measured at 7 am, 11 am and 5 pm decreased from 16.4±1.2 to 7.7±0.4 mmol/l. Aliquots of the heparinized blood were incubated for 15min at 37°C with insulin (0-400 nmol/l) to activate the IRK in the intact blood cells. The cells were then rapidly solubilized under conditions that preserved the "in situ"-activity state of the insulin receptors. The receptors were then immobilized to microwells coated with anti-insulin receptor antibody. IRK-activity towards recombinant insulin receptor substrate-1 and insulin binding activity were measured in these wells. Control experiments with leukocyte filters showed that insulin binding and IRK-activity stemmed almost exclusively from the erythrocytes. IRK-activities (expressed as amol P / min / fmol insulin binding activity) were similar in the nondiabetic (2.1±0.3 and 34.2±1.2 at 0 and 400 nmol/l insulin, respectively) and NIDDM subjects (2.1±0.3 and 35.1±1.4 at 0 and 400 nmol/l insulin, respectively). Activities were also similar before (2.4±0.4 and 32.2±2.0 at 0 and 400 nmol/l insulin, respectively) and after improvement of metabolic control (2.4±0.5 and 32.0±2.3 at 0 and 400 nmol/l insulin, respectively). There were also no differences at submaximal insulin concentrations. Conclusions: (1)Insulin stimulation of intact blood cells results in a ≈15-fold stimulation of IRK-activity. (2) IRK-activation in blood cells of NIDDM patients is not impaired. (3) Our data indicate that, at least in blood cells, hyperglycemia does not impair insulin-induced IRK-activation.

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ROLE OF THE JAK-STAT PATHWAY IN INSULIN-SECRETING CELLS

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It is well established that the JAK-STAT pathway is implicated in the intracellular signalling of a number of cytokines. We have already shown that two mitogenic factors for pancreatic β-cells, growth hormone (GH) and prolactin (PRL), activate JAK2 tyrosine kinase in the differentiated insulinoma cell line, INS-1. In this study, activation of the STAT proteins by these hormones was investigated in INS-1 cells, since differential activation of these proteins might determine the tissue-specific response to the ligands. Both GH and PRL were found to promote tyrosine phosphorylation of STAT1 and STAT5. Gel mobility shift assay was performed using oligonucleotides representing interferon (IFN)-γ-activation sites (GAS) to examine DNA-binding activities of STAT proteins in INS-1 cells stimulated with either GH or PRL. Both GH and PRL were capable of stimulating the DNA binding to β-CAS (β-casein gene promoter) GAS, a binding site of STAT5. Interestingly, DNA binding activity to IFN-regulatory factor 1 (IRF-1) GASprobe was rather decreased by GH or PRL, whereas no change was observed in the binding to the m67 sis-inducible element (SIE). In contrast to the two hormones, IFN-γ failed to promote tyrosine phosphorylation of JAK1 or JAK2, although this cytokine significantly inhibited glucose-induced insulin secretion and augmented the induction of inducible NO synthase by tumor necrosis factor-α in INS-1 cells. In conclusion, the JAK-STAT pathway, possibly through activation of JAK2 and STAT5, mediates the mitogenic effects of GH and PRL. The inhibitory effect of IFN-γ on insulin secretion as well as induction of iNOS induction are not associated with an activation of this pathway.

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EXPOSURE TO HIGH GLUCOSE LEVELS IMPAIR INSULIN ACTION IN THE L6 SKELETAL MUSCLE CELLS.

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The mechanisms responsible for the impairment of glucose metabolism by high glucose levels are still unclear. We have addressed this issue in differentiated L6 muscle cells expressing human insulin receptors ($L6_{hIR}$). Increasing glucose in the medium from 7.0 to 25.0 mM in the absence of insulin led to a 40% increase in total glucose disposal/12h by these cells ($P<0.01$). This effect was accompanied by an 80% increase in 2-deoxy-D-glucose (2-DG) uptake, and by a 2- and 0.5-fold increase in glycogen accumulation and glucose oxidation to CO_2, respectively ($P<0.001$). Following the exposure to the high glucose medium, the activities of glycogen synthase (GS) and pyruvate dehydrogenase (PDH) in the $L6_{hIR}$ also increased by 2- and 0.4-fold, respectively ($P<0.01$). In cells exposed to 7 mM glucose, insulin exposure (100nM; 12h) increased total glucose disposal, oxidation, glycogen accumulation and GS and PDH activities to a similar extent as 25 mM glucose alone. In contrast, in cells exposed to 25 mM glucose, insulin had no effect on any of these functions. With the $L6_{hIR}$, increasing glucose in the culture medium from 7.0 to 25.0 mM in the absence of insulin determined a 2-fold increase in IR kinase toward IRS-1 and 2 ($P<0.01$). Subsequent exposure of the cells to insulin did not further activate the receptor. hIR preparations from cells exposed to the high glucose concentration also exhibited little insulin responsiveness. Glucose effect on hIR kinase was maximum in 20 min and not duplicated by xylose. Thus, high glucose concentrations, *per se*, may activate glucose metabolism in muscle by affecting insulin signalling at the level of IR substrate phosphorylation. In the $L6_{hIR}$, however, the effect of high glucose concentration on glucose metabolism is accompanied by an inhibition of insulin action.

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VANADATE BUT NOT INSULIN STIMULATES MAP-KINASE IN INSULIN RESISTANT ADIPOCYTES FROM AGED RATS

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Vanadate is a well-known insulin mimetic agent both *in vivo* and *in vitro*. Although there is some controversy about its mechanism of action,available data suggest that,in the absence of insulin, it acts at a post insulin receptor level. This fact allows its use as a tool to explore specific changes in the insulin signaling cascade that result in cellular insulin resistance. We have previously shown a decreased insulin effect in adipocytes from old rats concerning the stimulation of glucose conversion to CO_2 and triglyceride, hexose transport, MAP-kinase(MAPK), and *in vitro* autophosphorylation of insulin receptor. Since vanadate is able to stimulate partially the hexose transport in this insulin resistant fat cells, we have investigated whether it also stimulates MAPK. Adipocytes were isolated from 3- and 24-month old rats and MAPK was determined as the ^{32}Pi incorporation into myelin basic protein. Fat cells were preincubated alternatively with or without 16 nM insulin or 1mM vanadate, for 5 and 20 min in both cases. Wortmannin (1μM) and PD98059(100μM),an inhibitor of MAPK kinase(MEK), were added in some experiments 10 and 30 min before the hormones, respectively. Phosphorylated MAPKs were immunodetected with an anti-phospho MAPK antibody. In young rats adipocytes insulin transiently stimulates MAPK (1.7 fold) whereas the stimulation induced by vanadate (1.8 fold) remains constant for at least 20 min. In fat cells from old animals the stimulation by insulin reaches 1.2 fold at 5 min and is not observable after 20 min of incubation. In contrast, vanadate fully stimulates MAPK (1.8 fold) and this effect is still relevant (1.55 fold) after 20 min. Preincubation with wortmannin does not modify the stimulation of MAPK in all cases studied whereas PD98059 abolishes the stimulatory effect of both, insulin and vanadate indicating that the later acts via MEK. Changes in MAPK activity correlated well with the amount of phosphorylated erk-1 and erk-2 proteins. These results strongly indicate that MAPK is fully activable in insulin resistant adipocytes from old rats and suggest that the impairment in insulin signaling to this enzyme must be located upstream of the stimulation of MEK and MAPK.

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INSULIN RECEPTOR TYROSINE-KINASE AND PC-1 CONTENT IN FIBROBLASTS OF HEALTHY NONOBESE NONDIABETIC SUBJECTS

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A reduced insulin receptor tyrosine-kinase activity (IR-TK) and an increased content of membrane glycoprotein PC-1 is found in both muscle and adipose tissue of healthy, nonobese (nonOb) nondiabetic (nonD) subjects with low insulin sensitivity, suggesting that these two defects are early events in the development of insulin (Ins) resistance. Aim: to verify whether these two defects are intrinsic and/or irreversible or acquired and/or reversible. Methods: In cultured fibroblasts from 17 nonOb (BMI<28), nonD (by OGTT) subjects with a wide range of Ins sensitivity, assessed by either i.v. Ins tolerance test (K_{itt} values:4.2-8.8, n=9) or euglycemic clamp (M values: 4.6-11.6, n=8). Ins effect (0-100 nM) on both glycogen synthesis (GS, 14C-glucose incorporation into glycogen) and IR-TK activity (autophosphorylation detected by ELISA), was measured as well as PC-1 content (by ELISA). Subjects were classified as high-sensitive (HS, n=8) or low-sensitive (LS, n=9) depending on K or M value, being higher or lower than the median value obtained in larger series of nonOb and nonD subjects studied by either ITT (n=67) or euglicemic clamp (n=64). Results: 1) At all concentrations tested ins stimulation of both GS and IR-TK was reduced in LS subjects ($p<0.05$ by 2-way ANOVA, for both measurements); moreover in LS a significantly higher ED50 of Ins stimulation of IR-TK was observed in LS (0.36±0.02 nM vs. 0.14±0.03, $p=0.01$). No difference of 125I-Ins binding was found between HS and LS. 2) PC-1 content was significantly higher in cultured fibroblasts from LS (57.2±6.15 ng/100 mg protein vs. 25.7±2.33, $p<0.01$) and correlated to the ED50 of Ins effect on IR-TK ($r=0.78$, $p<0.01$). In all these measurements, very similar data were obtained in HS vs. LS, whatever measurement of Ins sensitivity. Conclusions: cultured skin fibroblasts of healthy nonOb, nonD subjects having low Ins sensitivity have a reduced IR-TK and an increased PC-1 content, associated to a reduced Ins action on glucose metabolism. The present data demonstrate that these defects are intrinsic and/or irreversible. In accordance with data obtained in muscle and adipose tissue they suggest that a reduced IR-TK and an increased PC-1 content may play a primary, early role in the development of Ins resistance.

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INSULIN MODIFIED TGF-β ACTION IN ENDOTHELIAL CELLS AND SMOOTH MUSCLE CELLS.

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Insulin is known to have mitotic activity as well as regulating activity of glucose homeostasis. But, it is not clear whether hyperinsulinemia contributes to atheroscrelosis. Otherwise, transforming growth factor-beta (TGF-β) is a potent regulator of cell growth and differentiation. As the result from the inhibition of the smooth muscle cells proliferation, TGF-β may suppress progression of atherosclerosis. We have examined whether insulin modified TGF-β signaling cascade. We used a human endothelial cell line (PAIEC) or a human smooth muscle cell line (PAISMC) which were stably transfected with an expression construct containing a plasminogen activator inhibitor-1 promoter fused to luciferase reporter gene (PAI-1-Luc). TGF-β rapidly increased PAI-1-Luc activity ~3 to 5-fold at 6 to 12 h which subsequently declined to basal levels within 48 h following the treatment. There is no effect of insulin itself on PAI-1-Luc activity. Surprisingly, physiological dose of insulin was found to strongly inhibit the TGF-β stimulation of PAI-1-Luc activity. Next, we examined PAI-1 synthesis and secretion in the smooth muscle cells. Insulin also inhibit the PAI-1 synthesis and secretion in the presence of TGF-β. These results suggested that insulin accelerates atherosclerosis through inhibition of TGF-β activity. Currently we are investigating the relationship of insulin-modified PAI-1 activity to the SEK/p38 cascade.

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ALANINE SCANNING MUTAGENESIS OF INSULIN

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Although a large number of insulin analogs have been studies to date, no comprehensive analysis of the insulin side chains involved in receptor binding has been reported. We have applied alanine scanning mutagenesis to further elucidate the role of individual amino acid residues in receptor binding. A total of 21 new insulin constructs with alanine substitutions were expressed as single-chain insulin precursors in the yeast *Saccharomyces cerevisiae*. Yeast culture medium was treated with *Achromobacter Lyticus* protease to yield mature insulin and then used directly in the binding assay. Binding data on the new analogs revealed that the alanine mutations that were most disruptive for binding were at positions TyrA19, GlyB8, LeuB11, and GluB13 resulting in decrease in affinity of 1000 fold, 33 fold, 14 fold, and 8 fold respectively relative to wild-type insulin. In contrast alanine substitutions at positions GlyB20, ArgB22, and SerA9 resulted in an increase in affinity for the insulin receptor. The most striking finding is that B20Ala insulin retains high affinity binding to the receptor. GlyB20 is conserved in insulins from different species and in the structure of the B-chain it appears to be essential for the shift from the α-helix B8-B19 to the β-turn B20-B22. Thus substituting GlyB20 with alanine most likely modifies the structure of the B-chain in this region but this structural change appears to enhance binding to the insulin receptor. Compiling these data with data in the literature on alanine analogs allows an extensive overview covering the effect of single alanine substitutions at a total of 38 positions of the insulin molecule.

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EFFECTS OF BACTERIA TOXINS ON INTRACELLULAR PROCESSING OF INSULIN IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

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Guanine nucleotide binding proteins(G-proteins) are critically important mediators of many signal transduction systems in hormone action. Recently, possible role of G-protein in insulin action was proposed. So we studied effects of cholera and pertussis toxins on insulin binding, internalization of insulin-insulin receptor complex, and insulin receptor recycling in cultured vascular endothelial cells obtained from human umbilical veins. All experiments were performed on confluent cells attached to the 35-mm multi-well plates at a density of approximately 1 x 10^6 cells/well after one passage. The results were as follows ; 1. Specific insulin bindings to receptors of endothelial cells were time-dependent and reached their maximal levels after 60 min in all cells. The maximal specific insulin binding of control, cholera toxin-treated group, and pertussis toxin-reated group were 2.38± 0.24%, 2.28±0.18%, and 1.86±0.25%, respectively. 2. The internalization of ^{125}I-insulin into endothelial cells assessed by acid washing dissociation method occured rapidly. There was no significant difference in the internalized radioactivity of ^{125}I-insulin among three groups. 3. The recycling of insulin receptor of the pertussis toxin-treated group, was significantly decreased compared with that of control. But there was no significant difference in receptor recycling between control and cholera toxin-treated group. In conclusion, in view of the fact that pertussis toxin inhibited the recycling of insulin receptor, pertussis toxin sensitive G-protein may be closely associated with the insulin receptor and appears to be important in both insulin action and intracellular processing of insulin receptor in endothelial cells.

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α_2-HS-GLYCOPROTEIN INHIBITS TRYPSIN-ACTIVATED INSULIN RECEPTOR TYROSINE KINASE ACTIVITY

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α_2-HSG is a specific inhibitor of the mitogenic arm of insulin signaling at the insulin receptor tyrosine kinase (IR-TK) level. In our search for molecular basis of this effect, we took advantage of the previously shown trypsinization of IR. Trypsin treatment of intact cells and partially purified IR activates β-subunit autophosphorylation. Trypsin treatment cleaves the IR-α subunit at Arg^{576}-Arg^{577}, thereby making the β-subunit constitutively active. To understand the mechanism of α_2-HSG action, we compared its IR-TK inhibitory function on insulin- or trypsin-activated IR-TK. Confluent dishes of HIR_cB cells were treated either with PBS or 0.5 mg/ml trypsin at 4°C for 30 min. Trypsin treatment of cells prior to isolation of IR resulted in proteolytic modification of the α-subunit. Removal of insulin binding site by trypsin treatment was confirmed by lack of ^{125}I-insulin binding. From non-reducing gels, it was evident that trypsin treatment separated the α_2-β_2 heterotetramer into heterodimers. The holoreceptor from untreated cells had an M_r ~400 kDa compared to a M_r~ 116 kDa of trypsin-treated IR. Trypsin-activated IR's showed a 10-fold stimulation in basal IR-TK. Insulin did not increase trypsin-activated IR-TK any further. α_2-HSG (0.1-0.3 μM) completely inhibited both insulin-induced IR autophosphorylation and trypsin-activated IR autophosphorylation to the same extent. These findings indicate that the mechanism of IR-TKA inhibition by α_2-HSG is not by an alteration of the conformational change brought about by insulin binding. This study also suggests that α_2-HSG's inhibitory role might be mediated through the IR β-subunit.

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EFFCT OF PERTUSSIS TOXIN ON INSULIN-INDUCED SIGNAL TRANSDUCTION IN RAT ADIPOCYTES AND SOLEUS MUSCLES

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It has been reported that pertussis toxin (PTX) suppresses the function of trimeric GTP binding protein. We examined the effect of PTX on insulin-induced glucose uptake, diacylglycerol (DG) production, protein kinase C (PKC) activation and phosphatidyl-inositol (PI)3-kinase activation to clarify the role of trimeric GTP binding protein for insulin-mediated signal transduction mechanism in rat adipocytes and soleus muscles. Isolated adipocytes and soleus muscles were preincubated with 0.01~1 ng/ml PTX for 2 hr, followed by stimulated with 10~ 100nM insulin, 10~100nM IGF-I or 1 μM tetradecanoylphorbol 13-acetate (TPA). Pretreatment with PTX resulted in the dose responsive decreases in insulin- and IGF-I-stimulated [^{3}H]2-deoxyglucose (DOG) uptake, and unchanged TPA-stimulated [^{3}H]2-DOG uptake. In adipocytes insulin-induced DG production, PKC β translocation from cytosol to the membrane, and PI 3-kinase activation were suppressed when treated with PTX, despite of unchanged [^{125}I] insulin specific binding. When soleus muscle protein was incubated with [^{32}P]NAD and activated PTX, 40 kDa α-subunit of Gi was detected in autoradiography. These results suggest that Gi α may interact with phospholipase C, and G βγ may couple insulin receptor with PI 3-kinase activation in insulin sensitive tissues.

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REGULATION OF GLUCOSE UPTAKE AND GLUT4 GENE EXPRESSION BY CYCLIC AMP AND VANADATE IN 3T3-F442A CELLS.

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To further elucidate the mechanisms for cAMP induced-insulin resistance we investigated long-term effects of cAMP, the tyrosine phosphatase inhibitor vanadate or the combination of both in cultured 3T3-F442A adipocytes. Eight days after reaching confluence, 3T3-F442A cells were treated with insulin (10 mU/ml) for 16 h leading to a ~50% decrease in ^{125}I-insulin binding at the cell surface ($p<0.05$). The cAMP analog 8-bromo-cAMP (4 mM), in contrast, increased cell surface insulin binding ($p<0.05$), whereas vanadate (10 μM) alone had no effect. Insulin-stimulated ^{14}C-deoxyglucose (DOG) uptake was reduced by ~50%, whereas basal DOG uptake was increased (~2-fold, $p<0.05$) after cAMP pretreatment for 16 h. Neither short- (30 min) nor long-term (16 h) vanadate treatment altered basal DOG uptake, but insulin-stimulated DOG uptake was clearly enhanced by vanadate pretreatment (10 μM). Northern blots showed an enhancing effect of insulin (10 mU/ml) on GLUT4 mRNA level and this was found when a low (5.6 mM) but not high (25 mM) glucose was present in the culture medium, whereas 8-bromo-cAMP produced a marked, dose-dependent reduction in basal and insulin-stimulated GLUT4 mRNA. Vanadate (10 μM) alone stimulated GLUT4 gene expression, and was also found capable of preventing the cAMP-induced reduction in GLUT4 mRNA levels. Conclusions: 1) Tyrosine phosphorylation of insulin signalling peptides may be involved in GLUT4 gene expression and this may be opposited by cAMP leading to insulin resistance. 2) A low, physiological glucose concentration surrounding the cells is a prerequisite for the stimulating effect of insulin on GLUT4 gene expression. Insulin insensitivity following high glucose may partly be due to an impaired insulin effect on GLUT4 gene expression.

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FAILURE OF TUMOR NECROSIS FACTOR-α TO DIRECTLY AFFECT INSULIN-STIMULATED GLUCOSE METABOLISM IN RAT MUSCLE.

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To better understand the effects of tumor necrosis factor-α (TNFα) on insulin sensitivity *in vivo*, direct interaction of the peptide with isolated rat soleus muscle strips was investigated. Biological activity of TNFα was validated by accumulation of plasminogen activator inhibitor-1 in the supernatant of cultured human umbilical vein endothelial cells (ng/ml after 24h: control, 56±11 *vs.* 1.4 nmol/l TNFα, 275±23; $p<0.01$). Muscles were exposed to TNFα at concentrations ranging from 0.01 to 5 nmol/l and rates of insulin-stimulated glucose metabolism were determined after TNFα-pretreatment for 30 min, 6 h, and 24 h. Independent of exposure time, TNFα failed to exert any significant effect on the rates of ^{3}H-2-deoxy-glucose transport (stimulation by 100 nmol/l insulin after preincubation without *vs.* with 5 nmol/l TNFα, cpm/mg/h: 30 min, 779±29 *vs.* 725±29; 6 h, 652±56 *vs.* 617±60; 24 h, 911±47 *vs.* 936±31) or glucose incorporation into glycogen (μmol/g/h: 30 min, 5.19±0.22 *vs.* 5.25±0.41; 6 h, 2.08±0.10 *vs.* 2.09±0.17; 24 h, 2.51±0.21 *vs.* 2.41±0.26). In parallel, TNFα neither affected insulin-stimulated rates of CO_2 release (from μmol glucose/g/h: 30 min, 0.39±0.03 *vs.* 0.42±0.03; 6 h, 0.83±0.06 *vs.* 0.98±0.06; 24 h, 2.59±0.24 *vs.* 2.28±0.10) and lactate release (μmol/g/h: 30 min, 15.9±0.6 *vs.* 17.0±0.8; 6 h, 17.4±0.6 *vs.* 19.4±0.9; 24 h, 15.4±0.6 *vs.* 17.0±1.1), nor did it induce any change in muscle glycogen content (μmol glucosyl units/g: 30 min, 10.4±0.5 *vs.* 12.4±0.6, 6 h, 11.6±0.9 *vs.* 11.3±1.0, 24 h, 8.7±0.8 *vs.* 7.5±0.3). In conclusion, our findings do not support the hypothesis of muscle insulin desensitization by TNFα via autocrine or paracrine mechanisms. The obtained data favour the concept that TNFα-dependent muscle insulin resistance *in vivo* depends on indirect effects rather than direct interaction of the peptide with skeletal muscle.

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IN VIVO TISSUE-SPECIFIC MODULATION OF RAT INSULIN RECEPTOR GENE EXPRESSION BY MINERALOCORTICOIDS.

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We have recently reported that aldosterone decrease insulin receptor (IR) mRNA levels in U-937 human promonocytic cells. Nonetheless, there are no studies on the possible "in vivo" effects of mineralocorticoids on IR gene expression. For these reasons, in the present work we comparatively analyze IR gene expression at the mRNA level in liver, hindlimb skeletal muscle and epididymal adipose tissue of rats uninephrectomized and treated with deoxycorticosterone acetate (DOCA) 20 mg/kg, 2 times at week during four weeks ($n=8$) in relation with sham-rats ($n=6$). All the animals were killed without anesthesia. Trunk blood samples were collected, and the three tissues in study were immediately removed and frozen in liquid nitrogen. Rat body weights were unafected by DOCA treatment, and plasma insulin and glucose levels were decreased and increased respectively, as compared to sham rats. Moreover, the DOCA treatment induced hipokalemia with normal plasma sodium concentrations and decreased plasma protein. For Northern blot assays, RNA samples of the tissues were electrophoresed in agarose-formaldehyde gels, blotted onto nitrocellulose membranes, hybridized with a ^{32}P-labeled probe (the 0.98 kb rat IR specific EcoRI fragment of the prIR p16 clone) (Gift from Prof. Goldstein) and autoradiographed. The autoradiographs were scanned with a laser densitometer and the readings normalized with the amount of 28S RNA, as revealed by ethidium bromide staining. Northern assays revealed two IR mRNA species of approximately 9.5 and 7.5 kb, in the three tissues. DOCA treatment induced an approximately 50% increase in the levels of both RNAs in the liver, 20% decrease in adipose tissue but not changes in skeletal muscle. These results provide evidence for an "in vivo" tissue-specific regulation of IR gene expression at the mRNA level, in rats under an experimental condition of excess of mineralocorticoids.

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EFFECT OF TUMOR NECROSIS FACTOR-α(TNF-α) ON INSULIN SIGNAL TRANSDUCTION IN RAT ADIPOCYTES

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Although much evidence has been accumulated, suggesting that TNF-α is the important mediator of insulin resistance, the precise mechanism is still unclear. Recently, it has been reported that insulin-induced glucose uptake is mediated by each activation of insulin receptor substrate-1 (IRS-1) and phosphatidylinositol 3-kinase (PI3K), and diacylglycerol-protein kinase C (PKC).

We have examined the effect of TNF-α on insulin-induced glucose uptake, activation of IRS-1 and PI3K, and PKC activation in rat adipocytes. Pretreatment with 10^{-8} M TNF-α for 60 min resulted in a decrease in 10nM insulin- or 10^{-6} M phorbol ester (TPA)-induced [^{3}H] 2-deoxyglucose uptake without affecting basal glucose uptake. Insulin- or TPA-stimulated activation of IRS-1 and PI3K after immunoprecipitation with phosphotyrosine antibody, was suppressed by preincubation with TNF-α for 60 min.
TNF-α pretreatment also suppressed insulin- and TPA-induced increases in membrane-associated PKC β.

These results suggest that TNF-α inhibits insulin- or TPA-stimulated both tyrosine kinase, IRS-1 and PI3K activation, and PKC activation in rat adipocytes.

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INSULIN RESPONSIVENESS IN MICE LACKING LAR PROTEIN PHOSPHATASE ACTIVITY.

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Introduction: Insulin binding to the alpha subunit of the insulin receptor (IR) results in phosphorylation and activation of the intracellular beta subunits. Subsequent inactivation by dephosphorylation is achieved by protein tyrosine phosphatases (PTPs) and the cell adhesion molecule-like PTP LAR has been implicated in the inactivation of the IR on the basis of in vitro studies. Our aim was to test this involvement by studying the insulin response in mice that are deficient in LAR signalling. **LAR Knock-out:** Gene targeting in mouse embryonic stem cells generated mice lacking sequences encoding both LAR phosphatase domains (LAR-/-). **Blood glucose response:** Post-prandial LAR-/- (n=12; 38-44 weeks old) and age matched control (C57BL/6; n=10; 43 weeks old) male mice were subjected to an insulin tolerance test (ITT) (1 U/kg, s.c.). Blood samples were obtained 120 and 0 min pre-dose and at 30 min intervals for 2 hours post-dose enabling calculation of the Δglucose-AUC. LAR-/- mice showed no differences (t-test) in pre-dose (120 min) plasma glucose (8.8±0.3 vs 9.5±0.3 mmol/l in controls, ns) (all values are mean±SE) or insulin concentrations (316±54 vs 393±55 pmol/l in controls, ns) nor in their response to the ITT (-344±38 vs -452±93 min · mmol/l in controls, ns). **Lipogenesis:** Adipocytes isolated from epididymal fat pad were used to determine the ED_{50} for insulin in both control mice and LAR knock-out mice. No significant difference was observed, ED_{50} for both groups were approx. 100 pM. **Insulin Receptor Tyrosine Kinase (IRTK):** The basal IRTK was measured in hindlimb muscles using a microtitre well assay. No significant difference was seen between LAR-/- and controls (2.0±0.5 vs. 3.2±1.1). **Conclusion:** The data provide no evidence of increased insulin signalling in LAR-/- in vivo nor in vitro compared to controls suggesting that LAR is not solely responsible for down regulating the IR signal.

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A MOLECULAR BASIS FOR INSULIN RESISTANCE: ELEVATED SER/THR PHOSPHORYLATION OF IRS-1 AND IRS-2 INHIBITS THEIR BINDING TO THE JUXTAMEMBRANE REGION OF THE INSULIN RECEPTOR (IR) AND IMPAIRS THEIR ABILITY TO UNDERGO INSULIN-INDUCED TYR PHOSPHORYLATION.

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Tumor narcosis factor-α (TNF) gained recognition as a key mediator of insulin resistance in obesity and NIDDM. We have previously shown that one important mechanism by which TNF and other insulin-resistance-inducing agents interfere with insulin signaling is through increased Ser/Thr phosphorylation of insulin receptor substrate-1 (IRS-1). To unravel the molecular basis for these inhibitory effects we undertook to study the interaction of Ser/Thr -phosphorylated IRS-1 and IRS-2 with the insulin receptor. We could demonstrate that similar to IRS-1, IRS-2 also interacts with the juxtamembrane (JM) domain (a. a. 943-984) but not with the C-terminal region (a. a. 1245-1331) of IR, expressed in bacteria as (His)6-fusion peptides. Moreover, incubation of rat hepatoma Fao cells with TNF, sphingomyelinase, TPA, or other P-Ser/Thr-elevating agents, reduced insulin-induced Tyr phosphorylation of IRS-1 and IRS-2, elevated their P-Ser/Thr levels, and significantly reduced their ability to interact with the JM region of IR. Similar inhibitory effects were obtained when Fao cells were subjected to prolonged (20-60 min) treatment with insulin. These findings suggest that insulin resistance is associated with enhanced Ser/Thr phosphorylation of IRS-1 and IRS-2, which impairs their interaction with the JM region of IR. Such impaired interactions abolish the ability of IRS-1 and IRS-2 to undergo Tyr phosphorylation by the insulin receptor kinase, and further propagate insulin signaling.

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NATURAL IRS-1 MUTATIONS WITH IMPAIRED FUNCTION AT DIFFERENT STEPS OF INSULIN SIGNALING PATHWAY

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IRS-1 is one of the major substrates of insulin receptor (IR) tyrosine kinase and mediates various insulin signals downstream. To examine if the natural IRS-1 gene mutations contribute to the developement of NIDDM, we have studied the impact of three mutant IRS-1s (G971R, P170R and M209T in which Gly971, Pro170 and Met209 are substituted by Arg, Arg and Thr, respectively) on insulin signaling. 32D-IR cells, stably overexpresing human IR, were transfected with wild-type (WT) or three mutant IRS-1 cDNAs, and analyzed. Cells expressing three mutant IRS-1s exhibited significant decrease in insulin stimulated thymidine uptake as compared with WT. Upon insulin stimulation, cells expressing G971R showed 39% decrease ($p<0.005$) in PI3-K activity and 22% decrease ($p<0.05$) in MAP-K activity, while cells expressing P170R and M209T showed slight decrease [17% and 14% ($p<0.05$)] in PI3-K and greater decrease [41% and 43% ($p<0.005$)] in MAP-K. In addition, both P170R and M209T showed significant decrease in phosphorylation [41% and 43% ($p<0.005$)] and association with IR [32% and 38% ($p<0.05$)], while G971R showed normal phosphorylation and association with IR compared with WT.

In conclusion, two mutations, P170R and M209T, occuring in the PTB domain of IRS-1, showed impaired mitogenic response mainly due to reduced association with IR, while G971R showed impaired mitogenic response mainly due to reduced association with PI3-K. These data suggested the contibution of IRS-1 mutations to the development of NIDDM in humans.

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OXIDANT STRESS INDUCED IMPAIRMENT IN INSULIN ACTION IS DUE TO DEFECTS IN DISTAL COMPONENTS OF INSULIN SIGNALING CASCADE.

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Accumulating evidence suggest a link between increased oxidant stress in diabetes and insulin resistance. We have previously demonstrated that exposure of 3T3-L1 adipocytes to prolonged, low grade oxidant stress result in impaired insulin action. In this study we aimed at elucidating the cellular mechanisms underlying this observation. Insulin Receptor Substrate 1 (IRS-1) content was reduced dose-dependently by incubation with glucose oxidase (GO) for 18 hours, reaching 60±5% reduction with 50 mU/ml GO. Immunoreactivity of IRS-1 with anti-phosphotyrosine antibodies following insulin stimulation was not affected by GO, suggesting an increased ratio of phosphotyrosine per IRS-1 protein following oxidation. However, this effect of GO did not impair PI3 Kinase activation through its interaction with IRS-1, as PI3 Kinase activity in immunopercipitates of IRS-1 was unchanged following GO treatment. In addition, activation of MAP Kinase by insulin was not altered by GO treatment, as assessed by immunoblot with anti active MAP Kinase Antibodies. As defects in the early events of the insulin signaling cascade did not seem to explain the oxidant stress -induced reduction in insulin action, we assessed the status of the two glucose transporters isoforms GLUT1 and 4. GLUT4 protein and mRNA were reduced following GO treatment by 43±6% and 40±4%, respectively. Concomitantly, GLUT1 protein and mRNA were increased 3.5±0.2 and 4.2±0.3 fold, respectively. GLUT4 down-regulation appeared to represent an independent effect of oxidant stress, as it was observed under inhibition of GLUT1 elevation by co-administration of cycloheximide. Hence, both down-regulation of GLUT4 content and/or a defect in its translocation to the plasma membrane may explain the observed insulin hypo-responsiveness. In conclusion, prolonged oxidant stress appears to impair insulin stimuation of glucose transport by interference with distal effectors of the insulin signaling cascade.

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EFFECTS OF WORTMANNIN AND OKADAIC ACID ON INSULIN REGULATED GLUCAGON SECRETION

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We have previously demonstrated the insulin induced negative regulation of glucagon secretion and the expression of functional insulin receptors in glucagon secreting cell lines. However, the mechanism of these phenomenon is still unknown. In this study, we evaluated the involvement of insulin receptor signaling pathway in glucagon secretion from In-R1-G9 and α TC clone 6 cells, using okadaic acid (an inhibitor of tyrosine phosphorylation of IRS-1) and wortmannin (an inhibitor of phosphatidylinositol 3 kinase (PI3K)). In the presence of insulin (10^{-7} mol/l), the contents of glucagon secreted into culture medium significantly ($p<0.05$) decreased as compared with those without insulin (by 33, 59 and 48% in In-R1G9 cells at 2, 6 and 12 hours after incubation, and by 23, 43 and 33% in α TC clone 6 cells, respectively). On the other hand, in addition of wortmannin (100nmol/l), glucagon secretion returned to the almost same level as those without insulin (99, 102 and 104% vs. those without insulin at 2, 6 and 12 hours in In-R1-G9 cells, and 94, 91 and 97% in α TC clone 6 cells, respectively). Addition of okadaic acid (1μmol/l) also recovered the glucagon secretion to the almost same level as those without insulin (97, 87 and 99% vs. those without insulin at 2, 6 and 12 hours in In-R1-G9 cells, and 107, 89 and 88% in α TC clone 6 cells, respectively). In conclusion, insulin might be involved in the negative regulation of glucagon secretion through PI3K, which is an important component in insulin signaling pathway.

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INSULIN-INDUCED NITRIC OXIDE AND SUPEROXIDE FREE RADICAL PRODUCTION OF HUMAN PLATELETS

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Recent data support the possible role of nitric oxide (NO) in the development of insulin signaling. The aim of this study was to examine the effect of insulin on the NO production of platelets. We measured the chemiluminescence of platelet-rich plasma, prepared from the blood of healthy volunteers, in the presence of luminol. Detection of NO by luminol is possible in the form of peroxinitrite produced from NO and superoxide. The results are expressed in percent of area under curves (AUC), taking the AUC of 84 pmol/l of insulin as 100 % (Figure 1). In case of insulin + Nω-nitro-L-arginine methyl ester (L-NAME, inhibitor of nitric oxide synthase enzyme, Figure 2), or insulin + superoxide dismutase (SOD, Figure 3) the AUC of insulin was taken as 100 %. Data are shown as mean±SD, n=9.

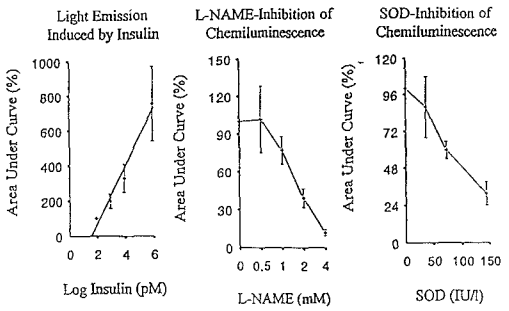

Figure 1.

Figure 2.

Figure 3.

Summarizing this data, insulin evoked a concentration dependent chemiluminescence increase, which was inhibited by L-NAME and SOD. As conclusion, we suppose that the insulin-induced increase of chemiluminescence was due to increased production of NO and superoxide free radicals forming peroxinitrite. This is the first direct evidence demonstrating stimulation of NO-release by insulin.

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INSULIN STIMULATES THE ASSOCIATION OF PI3-KINASE SERINE KINASE WITH THE INSULIN RECEPTOR AND PDE3 IS A SUBSTRATE.

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PI 3-kinase, a heterodimer consisting of a 85 kDa regulatory subunit and a 110 kDa catalytic subunit, is a dual enzyme with both lipid and serine kinase activities. The aim of this study was to elucidate the effect of insulin on PI 3-kinase lipid and serine kinase activities and the association with insulin receptor (IR) and IRS-1 in human adipocytes. Insulin stimulated the association of p85 and p110 with both IR and IRS-1. Insulin increased~20-fold the PI 3-kinase lipid kinase activity associated with IRS-1 but not with IR. Protein kinase activity was also assayed in the presence of Mn^{2+} in the same immunoprecipitates in the presence or absence of wortmannin. The results showed that IR, but not IRS-1 immunoprecipitates from cells treated with insulin contained a wortmannin-sensitive kinase that phosphorylated several proteins including p85, p110 and a 135 kDa protein To investigate if this protein could be cGI-PDE (PDE3) we performed protein kinase assays in IR immunoprecipitates using recombinant PDE3. Insulin stimulated the phosphorylation of cGI-PDE by the immunoprecipitates and this activity was inhibited by wortmannin. In conclusion: A) The p85 and p110 subunits of PI 3-kinase are associated with both IR and IRS-1 in response to insulin, B) Insulin stimulated only the PI 3-kinase lipid kinase activity associated with IRS-1, C) Insulin stimulated only the PI-3 kinase serine kinase activity associated with IR that phosphorylated several proteins including p85 and PDE3 suggesting a role of the PI 3-kinase serine kinase in the antilipolytic effect of insulin in human adipocytes.

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INSULIN INDUCES THE EXPRESSION OF HUMAN ENDOTHELIAL NITRIC OXIDE SYNTHASE

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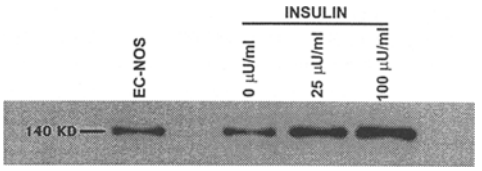
It has recently been shown that insulin induces vasodilatation in human arteries and human veins. This effect of insulin has been shown to be a direct one on the human vein. In view of these observations and the fact that insulin induced vasodilatation is impaired in insulin resistant states like Type II diabetes and obesity, we have now investigated the hypothesis that insulin may induce the expression of endothelial nitric oxide synthase (NOS) in endothelial cells grown from human aorta. Human aortic samples were obtained from transplant donors. Endothelial cells were harvested following collagenase digestion and were grown in 2% Fetal Bovine Serum (FBS) containing media. Identity of endothelial cells was confirmed by their cobblestone morphology, positive staining for EN4, Factor VIII and Ulex europaeous and negative smooth muscle α-actin staining. When the cells reached 90% confluency, media was changed to 10% charcoal/dextran treated FBS containing media and cells were induced with insulin. Media was changed every two days for six days. Cells were homogenized, electrophoresed and blotted. Blots were treated with monoclonal antibody for endothelial NOS. In four separate experiments it was clearly demonstrated that there was a dose dependent induction by insulin of NOS in the endothelium (see figure). We conclude that insulin induces a dose dependent induction of nitric oxide synthase in human aorta and endothelial cells and that this effect may be contributory to the overall vasodilatory effect of insulin directly at the vascular level.



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TAXOL® INHIBITS INSULIN ACTION WITH A DECREASE IN PHOSPHATIDYLINOSITOL-3-KINASE ACTIVITY.

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Paclitaxel (Taxol), a clinical anti-neoplastic agent, stabilizes microtubules, inhibiting intracellular vesicular trafficking. In our previous studies in dogs, interstitial concentration of 8 μmol/l Taxol caused a reduction in the rate of glucose disposal during hyperinsulinemic euglycemic clamps. However, this reduction was not due to reduced transendothelial transport of insulin. To study possible cellular mechanism(s) by which Taxol caused insulin resistance, we employed mouse 3T3-L1 adipocytes and studied the insulin signaling pathway for glucose uptake. Incubating cells with 100 nmol/l insulin increased [^{3}H] 2-deoxyglucose (2-DG) uptake 1.8-fold compared to basal. Preincubation of 3T3-L1 cells with Taxol resulted in a dose-dependent decrease in 2-DG uptake with insulin. At 10 μmol/l Taxol, there was complete suppression of insulin-stimulated 2-DG uptake ($p=0.36$ compared to basal). Insulin receptor (IR) tyrosine kinase activity was measured by immunoadsorption plate assay using poly (Glu, Tyr) (4:1) as an exogenous substrate, and IR tyrosine phosphorylation was measured by Western blot analysis with anti-phosphotyrosine antibodies (α-PY). Insulin stimulation resulted in 5.0-fold increase in IR tyrosine kinase activity and 2.7-fold increase in IR tyrosine phosphorylation. Taxol did not affect either IR tyrosine kinase activity or tyrosine phosphorylation ($p=0.31$ and 0.87, respectively). Since phosphatidylinositol-3-kinase (PI3K) activity has been reported to be crucial for insulin-stimulated glucose uptake, we measured insulin-stimulated PI3K activity in α-PY immunoprecipitates. There was a decrease in PI3K activity with Taxol with a linear correlation between decreases in 2-DG uptake and PI3K activity ($r^2=0.99$). These data suggest that Taxol inhibits insulin-stimulated glucose uptake via suppression of PI3K activity.

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CHOLESTEROL IS REQUIRED FOR SIGNAL TRANSDUCTION VIA GLYCOSYL PHOSPHATIDYLINOSITOL(GPI)-ANCHORED PROTEINS

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Glycosyl phosphatidylinositol(GPI)-anchored proteins are cell surface proteins which have no transmembrane domain but are anchored to the outer leaflet of the plasma membrane by a phospholipid moiety. GPI-anchored proteins can transduce signals to the cytoplasm after crosslinking on the cell surface or following release of inositol phosphoglycan which may also serve as an alternative second messenger for insulin action. Moreover, GPI-anchored proteins are clustered in distinct membrane domains, which are formed by an unique lipid composition requiring cholesterol, but the exact mechanism of signal transduction following crosslinking of GPI-anchored proteins is not yet elucidated. Therefore, we studied the influence of metabolic alterations of cellular cholesterol content on the calcium response via GPI-anchored proteins CD59 and CD48 in human Jurkat T-lymphocytes. Lowering cholesterol by different inhibitors of cellular cholesterol synthesis suppressed calcium response via GPI-anchored proteins by about 50% whereas stimulation via the transmembrane CD3 complex was only minimally affected. The decrease in overall calcium response via GPI-anchored proteins was reflected by an inhibition of the release of calcium from intracellular stores. Cell surface expression of GPI-anchored proteins was not changed quantitatively by the treatment, neither was the pattern of immunofluorescence in microscopic examination. In addition, the distribution of GPI-anchored proteins in detergent-insoluble complexes was not altered. In conclusion, cellular cholesterol is an important prerequisite for signal transduction via GPI-anchored proteins beyond formation of membrane domains.

Supported by the Austrian Science Foundation (P11404Med).

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ALTERED EXPRESSION OF INSULIN SIGNALLING COMPONENTS IN ADIPOCYTES FROM FETALLY MALNOURISHED MALE RATS.

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Epidemiological studies have suggested that there is a relationship between fetal growth and the subsequent development of non-insulin-dependent diabetes mellitus. In the present study insulin action on rat adipocytes was studied in the offspring of mothers who had been fed either a control (20 % protein) or a low (8 %) protein diet during pregnancy and lactation. Adipocytes isolated from low protein offspring had a significantly higher basal glucose uptake (1202 ± 116 pmol /min/mg protein compared to 584 ± 24 pmol/min/mg protein; $p < 0.01$) and a significantly higher insulin-stimulated glucose uptake (2002 ± 213 pmol/min/mg protein compared to 1598 ± 207 pmol/min/mg protein; $p < 0.05$) than controls. This may be related to a three-fold increase in insulin receptors in low protein adipocytes. Consistant with these observed changes in glucose transport, adipocytes from low protein animals had significantly higher basal ($p < 0.001$) and insulin stimulated ($p < 0.001$) IRS-1 associated PI 3-kinase activities. There was also more p85 associated PI 3-kinase activity in these adipocytes ($p < 0.005$). There was no difference in expression of the p85 regulatory subunit or the p110α catalytic subunit of PI 3-kinase. In contrast there was a six fold reduction in the p110ß catalytic subunit of PI 3-kinase in adipocytes from low protein animals. These results suggest that poor fetal nutrition during pregnancy and lactation can have long term effects on glucose transport and on the expression of key components of the insulin signalling pathway in adipocytes.

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THE EFFECT OF INSULIN ON POLYMERISATION OF ACTIN IN RAT SKELETAL MUSCLE CELLS

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We have shown that the effect of insulin on cell membrane dynamics is abolished by disrupting the cytoskeleton in cultured rat skeletal muscle cells (L6 myotubes) and human mononuclear leukocytes. The interaction between insulin and the cytoskeleton remains unclear though the microfilaments may be involved. We therefore developed a novel technique to study the effect of insulin on the polymerisation of filamentous (F) and globular (G) actin in L6 myotubes. L6 myotubes on glass coverslips were studied after 6-10 days in culture. The F and G actin were labelled with fluorescent phalloidin and DNase I respectively. The morphological distribution of the F and G actin in the cell membrane was examined by a confocal laser scanning microscope. The fluorescent intensities per unit area were measured and the ratio of F and G actin (F/G) was compiled using a computer controlled image analyser. The results are expressed as means [S.E.M.] from five experiments.

		Insulin			
	Control	10nM	100nM	500nM	1000nM
F/G	1.432	1.710	3.924[a]	2.719	1.473
	[0.359]	[0.711]	[0.191]	[0.465]	[0.343]

a: $p<0.01$ vs control ; paired t-test

Insulin (100nM) caused a significant change in the F/G actin ratio of L6 myotubes. A bell-shaped concentration effect of insulin on F/G actin was observed. This could be secondary to cross-reaction with other growth factor receptors at higher concentrations of insulin. Insulin has been shown to modulate membrane fluidity and cause a major reorganisation of the actin network in L6 myotubes. Our result suggests that changes in polymerisation of actin may be the underlying mechanism whereby insulin affects cell membrane dynamics in L6 myotubes.

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DIFFERENTIAL SUB CELLULAR LOCALIZATION OF p110 PI 3-KINASE SUBUNITS IN 3T3-L1 ADIPOCYTES

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In the current study we have investigated whether differential patterns of sub cellular location of the PI 3-kinase catalytic subunits exist in insulin responsive 3T3-L1 adipocytes. We have previously shown that differential regulation of PI 3-kinase at distinct sub cellular locations may explain differences observed in the PI 3-kinase dependent responses that can be stimulated by insulin compared with those that can be stimulated by other growth factors. In particular sub cellular fractionation studies have shown insulin and PDGF both stimulate PI 3-kinase activity and the level of the p85 adapter subunit in the plasma membrane (PM) while only insulin stimulates these in microsomal membranes (MM) that in 3T3-L1 adipocytes (Nave et.al. Biochem J 318 55-60). In the current study we have characterised antibodies specific for both p110α and p110β . Using these it was found both p110α and p110β are upregulated during fat cell differentiation. The subcellular distribution of p110β closely matched that of p85α with ≈ 10% present in PM, ≈ 20% present LDM and the the bulk being present in the cytosol. Surprisingly though approximately 70% of the immuno-reactive p110α was found in the MM fraction with less than 10% present in the PM and 20% present in the cytosol. The implications for insulin signalling of this differential distribution of p110α and p110β is being investigated.

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EXTRAPANCREATIC EFFECT OF GLIBENCLAMIDE ON PYRUVATE DEHYDROGENASE ACTIVITY.

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To know the mechanisms of extrapancreatic action of sulfonylurea, pyruvate dehydrogenase(PDH) activities and 2-deoxy glucose uptake were measured in mouse adipose tissues. Glibenclamide (3mg/kg/day. SU) was orally adminstrated for 10 days prior to the experiment. Basal PDH activity was 5- 6 folds increased in SU treated mouse and PDH activity by the incubation with insulin(250 μU/ml) was 2 times higher in (SU) compared to that in control. 2-deoxy glucose uptake was significantly increased in the adipocytes from (SU) in the presence of high concentration of insulin (0.1-1mU/ml), but there were no effects in low concentration of insulin (0-25 μU/ml). Addition of phlorizin(4mM) did not inhibit insulin-stimulated PDH activity in (SU). No effect of direct addition of SU on PDH activity was observed in the control adipose tissue.There were no differences in insulin binding activity in adipocytes and blood IRI levels between (SU) and control. These results suggest that SU acts on post-insulin receptor systems in insulin action.

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EFFECT OF TROGLITAZONE ON TROMBIN-INDUCED PLATELET AGGREGATION MECHANISM IN VITRO

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Troglitazone(CS-045), antidiabetic thiazolidinedione, is known to improve insulin resistance. However, it is not unclear the effect of troglitazone on platelet aggregation. We studied the effect of troglitazone on thrombin-induced platelet aggregation, phosphoinositide metabolism, protein phosphorylation and phosphatidylinositol 3-kinase in vitro in human platelet.

Maximum platelet aggregation by ADP, collagen and thrombin was suppressed by the pretreatment with 0.1～1 μM troglitazone for 60min. Pretreatment with troglitazone resulted in decreases thrombin-induced phosphatidic acid production, hydrolysis of phosphatidylinositol 4,5-bisphosphate by phospholipaseC, and 47kDa protein phospholylation. Troglitazone alone stimulated phosphatidylinositol 3-kinase(PI3K) activity, but thrombin-induced PI3K activation was suppressed by the pretreatment with troglitazone for 60 min.

These results suggest that troglitazone has a potent inhibitory effect of platelet aggregation via suppression of both thrombin-induced activation of phosphoinositide metabolism and PI3K activation in human platelet.

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INSULIN STIMULATES GLYCOGEN SYNTHESIS IN BAKERS' YEAST

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Yeast harbors homologs of many human genes involved in transmembrane signalling. This study was performed to test for the putative existence of elements of insulin signaling in yeast. 1) The effect of insulin on glucose metabolism of wild type yeast cells grown in rich glucose medium in the absence or presence of 1 μM human insulin during the transition from late exponential to stationary phase was assayed. Glycogen accumulation was increased by 40-60% in the insulin-induced (85±11 mg/g cells) compared to basal cells (53±8 mg/g cells). The decrease of glycogen phosphorylase activity (45.9+3.8 mU/mg protein) was significantly more pronounced in insulin-induced (5.5±0.9) compared to basal cells (12.0±1.9). Glycogen synthase activity ratio (68.7±7.6 %) declined to 34±8 %, only, in the presence of insulin *vs.* 18±5 % in its absence. Thus, insulin (EC_{50}=150-250 nM) present during logarithmic growth, already, causes glycogen phosphorylase and synthase to become more sensitive and resistant, respectively, to inactivation when the culture reaches saturation. 2) A putative insulin receptor-like protein was partially purified from solubilized plasma membranes by wheat germ agglutinin and insulin-agarose affinity chromatography. It was specifically crosslinked to ^{125}I-labelled porcine insulin using a bifunctional crosslinker. Equilibrium binding using precipitation and subsequent filtration of the receptor revealed specific binding of human insulin and mouse IGF-1 (K_d=1.5±0.5 μM and 0.6±0.3 μM, respectively). 3) Western blotting with anti-phosphotyrosine antibodies of receptor incubated *in vitro* with ATP demonstrated stimulation of autophosphorylation on tyrosine residues in response to 0.5 μM insulin (3.8±0.9-fold *vs.* basal), which was blocked by 0.1 mM Na_3VO_4. Understanding the interaction of the insulin receptor-like protein with downstream components in yeast may help to understand metabolic insulin signalling in mammalian cells.

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GLUCOSE OXIDASE IMPAIRS INSULIN STIMULATED GLUCOSE TRANSPORT IN DIFFERENTIATED 3T3-L1 ADIPOCYTES.

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In 3T3-L1 adipocytes, various inducers of insulin resistance including prolonged exposure to insulin and TNF-α were shown to reduce expression of the insulin sensitive glucose transporter GLUT4, while increasing GLUT1 expression. As oxidant stress was recently suggested to be linked to impairment in insulin action, we assessed the effect of prolonged exposure of 3T3-L1 adipocytes to an enzymatic H2O2 generating system. We observed that 18 hours exposure of 3T3-L1 adipocytes to glucose oxidase (GO) resulted in a reduced capacity of insulin to increase glucose transport above basal activity. This could not be attributed to defects in early events of the insulin signaling cascade, but was associated with a 45% reduction in the expression of GLUT4 protein and mRNA, accompanied by a 3.5 -fold increase in GLUT1 protein and mRNA content. In this study we assessed whether this impairment in insulin stimulated glucose transport (ISGT) can be attributed to the alterations in expression of the two glucose transporteres isoforms. 3T3-L1 adipocytes were exposed to GO for up to 4 hours. While exerting no significant effect on basal glucose transport activity, ISGT was reduced by 50% following 1or 2 hour exposure to 100 or 50 mU/ml GO, respectively. ISGT was completely abolished by increasing exposure time to 2 and 4 hours, respectively. Under these conditions, no significant changes in total membrane GLUT1 and GLUT4 content were observed. This effect of GO could not be inhibited by co-administration of okadaic acid (3 nM), H7 (0.1 or 1 mM), or MAP Kinase inhibitor (PD98059, 10 μM), suggesting that neither activation of class 1 phosphatases, PKC or the MAP kinase cascade explains oxidant stress induced ISGT defect. In conclusion, GO induces insulin resistance in 3T3-L1 adipocytes. This relatively early effect appears to represent impaired insulin stimulated translocation of existing glucose transporters to the plasma membrane, by mechanism which remain to be elucidated.

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THE EFFECT OF ACUTE HYPERGLYCAEMIA AND GLUCOSAMINE ON gp160 AND PI3-KINASE DISTRIBUTION

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We have previously shown that high glucose (HG) and to a greater extent glucosamine (GLN) can acutely cause the internalisation of GLUT4, an effect mediated via the hexosamine biosynthesis pathway, however how this occurs is not understood. Two possible regulators that may be involved include: 1) gp160, a glycoprotein found exclusively on GLUT4-containing vesicles in rat adipocytes and 2) PI3-kinase, which is believed to be required for insulin-stimulated GLUT4 translocation. The aim of this study was to investigate the effect of acute hyperglycaemia and GLN on the intracellular distribution of gp160 and PI3-kinase. GLUT4 and gp160 levels were measured in total lysates, high density microsomal (HDM), low density microsomal (LDM) and plasma (PM) membranes, while PI3-kinase activity was measured in cytosolic and particulate fractions of rat adipocytes pre-incubated with HG (30 mM) in the presence of insulin (0.7 nM) or GLN (3 mM) for 2 hours. GLUT4 and gp160 levels were detected by Western blotting using rabbit polyclonal antibodies. PI3-kinase activity was detected by thin layer chromatography of a ^{32}P-labelled phosphatidyl inositol substrate. HG and GLN reduced the basal level of GLUT4 on the PM by 50 ± 9% ($p < 0.005$ n=5) and 80 ± 5% ($p < 0.005$ n=5) respectively, with a concomitant increase in the LDM, suggesting intracellular retention of GLUT4. Lysates and HDM GLUT4 levels were unaltered. HG and GLN reduced the basal level of gp160 by 72 ± 2% ($p < 0.001$ n=4) and 71 ± 2% ($p < 0.0005$ n=4) respectively in LDM, with a concomitant increase in HDM, suggesting retention of gp160 in the endoplasmic reticulum. Lysate gp160 levels were unaltered, suggesting that total gp160 was unchanged. The glycosylation rate of gp160, measured using ^{3}H-mannose incorporation was normal in adipocytes treated with GLN. GLN caused a redistribution of PI3-kinase activity from the particulate, (decreasing by 55 ± 8%, p=0.01 n=4) to the cytosolic fraction. It is concluded that the intracellular retention of GLUT4 caused by acute hyperglycaemia is associated with a hexosamine biosynthesis pathway-mediated decrease of gp160 in LDM and with a redistribution of PI3-kinase activity to the cytosol. It remains to be determined if these events are causally related.

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EFFECT OF TRIYODOTIRONINE ON GLUT1 mRNA EXPRESSION AND ON ITS SUBCELLULAR DISTRIBUTION IN 3T3-L1 ADIPOCYTES.

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Triyodotironine (T_3) has an important role in the regulation of glucose metabolism. The aim of this work was to know the effect of T_3 on glucose transport and on the glucose transporter GLUT1 in 3T3-L1 adipocytes. Differentiated cells were incubated in the absence and presence of T_3 (50 nM) for three days. [^{3}H]-2 deoxy-glucose uptake was measured in basal situation. Plasma membranes, low density microsomes and high density microsomes were isolated in order to see the GLUT1 subcellular distribution. Protein GLUT1 was measured by Western-blot and its mRNA levels by Northern-blot. T_3 produced a 4-fold increase (0.5±0.12 vs 0.13±0.05 nmol/500,000 cell/6 min, Mean±EEM, $p<0.05$, n=10) in the basal glucose uptake. Cells treated with T_3 had higher GLUT1 protein levels (761±106 vs 406±108 AU/15 μg of protein, $p<0.05$, n=5) although its percentage in the three subcellular fractions was not modified by this hormone. The specific mRNA levels were similar in the absence and presence of T_3 (2.6±0.7 vs 1.6±0.3 AU/15 μg total RNA, n.s., n=4). In conclusion, thyroid hormone stimulates glucose uptake due, at least in part, to an increase in GLUT1 protein content. The striking discrepancy between GLUT1 mRNA and GLUT1 protein abundance indicates the importance of post-transcriptional regulatory mechanism in the control of GLUT1 expression.

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5-AMINO-4-IMIDAZOLECARBOXAMIDE RIBOTIDE IS A POTENTIAL MEDIATOR OF GLUCOSE TOXICITY

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Marshall and colleagues demonstrated that a co-product of glucose and glutamine metabolism mediates glucose-elicited desensitization of the glucose uptake system, and identified the likely mediator as glucosamine or a metabolite. Key to their reasoning were the facts that azaserine, an inhibitor of *de novo* glucosamine biosynthesis, blocked desensitization while exogenously added glucosamine induced desensitization in the absence of glucose. Glucose and glutamine metabolism interact at a number of additional azaserine-inhibitable reactions, including key steps in the *de novo* biosynthesis pathway of purines. 5-amino-4-imidazolecarboxamide ribotide (ZMP), an intermediate in this pathway, is a structural analogue of AMP that has significant effects on intermediary metabolism. To learn whether ZMP desensitizes the insulin-stimulated glucose uptake system, primary rat adipocytes were incubated for 5 hours in HEPES buffered salt solution (HBSS) containing 20 mM glucose without or with either 10 ng/mL insulin or 500 μM 5-amino-4-imidazolecarboxamide riboside (AICAR, the dephosphorylated precursor of ZMP), and insulin-stimulated glucose uptake was measured following a 30 minute recovery period in HBSS alone. Incubation with either AICAR or insulin during the induction period reduced subsequent insulin-stimulated glucose uptake by 70% (from 1300 (HBSS w/o AICAR or insulin) to 325 (+AICAR) or 400 (+insulin) dpm, Ave of 3 independent experiments). AICAR was similarly effective in HBSS without added glucose. The AICAR effect was dose-dependent, with an ED_{50} of 250 μM, and the $T_{1/2}$ was 30 minutes. By contrast the $T_{1/2}$ for glucose+insulin mediated desensitization ranged from 50 to 65 minutes (3 independent experiments). The effects of maximal insulin and AICAR were not additive. These data suggest that ZMP may contribute to glucose toxicity as an additional intracellular mediator of glucose-induced desensitization of the glucose transport system.

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MECHANISMS OF INSULIN RESISTANCE IN OBESE ZUCKER RATS- TYROSINE PHOSPHORYLATION OF IRS-1 AND PI3-KINASE BINDING AND ACTIVATION ARE REDUCED

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The aim of this study was to elucidate the molecular mechanisms for the insulin resistance in obese Zucker rats. Glucose uptake, Glut4 translocation, cellular content of key proteins in the insulin signaling cascade as well as PI3-kinase binding and activity were measured in fat cells from lean and obese Zucker rats of similar age. Insulin increased glucose uptake 4-5 fold in lean animals while obese cells were virtually completely unresponsive to insulin. Glut4 protein content was also reduced accounting for the impaired translocation of the transporters to the plasma membrane in response to insulin. The most pronounced difference in proteins involved in the insulin signaling cascade between lean and obese cells was the marked impairment in insulin-stimulated tyrosine phosphorylation of IRS-1. This impairment far exceeded the slight reduction in IRS-1 protein content in obese cells. Insulin-stimulated PI3-kinase activity was also markedly impaired in the obese cells. Conclusion: Cells from obese Zucker rats exhibit several perturbations in proteins involved in insulin action. However, the most prominent are the low Glut4 protein content and the marked impairment in tyrosine phosphorylation of IRS-1 which in turn is associated with a low binding and activation of PI3-kinase.

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HIGH PALMITATE, HIGH GLUCOSE, AND HIGH INSULIN ADDITIVELY BLUNT INSULIN ACTION IN ISOLATED RAT SKELETAL MUSCLE.

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Circulating factors believed to contribute to insulin resistance prevailing in obesity and diabetes mellitus include high concentrations of free fatty acids, glucose, and insulin. After 5 h preexposure to high palmitate (1.5 mM), isolated rat soleus muscle strips exhibited decreased rates of insulin-stimulated (10 nM) ^{3}H-2-deoxy-glucose transport (cpm/mg/h, "**GT**": control *vs.* high palmitate, 667±41 *vs.* 529±27, p<0.05) and glycogen synthesis (μmol glucose incorporated into glycogen/g/h, "**GS**": control *vs.* high palmitate, 2.09 ±0.16 *vs.* 1.64±0.18, p<0.02). Insulin desensitization was also found after pretreatment with high glucose (50 mM) (control *vs.* high glucose: **GT**, 620± 41 *vs.* 532±29, p<0.005; **GS**, 1.68±0.14 *vs.* 0.90±0.08, p<0.001) or high insulin (10 nM), which affected glycogen synthesis only (control *vs.* high insulin: **GT**, 633±22 vs. 585±37, n.s.; **GS**, 2.70±0.37 *vs.* 0.89±0.05, p<0.005). We examined the mode of interaction of these factors, and observed a further decrease in insulin action in muscle strips rendered insulin resistant by high palmitate, when additionally exposed to high glucose (high palmitate *vs.* high palmitate + high glucose: **GT**, 608±31 *vs.* 458±23, p<0.001; **GS**, 1.55±0.18 *vs.* 0.97±0.10, p<0.005) or high insulin (high palmitate *vs.* high palmitate + high insulin: **GT**, 520±25 *vs.* 533±36, n.s.; **GS**, 1.76±0.21 *vs.* 1.03±0.09, p<0.01). Likewise, high insulin induced a further decrease in insulin action, when combined with high glucose preexposure (high glucose *vs.* high glucose + high insulin: **GT**, 556±30 *vs.* 436±38, p<0.05; **GS**, 1.69±0.08 *vs.* 0.60±0.05, p<0.001). In conclusion, high concentrations of palmitate, glucose, and insulin decrease insulin-stimulated muscle glucose metabolism in an additive fashion, and will thus independently contribute to insulin resistance *in vivo*.

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Glucose transporter levels in a spontaneously NIDDM model rat of the male Otsuka Long-Evans Tokushima Fatty (OLETF) strain

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OLETF rats are NIDDM model animals. To evaluate the role of glucose transporter (GLUT) in the development of diabetes in this model, we examined the action of insulin on the translocation of GLUT4 in isolated adipocytes, GLUT4 protein and GLUT4 mRNA levels in muscles. Long-Evans Tokushima Otsuka (LETO) rats were used as a control strain. In the adipocytes, GLUT4 protein levels in OLETF rats at 30 weeks of age and at a diabetic stage were considerably lower than for the LETO rats. In the prediabetic stage (7 weeks), there were no significant changes in GLUT4 protein levels for either LETO and OLETF rats. The degree of GLUT4 translocation in OLETF rats was lower than that in LETO rats at 7 weeks. In muscles, the decrease in GLUT4 protein was observed in OLETF rats at 30 weeks of age. However, there was no marked difference in GLUT4 mRNA levels between OLETF and LETO rats. The issue of whether such a change is under the influence of hyperglycemia was also examined using rats which were rendered diabetic by 70% pancreatectomy(Px). OLETF rats aged 7 weeks were assigned to partial Px and sham. 4 weeks after surgery, GLUT4 decrease was observed for both tissues of hyperglycemic Px rats compared with euglycemic sham. These findings suggest a defect early in the insulin resistance of OLETF rats probably reflects impaired GLUT4 translocation, intrinsic activation, and/or insulin signaling pathway leading to glucose transporter translocation. GLUT4 decrease, which occurs later in the process appears to be a consequence, rather than a cause of diabetes in OLETF rats.

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AMYLIN ACTS AS A GROWTH FACTOR DURING THE DEVELOPMENT OF THE RAT KIDNEY

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Amylin is a 37-amino acid peptide expressed principally in pancreatic ß-cells in the adult. We have previously demonstrated high affinity binding sites for amylin in the rat renal cortex which have been localised to the proximal tubules. The aim of the present study was to assess gene and protein expression of amylin during embryogenesis with a particular focus on the developing kidney using *in situ* hybridisation and immunohistochemical techniques. Amylin mRNA was transiently expressed between embryo day 17 and postnatal day 7. The location of these amylin gene transcripts was below the nephrogenic zone, associated with the primitive tubules of the developing nephrons. There was no evidence of expression in the normal adult kidney. In the developing kidney (metanephros), amylin peptide could also be detected by immunohistochemistry using a rabbit polyclonal anti-rat amylin antibody (1:2,500), in these primative tubules. In primary cultures of tubular epithelial cells isolated from postnatal day 4 pups, amylin (10^{-7}M) stimulated proliferation of these cells by 125±6% versus control as assesed by incorporation of tritiated thymidine. This stimulation was inhibited by the amylin peptide receptor antagonist, AC512 (91±7% of control). In conclusion amylin is biosynthesized in the developing proximal tubules and acts to stimulate the proliferation of epithelial cells. This role for amylin has implications for our understanding of developmental and pathological processes in the kidney including diabetic nephropathy.

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ACTIVATION BY NORADRENALINE OF GLUCOSE TRANSPORT INTO CULTURED BROWN ADIPOCYTES FROM RATS.

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Glucose uptake into brown adipose tissue has been shown to be enhanced directly by noradrenaline (NA) released from sympathetic nerves. In this study we analyzed the mechanism of NA-induced increase in glucose transport using cultured brown adipocytes, which responds to NA as well as insulin. NA and insulin independently stimulated glucose uptake. The effect of NA was mimicked by the β_3-adrenergic agonist, BRL37344, at concentrations much lower than NA. Dibutyryl-cAMP also mimicked the stimulatory effect of NA, and the antagonist of cAMP, cAMP-S Rp-isomer, blocked the NA effect. Insulin caused the increase in the content of GLUT4 glucose transporter in the plasma membrane, whereas NA did not affect the subcellular distribution of GLUT4 as well as that of GLUT1. NA decreased pronouncedly the *Km* value for glucose uptake. Photoaffinity labelling of the exofacial glucose binding sites of GLUT1 and GLUT4 with a membrane-impermeant bismannose derivative revealed that NA increased the labelling of cell surface GLUT1, but not of cell surface GLUT4, without increase in the GLUT1 protein in the plasma membrane. The NA-induced increase in photoaffinity labelling of GLUT1 was completely suppressed by the cAMP antagonist. These results suggest that the mechanism by which NA stimulates glucose transport into brown adipocytes is not due to the recruitment of GLUT1 or GLUT4, but due to an increase in the functional activity of GLUT1, which is mediated by a cAMP-dependent pathway.

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B CELL PEPTIDES INFLUENCE ISLET AMYLOID POLYPEPTIDE (IAPP) FIBRIL FORMATION *IN VITRO*

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Amyloid deposits in pancreatic islets in noninsulin-dependent diabetes mellitus (NIDDM) consist mainly of islet amyloid polypeptide (IAPP). The mechanism of the IAPP fibril formation is unclear. We examined whether the B cell granule peptides, insulin, C-peptide and pancreastatin affect the formation of ß-pleated sheet fibrils *in vitro* from human IAPP. Samples of 32 µmol/l IAPP in Tris buffered saline, pH 7.4, with or without each of the three peptides, were incubated for 48 hrs and fibril formation was examined by electron microscopy, by measuring the radioactivity of soluble IAPP after adding trace amount of ^{125}I-labelled IAPP and by thioflavine (ThT) fluorescence spectroscopy. Under control conditions, IAPP spontaneously formed fibrils, as evident by all three techniques. As judged by electron microscopy, insulin and pancreastatin at ratios 10:1 or 100:1 (peptide:IAPP) inhibited fibril formation, whereas C-peptide had no effect. In contrast, at ratios 1:10 or 1:100, i.e., IAPP in excess, all three peptides increased fibril formation. As quantified by radioassay and ThT fluorescence spectroscopy, insulin, C-peptide and pancreastatin inhibited fibril formation by 60-100% at ratio 100:1 (peptide:IAPP), whereas a potentiated IAPP fibril formation was observed at ratios of each of the three peptides to IAPP of 1:10 and 1:100, i.e., IAPP in excess. We conclude that IAPP fibrillisation is influenced by other B cell secretory granule peptides in a molar ratio dependent manner, which might be of importance for IAPP fibril formation and the cause of islet amyloidosis in NIDDM.

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DIRECT STIMULATION BY α-LIPOIC ACID OF GLUCOSE TRANSPORT IN SKELETAL MUSCLE OF ZUCKER RATS.

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α-Lipoic acid (ALA), a potent biological antioxidant, improves insulin action of skeletal muscle glucose transport and metabolism in both human and animal models of insulin resistance. In order to obtain further insight into the potential intracellular mechanisms for the action of ALA on insulin-stimulated glucose transport in skeletal muscle, we investigated the effects of direct incubation with ALA on 2-deoxyglucose (2-DG) uptake by epitrochlearis muscle from either insulin-sensitive lean (*Fa/-*) or insulin-resistant obese (*fa/fa*) Zucker rats. ALA (2 mM) stimulated 2-DG uptake in muscle of lean animals by 76%, whereas ALA stimulated 2-DG uptake by only 48% ($p<0.05$ vs. lean) in muscle from the obese animals. The stimulation of 2-DG uptake due to ALA was enhanced 30-55% in the presence of insulin in muscle from lean and obese animals. In contrast, ALA action on 2-DG uptake was not additive with the effects of electrically-stimulated muscle contractions in either insulin-sensitive or insulin-resistant muscle. Wortmannin (1 µM), an inhibitor of phosphotidyl-inositol-3-kinase, completely inhibited insulin action on 2-DG uptake, but had no effect on contraction-stimulated 2-DG uptake. Wortmannin inhibited ALA action on 2-DG uptake by only 25% in muscle from either lean or obese Zucker rats. Collectively, these results indicate that although a portion of ALA action on glucose transport in skeletal muscle is mediated via the wortmannin-sensitive insulin signal transduction pathway, the majority of the direct effect of ALA on this process is insulin-independent.

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IS NON-INSULIN-DEPENDENT DIABETES MELLITUS ALZHEIMER'S DISEASE OF THE ISLET?

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Brain dysfunction in Alzheimer's disease (AD) is associated with loss of cortical neurons with deposition of amyloid deposits of a locally expressed protein (ß protein). Impaired insulin secretion in non-insulin-dependent diabetes mellitus (NIDDM) is associated with loss of ß cells with deposition of islet amyloid polypeptide derived amyloid deposits. Both AD and NIDDM have strong genetic components with increased phenotype expression with aging. It has been proposed that islet and cerebral amyloidosis are cytotoxic and important in pathogenesis of these diseases. A defect may be common to both diseases. We therefore asked the question is islet amyloid increased in patients with AD? We studied pancreas obtained at autopsy from 28 AD cases (diagnosed in life and confirmed at death) versus 21 cases without AD (non-AD, also diagnosed in life and at death) matched for age but selected at random for presence or absence of diabetes. Prevalence (%) and extent (scale 0-4) of islet amyloid was compared on Congo red sections of the tail of pancreas. Islet amyloid was more prevalent (13.9 vs. 4.4, $P < 0.05$) and extensive (0.40 vs. 0.10, $P < 0.05$) in AD versus non-AD despite a lower BMI (24.3 vs. 27.1 kg/m^2 AD vs. non-AD, $P = 0.06$). Prevalence of NIDDM and mean fasting plasma glucose were slightly (but not significantly) higher in AD than non-AD. We conclude that islet amyloid is increased in patients with Alzheimer's disease implying that there may be an underlying defect in protein trafficking in AD and NIDDM.

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BRADYKININ ENHANCES GLUT4 TRANSLOCATION IN DOG SKELETAL MUSCLES AND RAT L6 MYOBLASTS

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We previously demonstrated that bradykinin infusion could increase glucose uptake into dog peripheral tissues(Diabetologia 37:300-307), and that bradykinin could potentiate insulin induced glucose uptake through GLUT4 translocation in dog adipocytes(Diabetologia 39:412-420). However, it is well known that skeletal muscle is the predominant tissue for insulin mediated glucose disposal. In this study, we evaluated whether bradykinin B2 receptor was expressed in dog skeletal muscles and rat L6 myoblasts, and how bradykinin can affect glucose uptake especially in the insulin signalling pathway and translocation of GLUT4. The bradykinin receptor binding studies revealed that dog skeletal muscles and L6 myoblasts possessed significant numbers of bradykinin receptors (Kd = 88, 76 pmol/l, Bmax=2.5, 20 fmol/mg protein, respectively). An RT-PCR amplification showed the mRNA specific for bradykinin B2 receptor in each cells. Bradykinin significantly increased 2-deoxyglucose uptake in a dose dependent manner in the presence of insulin (10^{-7} mol/l). Bradykinin also enhanced insulin stimulated GLUT4 translocation, and insulin induced phosphorylation of insulin receptor β subunit and IRS-1 without affecting the binding affinities or numbers of cell surface insulin receptors in both cells.

It is concluded that bradykinin could potentiate the insulin induced glucose uptake through GLUT4 translocation. This effect could be explained by the potency of bradykinin to upregulate the insulin receptor tyrosine kinase activity which stimulates phosphorylation of IRS-1, followed by GLUT4 translocation.

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INTACT BUT NOT TRUNCATED des(27-31) RAT C-PEPTIDE STIMULATES β-CELL Na^+,K^+-ATPase ACTIVITY.

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Recent experiments have demonstrated that C-peptide exerts a stimulatory effect on Na^+,K^+-ATPase activity in several tissues including renal, β- and nerve cells. It has also been found that C-peptide (produced during conversion of proinsulin to insulin) may be truncated in secretory granules and this truncated form lacking the last 5 amino acids (des-(27-31)C-peptide) is released from β-cells in response to classical secretogogues. In the present study the possible effect of such truncation on C-peptide's ability to stimulate Na^+,K^+-ATPase was examined. Single microdissected islets from adult normoglycemic *ob/ob* mice were used as they are made up almost exclusively of β-cells. Na^+,K^+-ATPase activity was determined from the hydrolysis of ^{32}P-ATP in the presence and absence of ouabain. When islets were incubated with synthetic rat C-peptide II there was a linear, concentration dependent stimulation of Na^+,K^+-ATPase activity in the range 10^{-10} - 10^{-7}M. Thus, 10^{-7}M C-peptide increased Na^+,K^+-ATPase activity 2.3 ± 0.1 fold above basal ($P < 0.01$). In contrast, synthetic rat des(27-31) C-peptide II exerted no detectable stimulatory effect (2±2% of C-peptide's effect) even at 10^{-7}M. These findings suggest that truncation of C-peptide in granules prior to secretion may be a physiological mode of C-peptide inactivation.

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GLUCOSE METABOLISM IS STIMULATED BY PRIOR CONTRACTION IN INSULIN RESISTANT SKELETAL MUSCLE

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Both during and after exercise there is increased glucose utilization in skeletal muscle. The mechanism(s) responsible for stimulation of glucose metabolism post-contraction (PC) require(s) identification. The aim of this study was to determine whether glucose metabolism is altered in skeletal muscle by prior contraction. We measured rates of glycogen synthesis (from [^{14}C]glucose, μmol/h/g dry wt.) in soleus (Type I fibres) and epitrochlearis (Type II fibres) muscles rested for 1 h, after being contracted *in vitro* for 30 min. The greatest effects on the rates of glycogen synthesis were by PC in epitrochlearis muscles and by insulin in soleus muscles. The effects of insulin and PC were additive in both muscle preparations.

	[Epitrochlearis]	[Soleus]	
Basal (no insulin)	2.2±0.3	3.3±0.4	
Insulin (10000 μU/ml)	16.6±1.4*+	34.5±1.7*	*P<0.0001 from basal
PC	19.9±1.7*+	11.9±1.0*	+P<0.0002 from soleus
PC and Insulin	30.1±2.4*+	41.2±0.9*	

The PC effects on glucose transport (^{3}H-2-deoxyglucose uptake, 2-DOG) were determined in insulin sensitive (lean, *Fa/?*) and resistant (obese, *fa/fa*) epitrochlearis muscles isolated from Zucker rats. In the presence of a basal insulin concentration (10 μU/ml), rates of 2-DOG transport (all results given as dpm/h/mg wet wt.; n≥5) were lower in epitroclearis muscles from obese (127±8, P<0.001) versus lean (206±18) rats. The rates of 2-DOG transport were significantly increased in resting muscles during the hour after 30 min of contraction for both lean (393±34, PC, versus 206±18, not stimulated, P<0.001) and obese (192±8, PC, versus 127±8, not stimulated, P<0.001) rats. This demonstrates that PC effects occur in both insulin sensitive and insulin resistant skeletal muscle. The present study highlights the significant post-contraction effects on glucose metabolism in muscles with Type II fibres that causes increased rates of 2-DOG transport in insulin resistant muscle.

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C-PEPTIDE FRAGMENTS STIMULATE RENAL TUBULE Na^+,K^+-ATPase ACTIVITY.

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C-peptide has been shown to stimulate Na^+,K^+-ATPase (NKA) activity in tissues including renal tubule cells, glomeruli, β-cells and nerve. Active sites in the C-peptide molecule have not been determined. Consequently, in the present study the influence of several different fragments of C-peptide on rat renal tubule NKA activity was examined. NKA activity was assessed as ouabain-sensitive ATP hydrolysis in single proximal tubule segments dissected from collagenase perfused rat kidneys. Rat C-peptide elicited a dose dependent increase in NKA activity in the range 10^{-8} - 10^{-6}M. The C-terminal penta (EVARQ) and tetrapeptides (VARQ) of C-peptide also stimulated NKA activity at $5{\cdot}10^{-7}$M, possessing 103 ± 5% and 92 ± 6%, respectively, of the equimolar intact C-peptide's activity. Likewise, fragments from the middle portion of the C-peptide molecule (ELGG, 36 ± 3%; ELGGGP, 46 ± 5%; ELGGGPEAG, 80 ± 7%) were able to significantly stimulate NKA activity. The non-native d-form of the dipeptide LG but not its l-form stimulated NKA activity. The findings are consistent with specific activity and binding interaction of a C-terminal segment of C-peptide as well as other non-specific effects possibly reflecting the complex enzyme assay.

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EFFECTS OF GLUCOCORTICOIDS ON THE SENSITIVITY OF GLUCOSE DISPOSAL TO INSULIN IN SKELETAL MUSCLE

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Glucocorticoids induce resistance of glucose disposal (GD) to insulin, but the mechanisms are unclear. We examined the effects of in-vivo injections of dexamethasone (DX) to rats (0.5 mg/day i.p., 5 days) on the sensitivity to insulin of glucose transport (GT, 3-O-methyl[^{3}H]glucose), glycogen synthesis (GS, [^{14}C]glucose), glucose oxidation (GO, [^{14}C] glucose) and on the total content (CGLUT4, Western blots, [WB]) and insulin-stimulated (100 mU/l) translocation of GLUT4 transporters to the plasma membrane (TGLUT4, surface biotinylation of muscle and WB) in soleus muscle incubated in-vitro. DX decreased GT (2.1±0.1 and 2.6±0.1 vs 3.0±0.2 and 3.9±0.3 µmol/h/g), GS (1.0±0.1 and 1.6±0.1 vs 2.6±0.2 and 4.0±0.2 µmol/h/g), and GO (0.4±0.05 and 0.3±0.01 vs 0.5±0.07 and 0.7±0.03 µmol/h/g) vs control at 10 and 100 mU/l insulin respectively, $p<0.01$; all rates at 1000 mU/l insulin were normal. CGLUT4 was not decreased after DX (scanning analysis of WB band densities, arbitrary units: 1089±30 vs 1226±39 in control for the 54 kDa and 413±59 vs 461±30 for the 44 kDa GLUT4); however, TGLUT4 was lessened by 60% (fold-increase in WB band density: 1.68±0.04 and 1.77±0.06 in control and 1.07±0.06 and 1.08±0.10 in DX for the 54 kDa and 44kDa GLUT4 respectively, $p<0.01$). **Conclusions:** 1) Glucocorticoids decrease GD in muscle by inducing resistance of GT, GS and GO to insulin. 2) The effect on GT is due to a decrease in insulin-stimulated translocation of GLUT4 transporters to the plasma membrane.

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GIP stimulates the secretion of GLP-1 from canine L-cells through a Protein Kinase A dependent pathway.

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We have studied the secretion of Glucagon Like Peptide 1 (GLP-1) from canine L-cells in short term culture. The response of the cells to increasing concentrations of Glucose-dependent Insulinotropic Peptide (GIP) was compared with response to Forskolin and Phorbol Myristate Acetate (PMA) in the presence or absence of Protein Kinase A inhibitor H8 and Protein Kinase C inhibitor Staurosporin, respectively. Single L-cells were isolated by centrifugal elutriation from epithelial cell preparations of canine ileum (Beagle). The cell fractions enriched for GLP-1 content were maintained in culture for 2 days in growth medium prior to the initiation of the release experiments. The cells were then serum-starved for 2 h and then incubated for 2 h under the following conditions: GIP (10^{-12}-10^{-7} M); PMA (10^{-10}-$4x10^{-7}$ M) in the presence and absence of Staurosporin (10^{-6} M); GIP (10^{-7} M) in the presence and absence of Staurosporin (10^{-6} M) and PMA ($4x10^{-7}$ M); Forskolin (10^{-9}-10^{-6} M) in the presence and absence of H8 (10^{-6} M); GIP (10^{-7} M) in presence and absence of H8 (10^{-6} M) and Forskolin (10^{-6} M). The glucose concentration was 5.5 mM in all experiments. Immunoreactive GLP-1 in supernatant and in extract from attached cells was measured and the results presented as percent of total GLP-1 normalised to either basal or GIP (10^{-7} M). Compared to basal, GLP-1 was dose-dependently released from the L-cells by GIP (2 fold at 10^{-7} M), PMA (4 fold at $4x10^{-7}$ M) and Forskolin (3 fold at 10^{-6} M). The two latter were significantly inhibited by Staurosporin and H8, respectively. The effect of GIP (10^{-7} M) was additive to PMA and not changed by Staurosporin. The combined effect of the GIP and PMA was reduced by Staurosporin. The effect of GIP (10^{-7} M) was not additive to Forskolin but significantly inhibited by H8. The inhibition of GIP effect was not altered by the presence Forskolin. Based on these data we suggest that the secretion of GLP-1 from canine L-cells can be stimulated both through a Protein Kinase C and A dependent pathway, and that the effect of GIP is mediated through a Protein Kinase A dependent pathway.

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EFFECT OF THE ADDITION OF GLICLAZIDE TO INSULIN TREATMENT ON MUSCLE GLUCOSE UPTAKE FROM DIABETIC RATS

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We have previously shown that gliclazide directly stimulates glucose uptake in perfused rat hindquarters. The aim of this work was to determine whether the addition of gliclazide at the insulin treatment in diabetic rats leads to an increase in muscle glucose uptake. Streptozotocin diabetic Wistar rats were treated for 12 days with either insulin alone (3 units NPH s.c./day, Group I, n=5) or oral gliclazide (5 mg/Kg twice a day p.o.) plus 3 units insulin/day, Group G+I, n=5). A non-diabetic group was used as a control (Group C, n=11). Mean glycemic value throughout the treatment period was lower in Group G+I than in Group I (98±5 vs 139±15 mg/dl, mean±SEM, $p<0.05$) without changes in serum C peptide levels. After 12 days of treatment, both basal and insulin-stimulated (10^{-9} and 10^{-7} M) glucose uptake by the perfused hindquarters were measured. Basal glucose uptake in both groups of diabetic rats were similar to the control group. Insulin stimulated glucose uptake in Group G+I was significantly higher than Group I (Ins 10^{-9}: 8.0±0.3 vs 5.8±0.6, Ins 10^{-7}: 12.0±0.6 vs 8.5±0.6 µmol/g/h, $p<0.05$) and similar to Group C (Ins 10^{-9}: 9.2±0.6, Ins 10^{-7}: 13.4±0.9 µmol/g/h). In partially purified solubilized receptors from skeletal muscle, the insulin binding and the tyrosine kinase activity were similar in the two groups of diabetic rats. In conclusion, the addition of gliclazide to the insulin treatment in diabetic rats normalized the insulin-stimulated glucose uptake by a post-receptor mechanism.

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EFFECTS OF GLP-1 ON THE KINETICS OF GLYCOGEN SYNTHASE ACTIVITY IN HEPATOCYTES FROM NORMAL AND DIABETIC RATS.

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Glucagon-like peptide 1(7-36)amide (GLP-1) was previously found to activate glycogen synthase in hepatocytes from both normal and diabetic rats. In the present study, the kinetic aspects of such an activation were investigated. Hepatocytes were isolated from overnight fasted male normal Wistar rats and animals that had been injected with streptozotocin either during the neonatal period or at the adult age. The hepatocytes were incubated for 15-20 min, being exposed to 16.7 mM glucose and, as required, either insulin or GLP-1 during the last 5-10 min of incubation. The reaction velocity was measured in cell homogenates over 15 min incubation, at which time it had reached a steady-state value. In both normal and diabetic rats, the increase in glycogen synthase activity caused by GLP-1 was concentration related in the 10^{-12} to 10^{-8} M range, progressively rising from 18±3 to 37±3%. At all hormone concentrations the relative extent of enzyme activation was lower in fed rats than in overnight fasted animals, the former value averaging 63.8±2.5 % of the latter one. In the starved rats, the relative extent of synthase activation by either insulin or GLP-1 progresively decreased when the glucose concentration of the incubation medium was lowered to 8.8 mM or less. At high concentrations of the hormones, activation of synthase reached a steady-state value within 1-5 min exposure of the hepatocytes to either insulin or GLP-1, in both normal and diabetic rats. At increasing concentrations of UDP-glucose (0.1 to 5.0 mM), the reaction velocity yielded a Km close to 0.4 mM. The affinity of synthase *a* for UDP-glucose tended to be higher in diabetic than in normal rats, and to be lowered by GLP-1. These findings indicate that the activation of liver glycogen synthase by GLP-1 displays attributes of rapidity, sensitivity and nutritional dependency that are well suited for both participation in the physiological regulation of enzyme activity and therapeutic purpose.

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CONCOMITANT EXPRESSION OF TWO RAB PROTEINS IS REQUIRED FOR INSULIN-STIMULATED GLUCOSE TRANSPORT IN CARDIAC MUSCLE CELLS

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The major mechanism mediating insulin induced glucose uptake in adipocytes, skeletal and cardiac muscle is known to be translocation of the glucose transporter isoform GLUT4 from an intracellular storage site to the plasma membrane. Several lines of evidence suggest that Rab-proteins could also be involved in the regulation of this mechanism, however, functional implications of these small GTP-binding proteins in cardiac glucose uptake have not been investigated so far. H9c2 cardiac myoblasts stably overexpressing the glucose transporter isoform GLUT4 (H9c2-K6) were transiently transfected with Rab4A-cDNA, Rab3C-cDNA or co-transfected with both Rab-cDNAs and basal and insulin stimulated 2-deoxyglucose (2-DOG) transport was assayed. In transfected cells Rab4A and Rab3C were markedly overexpressed, both were not detectable in the controls transfected with blank vector. These controls exhibit basal 2-DOG uptake increased by about 100 % with no further effect of insulin. In cells transfected with Rab4A-cDNA basal 2-DOG uptake was decreased by about 25 % and 60 min insulin stimulation had no significant effect. In contrast, myoblasts transfected with Rab3C-cDNA showed no significant decrease in basal 2-DOG uptake but insulin stimulation resulted in a slightly increased transport rate. Under basal conditions the co-transfection of Rab4A- and Rab3C-cDNAs together resulted in a 55 % decreased 2-DOG uptake in the GLUT4-overexpressing clone in contrast to control H9c2-myoblasts, which showed no change in glucose transport under the same conditions. Furthermore stimulation with insulin resulted in a 2fold increase in 2-DOG uptake in the clonal cell line transfected with both Rab-cDNAs.

In conclusion, our data show that neither Rab4A nor Rab3C alone are sufficient to mediate insulin stimulated glucose uptake but that co-expression renders the cells insulin sensitive. Thus, we suggest that these Rab isoforms are involved in the regulation of both basal and insulin activated glucose transport in a coordinated and synergistic fashion. (Supported by DFG, SFB 351 C2)

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EFFECT OF GLP-1 ON GLUCOSE TRANSPORTERS IN LIVER, MUSCLE, AND ADIPOSE TISSUE OF DIABETIC RATS

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GLP-1(7-36)amide (GLP-1) has been proposed as a therapeutical agent in NIDDM, and it was found that the peptide exerts insulin actions in some extrapancreatic tissues. In this work we have explored, by Dot-Blot analysis, whether GLP-1 treatment affects the levels of glucose transporters in liver (GLUT-2 and GLUT-1), muscle and adipose tissue (GLUT-4 and GLUT-1) membranes, from rats treated with streptozotocin in the adult age (IDDM) or at day 1 of birth (NIDDM), receiving no treatment or either GLP-1 (0.4 nmol/kg/h) or insulin (450 mU/kg/h) through an osmotic pump during three days. Tissues from untreated normal rats were included as controls. **In the liver**, GLUT-2 was augmented in IDDM (126 ± 11% of normals, $n=3$, $p<0.05$) and decreased in NIDDM (39 ± 5, $n=3$, $p<0.05$), and its levels were normalized in both types of diabetes by either GLP-1 or insulin treatment; GLUT-1 was decreased in NIDDM rats (55 ± 4%, $n=3$, $p<0.05$) and neither treatment modified its level, while in IDDM, insulin treatment increased by 2-fold the GLUT-1 content. **In the muscle**, GLUT-4 was reduced in IDDM rats (55 ± 3%, $n=6$, $p<0.001$), and insulin or GLP-1 treatment normalized its level, and induced a 2-fold increase in GLUT-4 and GLUT-1 content in NIDDM rats. **In adipose tissue**, GLUT-4 levels were decreased in IDDM (50 ± 10%, $n=3$, $p<0.001$) and in NIDDM (24 ± 4%, $n=3$, $p<0.001$) rats, and either insulin or GLP-1 treatment normalized them; GLUT-1 was also decreased in IDDM (59 ± 4%, $n=3$, $p<0.001$) and NIDDM (46 ± 7%, $n=3$, $p<0.001$) rats, and the treatment with insulin decreased, even more, the GLUT-1 content in NIDDM rats. These results add support to the role of GLP-1 in the *in vivo* overall glucose disposal.

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INSULIN AND CYCLIC AMP REGULATION OF GLUT4 TRANSLOCATION TO THE CELL SURFACE OF INTACT ADULT MYOBLASTS

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Insulin and contraction are the major physiological stimuli of the glucose transport system in skeletal muscle. They each act mainly by recruiting GLUT4 transporters from intracellular vesicles to the plasma membrane, but apparently by different, and as yet unknown, mechanisms. We investigated the regulation of glucose transport and GLUT4 translocation in C2C12 adult murine skeletal muscle cells stably expressing myc-epitope tagged GLUT4 (GLUT4myc). We measured in parallel 2-deoxyglucose (2-DG) uptake and cell surface GLUT4myc content (by myc-antibody binding) in intact cells. Insulin stimulated 2-DG uptake in C2C12 myoblasts expressing GLUT4myc in a concentration-dependent manner from 3.7 ± 0.6 nmol/mg protein/min without insulin to 5.9 ± 0.7 nmol/mg protein/min with 10^{-6} M insulin for 20 min incubation (in parental myoblasts which have no endogenous GLUT4, the corresponding values were 1.9 and 2.6 nmol/mg protein/min). In C2C12-GLUT4myc myoblasts, insulin increased cell surface GLUT4myc content in a time- and concentration-dependent manner with a maximum 2.7 ± 0.3-fold increase after 20 min incubation with 10^{-6} M insulin. We then examined the effect of cyclic AMP (cAMP) levels on GLUT4 translocation using forskolin, a direct activator of adenylate cyclase. Increasing concentrations of forskolin stimulated concomitantly intracellular production of cAMP (2.7 ± 0.3-fold increase at 10^{-5} M forskolin) and cell surface GLUT4myc content (1.6 ± 0.1-fold increase at 10^{-5} M forskolin). Furthermore, the forskolin effect on GLUT4 translocation appeared to be additive to the effect of insulin (3.6 ± 0.1-fold increase with forskolin 10^{-5} M plus insulin 10^{-7} M vs 2.2 ± 0.1-fold increase with insulin alone). In addition, wortmannin, an inhibitor of phosphatidylinositol 3-kinase, suppressed the action of insulin on 2-DG uptake and GLUT4 translocation in a dose-dependent manner (maximum effect 50-100 nM), but not the action of forskolin. Forskolin and cytochalasin B, which both bind to glucose transporters, inhibited 2-deoxyglucose uptake, however cytochalasin B did not increase cell surface GLUT4myc content. Thus, our data suggest that cAMP stimulates GLUT4 translocation to the plasma membrane independently of insulin, and that the C2C12-GLUT4myc myoblasts are a useful system for studying different signalling mechanisms leading to activation of glucose transport.

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SOMATOSTATIN BUT NOT INSULIN IS AN IMPORTANT PARACRINE REGULATOR OF GLUCAGON SECRETION IN RAT A-CELLS

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High-resolution capacitance measurements were used to explore the effects of somatostatin and insulin on Ca^{2+}-dependent exocytosis in glucagon-secreting rat pancreatic A-cells obtained by fluorescence-activated cell sorting. This was investigated using the perforated patch whole-cell configuration and increases in cell capacitance were recorded in response to 500 ms voltage-clamp depolarizations from -70 mV to 0 mV applied at 2 min interval. Membrane depolarization was associated with activation of voltage-gated Ca^{2+} current, increased cytoplasmic free Ca^{2+} ($[Ca^{2+}]_i$) and stimulation of Ca^{2+}-dependent exocytosis. Addition of the β-adrenergic agonist isoprenaline or the gut hormone glucagon-like peptide 1(7-36)amide [GLP-1(7-36)amide] potentiated Ca^{2+}-dependent exocytosis by >3-fold. The stimulatory actions of isoprenaline and GLP-1(7-36)amide were completely antagonized by somatostatin (400 nM) which was only associated with approximately 25% reduction in the whole-cell Ca^{2+} current and the increase in $[Ca^{2+}]_i$. The strong inhibition of somatostatin was reproduced in the standard whole-cell patch-clamp configuration in which the cytoplasmic cAMP concentration was clamped to 0.1 mM. This suggests that the ability of somatostatin to suppress exocytosis is unlikely to reflect inhibition of adenylate cyclase or involves a diffusional messenger. Interestingly, insulin (10-100 nM) had no effect on either depolarization-evoked whole-cell Ca^{2+} current or the associated exocytotic response. We propose that somatostatin is an important paracrine regulator of glucagon secretion from the A-cell which principally reflects interference with a late step in the stimulus-secretion coupling and that release of somatostatin from the D-cells, rather than insulin, accounts for the reported inhibitory action of GLP-1 on glucagon secretion in vivo.

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IMPAIRED STIMULATION OF THE NITRIC OXIDE/cyclicGMP SYSTEM AND GLUCOSE UPTAKE IN INSULIN RESISTANT MUSCLE

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Nitric oxide, which is generated in skeletal muscle by nitric oxide synthase, stimulates glucose transport and metabolism. Nitric oxide release from skeletal muscle is stimulated by contraction. Nitric oxide probably stimulates glucose utilisation by activation of guanylate cyclase leading to increased cyclicGMP formation. Zaprinast inhibits the enzyme, cyclicGMP phosphodiesterase (which hydrolyses cyclicGMP), thus leading to an increase in cyclicGMP levels in skeletal muscle. Since increasing cyclicGMP levels are associated with increased rates of glucose utilisation the main aim of the present study was to investigate the effects of zaprinast (27 μM) on glucose utilisation in soleus muscles isolated from insulin sensitive (lean Zucker) and insulin resistant (obese Zucker) rats. Zaprinast significantly increased the rates (all results given as units μmol/h/g wet wt.) of ^{14}C-lactate release (a good indicator of glucose transport) (7.38±0.68 (6), zaprinast (Z) versus 4.68±0.47 (6), control (C); $P<0.01$) and glycogen synthesis (3.42± 0.39 (Z) versus 1.79±0.05 (C); $P<0.001$) in soleus muscle preparations from lean Zucker rats . Zaprinast failed to stimulate ^{14}C-lactate release (3.11±0.32 (Z) versus 2.59±0.23 (C)) or glycogen synthesis (1.02±0.08 (Z) versus 0.99±0.13 (C)) in incubated soleus muscle preparations isolated from obese Zucker rats. Zaprinast caused a significant increase in cyclicGMP content (80%) in soleus muscles isolated from lean rats, but zaprinast had no effect on cyclicGMP content in soleus muscles isolated from obese rats. These results suggest that in resting skeletal muscle from obese Zucker rats the activity of the endogenous nitric oxide/cyclicGMP system is altered. Future studies will establish if this system can be activated in insulin resistant skeletal muscle (e.g. by contraction) to yield increased rates of glucose utilisation.

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CATECHOLAMINE MODULATION OF GLUCAGON SECRETION IN CLONAL PANCREATIC ALPHA CELLS

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In diabetes, the frequent and often dangerous occurrence of hypoglycemia is attributable to impaired glucose counterregulation, which is normally mediated by several hormones including the catecholamines and glucagon. The difficulty in using normal pancreatic α-cells and the lack of good alternate *in vitro* models have retarded our understanding of the hormonal regulation of α-cell glucagon secretion. In earlier studies, I have established that the clonal cell line αTC-6, is a useful practical tool for studying the regulation of α-cell glucagon secretion, by demonstrating that the physiological secretagogues arginine and potassium modulate glucagon release in these cells. In the present study I have further characterized the physiology of αTC-6 cells by examining the effects of amino-acids and the catecholamine, epinephrine. The αTC-6 cells were plated in conventional tissue culture- ware and glucagon secretion monitored in static incubation assays. Arginine stimulates glucagon release in a dose-dependent fashion (1-10 mM) with a maximal 2-fold increase over basal levels at 10 mM arginine. An amino-acid mixture consisting of alanine, glutamine and arginine (total 6 mM) also enhances glucagon release. The amino-acid mixture induced a 1.6-fold increase in secretion over basal levels (basal: 20.0 ± 5.8 (S.E.), amino-acid mix: 31.3 ± 2.9 pg/min/well). Epinephrine (1 μM) by itself had no appreciable effect on basal glucagon release (20.3 ± 7.5 pg/min/well). However, in the presence of the amino-acid mixture, epinephrine induced a synergistic effect with a potentiation of stimulated glucagon release to a 2-fold increase over basal levels (40.6 ± 7.0 pg/min/well). In contrast, epinephrine inhibited insulin secretion from clonal beta (HIT) cells. These results are consistent with earlier observations in purified alpha cells by Pipeleers and coworkers. Our results demonstrate the interplay between counterregulatory hormones and further studies with this alpha cell model (αTC-6) may enhance our understanding of the molecular mechanisms involved in the glucose-counterregulatory response and its aberration in diabetes.

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ROLE OF OXIDATIVE STRESS IN THE REGULATION OF GLUCOSE TRANSPORT IN VASCULAR SMOOTH MUSCLE CELLS

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This study was designed to investigate the mechanism by which high D-glucose affects the glucose transport system in bovine aortic smooth muscle cells (SMC). High glucose levels downregulate the rate of glucose transport and the plasma membrane content of the glucose transporter GLUT-1 in these cells. Inhibition of 12- and 15-lipoxygenase by esculetin reverses the effects of high glucose by increasing the total cell content of GLUT-1 and its plasma membrane content. Moreover, the free radical scavenger nitroxide inhibits the glucose-induced downregulation of the hexose transport system when added together with 20 mM glucose to the cells. Treatment of SMC maintained at 20 mM glucose with both esculetin and nitroxide combines the effects of both agents: While nitroxide abolishes the glucose induced downregulation of the glucose transport system, esculetin increases the rate of hexose transport to the control level observed in cells maintained at 5 mM glucose. We suggest that free radical formation by high glucose levels increases the activity of 12- and 15-lipoxygenase. The products of these enzymes (12- and 15-HETE and/or lipoxins) affects the expression and cellular distribution of GLUT-1. Thus, elimination of free radicals with nitroxide abolishes this effects of high glucose.

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A MUTANT HUMAN IGF-I RECEPTOR AS A DOMINANT NEGATIVE MUTANT IN L6 MYOBLASTS

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We previously demonstrated that L6 myoblasts underwent glucose uptake through GLUT1 with the activation of endogenous IGF-I receptor. To clarify the IGF-I signal transduction, mutant human IGF-I receptor was generated to stably transfect into L6 myoblasts. 950 tyrosine in NPXY motif localized in juxta-membrane domain of the IGF-I receptor was substituted for alanine, resulting in failure of the phosphorylation of IRS-1 molecule. The receptor was increased in number at 2.8 fold in wild type expressing L6 transfectants and at 3.4 fold in mutant expressing transfectants. IGF-I stimulation to wild type transfectants induced both IGF-I receptor β-subunit and IRS-1 phosphorylation. In contrast, for the mutant transfectants, IGF-I did not induce IRS-1 phosphorylation but β-subunit phosphorylation. 2DOG uptake was assessed after 24 hours IGF-I stimulation to these transfectants. As compared to the untransfectants at 2.1 nM with ED_{50}, wild type transfectants showed 1.1 nM and mutant transfectants 3.3 nM with ED_{50}. These results indicate that 950 tyrosine is essential for the phosphorylation of IRS-1, and the IRS-1 is required for IGF-I signaling to glucose uptake in L6 myoblasts. The finding that mutant transfectants were less sensitive to IGF-I than that of untransfectants suggests that the 950 tyrosine mutant receptor may play as a dominant negative mutant against to the endogenous intact rat IGF-I receptor by the mechanism of wild-mutant hybrid formation.

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CHRONIC HYPERLACTATEMIA DECREASES MUSCLE GLUCOSE UPTAKE AND GLUT4 mRNA IN FREELY MOVING RATS.

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An increased basal plasma lactate concentration is present in obesity and diabetes. It has been hypothesized that hyperlactatemia may influence glucose metabolism, thus playing a role in the development of insulin resistance. We previously demonstrated that acute lactate infusion in normal rats is able to induce insulin resistance at muscular level. The present study was carried out to elucidate the mechanism of the appearence of insulin resistance. In freely moving, cronically catheterized rats, a 24 hrs sodium lactate or sodium bicarbonate infusion was performed. To study the glucose uptake in different skeletal muscles, a bolus (30 μCi) of 2-deoxy-^{3}H-glucose was injected in basal condition and at the end of a euglycaemic hyperinsulinemic clamp. GLUT4 and PDH mRNA levels were assessed by Northern blot in skeletal muscles. Our results show that hyperlactatemia decreases glucose uptake in skeletal muscles (i.e. soleus: lactate 6.15±1 vs control 16.55±1.7 $p<0,0005$). Besides, the GLUT4 mRNA levels were decreased in the same tissues (i.e. soleus: lactate 13671±7154 vs control 37327±4603 $p<0,005$), whereas the PDH mRNA risulted significantly increased in lactate infused animals. These results indicate that lactate deeply influences peripheral glucose metabolism and suggest a role in the development of insulin resistance.

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4-HYDROXY-2-NONENAL DOES NOT AFFECT GLYCOLYSIS

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4-Hydroxy-2-nonenal (HNE) is one of the major products of the peroxidative decomposition of polyunsaturated fatty acids of membrane lipids. HNE possessing a number of adverse biological effects is increased under conditions, e.g. diabetes, of increased oxidative stress. It is possible that the compound is involved in the vicious cycle of hyperglycemia in diabetes. To address this issue, we examined whether HNE exerts an inhibitory effect on glycolysis. HNE inactivated the rate-limiting enzymes (from animal sources) of the glycolytic pathway and the pentose phosphate pathway when incubated at 37°C for 1 h in the absence of glutathione (GSH). The HNE concentration for half-maximal inactivation of 6-phosphofructokinase (PFK) and glyceraldehyde-3-phosphate dehydrogenase was 3-10 μM; and that value for pyruvate kinase, glucose-6-phosphate dehydrogenase, and hexokinases I and II was 0.15-0.6 mM. In the presence of 5 mM GSH, however, only PFK, irrespective of the source (muscle, liver, or erythrocyte), was inactivated by 40-50% by 0.1 mM HNE. Even PFK was not inactivated in the presence of both GSH and its substrate, ATP (2 mM). Glycolysis in the human erythrocyte as a model cell was not affected by treatment of cells with 0.1 mM HNE at 37°C for 30 min. The results indicate that HNE, at concentrations observable under physiological and pathological conditions, does not affect glycolysis in cells.

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THE ROLE OF GLUCOSE TRANSPORTERS AND IGF1 RECEPTORS IN IMPAIRED BONE GROWTH IN DIABETIC MICE.

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Uncontrolled IDDM children are characterized by a slow growth rate, which improves upon adequate therapy. While skeletal growth is an energy consuming process involving high glucose utilization, the role of glucose transporters (GLUT) and their regulation in the bone formation process, are not yet fully understood. Thus, we studied both *in vivo* and *in vitro* early endochondral bone formation in control and in STZ- induced young diabetic mice before and after insulin therapy. Using in situ hybridization and immunohistochemistry techniques, we demonstrated the existence of GLUT4, the insulin sensitive GLUT, as well as GLUT1, in neonatal-derived murine mandibular condyles and in the humerus growth plate - two models for endochondral bone formation. Compared to control, while the expression (mRNA and protein) of the core protein of cartilage specific proteoglycans (CSPG) were diminished in diabetic growth centers, osteocalcin gene expression were enhanced. This presents an uncoupling between the chondrogenesis and osteogenesis processes. Further, in the bones of diabetic mice GLUT4 levels, IGF-I and its receptor, but not GLUT1, were markedly reduced and associated with severe histological changes in the mandibular condyles and humerus growth plate. Thus, we propose that GLUT4 and IGF-I receptor have an important role in early bone growth.

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IDENTIFICATION OF THE MECHANISMS INVOLVED IN THE POTENTIATING EFFECTS OF METFORMIN ON INSULIN-INDUCED GLYCOGEN SYNTHASE ACTIVITY IN *XENOPUS* EGGS.

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In this study, we focused our attention towards the ability of metformin to improve the biological function of insulin in *Xenopus laevis* oocytes. Addition of metformin alone (20μM) to oocytes had no effect on the basal rates (n=4) of glucose uptake (143±16.5pmol/h/oocyte versus 129.1±8.9pmol/h/oocyte) or of glycogenesis, measured by [^{14}C] glucose incorporation into glycogen (14.8±1.7pmol/h/oocyte versus 11.4±0.2pmol/h/oocyte). In contrast, the drug clearly enhanced insulin action on both metabolic events (glucose uptake: 426.8±29.3pmol/h/oocyte versus 301.8±28.2pmol/h/oocyte and glycogen synthesis: 92.6±8.1pmol/h/oocyte versus 51.3±4.9pmol/h/oocyte, n=4). A short term preincubation of oocytes with metformin, but in the absence of glucose, also increased the percentage of glycogen synthase (GS) active form above insulin(50nM)-induced level (basal 17.4±5.7%, metformin 21.3±4.1%, insulin 31.2±4.6%, metformin + insulin 62.7±4.2%, n=5). Interestingly, metformin microinjected into eggs (at a final concentration of 20nM) amplified equally the same biochemical process, whereas a previous injection of tyrphostin B46 (a specific inhibitor of receptor tyrosine kinase activity) prevented the stimulatory effect of this biguanide. As the activation of GS in oocytes might involve a kinase/phosphatase cascade initiated by insulin at the level of its receptor, we further investigated the role of metformin within the insulin signaling pathway. While having no effect alone, metformin together with insulin was able to decrease significantly ($P<0.005$) the level of cAMP in the oocytes (basal 1.83±0.52pmol/oocyte, metformin 1.63±0.49pmol/oocyte, insulin 1.42±0.35pmol/oocyte, metformin + insulin 0.69±0.2pmol/oocyte, n=9). This reduction of cAMP content might lead to the dephosphorylation of GS and hence to its activation. Our results strongly suggested that the potentiating effect of metformin on GS activity was more closely correlated to an alteration of pathways regulating the activity of this rate-limiting enzyme rather than to a secondary effect dependent upon an increased glucose uptake. Together with other data from recent literature, this allows us to conclude that metformin could act at an intracellular site most likely linked to the tyrosine kinase domain but located before the branching of individual signaling pathways of insulin.

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STUDIES ON THE MOLECULAR EVOLUTION OF GLUCOSE TRANSPORTER GENE FAMILY

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Glucose transporter gene family which was involved in the glucose metabolism in the body has been considered as candidate gene in the development of diabetes mellitus. We compared the nucleotide and amino acid sequences of the family in different species; deduced the hydrophilicity and hydrophobicity distribution of the amino acid; calculated the genetic distance and constructed the phylogenetic trees by UPGMA and N-J method. The results show that the member of glucose transporter gene family are homologous; all the members of this family have a similar topology; It is suggested that the family may derived from a remote common ancestor in which the gene duplication events of different origins took place; this evolutionary process is advantageous to the stability of the structure. The different branch length in the phylogenetic trees constructed by the N-J method indicated difference of evolutionary rates in the process of evolution. Also, the difference between the trees at protein and nucleotide level may result from the hidden substitution in the genome.

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NITRIC OXIDE LEVELS ARE RESPONSIBLE OF GLUCOKINASE ACTIVITY IN CULTURED RAT HEPATOCYTES. G. Valsecchi, L.D. Monti, P.M. Piatti, S. Costa, C. Stangalini, V.C. Phan, E. P. Sandoli, C. Socci and G. Pozza. Istituto Scientifico H. San Raffaele, Milan, Italy.

Aim of the study was to evaluate the influence of endothelial factors (endothelin-1 and nitric oxide) on glucokinase (GK) activity, a key regulatory enzyme on hepatic glucose fluxes. To this scope, we evaluated the effects of endothelin-1 (ET-1, 2.0 nmol/l) and L-NAME ($4x10^{-5}$ mol/l), an inhibitor of nitric oxide synthase, on GK activity and glycogen content in cultured rat hepatocytes. Nitric oxide levels significantly decreased by 20% both after ET-1 ($p<0.01$) and L-NAME ($p<0.01$). Concomitantly, GK activity decreased by 52% after ET-1 (from 0.56±0.07 to 0.29±0.02 nmol/μg protein/h; $p<0.05$) and by 43% after L-NAME (from 0.56±0.07 to 0.32±0.02 nmol/μg protein/h; $p<0.05$). On the contrary, ET-1 was less effective than L-NAME in decreasing glycogen content (from 47.3±2.2 to 40.5±5.1 mg/g tissue with ET-1, NS; and to 35.4±0.8 mg/g tissue with L-NAME, $p<0.001$ vs basal; NS vs ET-1). When ET-1 and L-NAME were added together in the culture medium, no synergistic effects were observed. In fact, under these conditions, nitric oxide levels, GK activity and glycogen levels decreased by 17%, 45% and 30%, respectively (NS vs ET-1 and L-NAME). L-Arginine (1.72 mmol/l), by increasing nitric oxide levels, was able to report GK activity and glycogen content to basal levels (0.67±0.1 nmol/μg protein/h; NS and 42.1±3.6 mg/g tissue; NS; respectively). In conclusion, nitric oxide pathway seems to be an important regulator of GK activity and glycogen content in cultured rat hepatocytes. ET-1 seems not active *per se* but its activity seems mediated by a decrease in nitric oxide levels.

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CHARACTERIZATION OF GLUT1-CONTAINING MEMBRANE VESICLES DERIVED FROM FIBROBLASTS OF A PATIENT WITH DEFECTIVE INSULIN-STIMULATED GLUCOSE TRANSPORT ASSOCIATED WITH MALINSERTION OF GLUT1 INTO THE PLASMA MEMBRANE.

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The aim of this study was to prepare and characterize GLUT1-containing membrane vesicles derived from fibroblasts of a patient with clinical features of Werner syndrome and defective insulin-stimulated glucose transport associated with malinsertion of GLUT1 into the plasma membrane in an attempt to examine the molecular cause of the defective docking/fusion process. Microsomal membrane fractions (200 μg) were incubated with protein G-beads for 16h at 4°C. After centrifugation anti-GLUT1 antiserum was added to the supernatant and the mixture was further incubated for 5h at 4°C. Nonspecific adsorption was monitored by identical treatment with a preimmune serum. The precipitate was pelleted, resuspended and incubated with beads for further 16h at 4°C. After centrifugation vesicle proteins attached to beads were washed and subjected to SDS-PAGE, blotted to nitrocellulose and the following proteins were immunodetected: VAMP-2, SNAP-25, Synaptotagmin and interestingly the p85 subunit of PI-3'kinase. Preliminary results suggest that the expression of p85 subunit of PI-3'kinase was reduced in GLUT1-containing membrane vesicles prepared from fibroblasts derived from the patient VH when compared to controls. In conclusion, GLUT1-containing membrane vesicles include VAMP-2, SNAP-25, Synaptotagmin as well as the p85 subunit of PI-3'kinase. Further study is necessary to investigate whether the decreased expression of p85 of PI-3'kinase in vesicles from the patient VH is responsible for the defective insertion of GLUT1 into the plasma membrane.

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PRESENCE OF ALDOSTERONE RECEPTORS IN U-937 HUMAN PROMONOCYTIC CELLS.

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Earlier studies of our laboratory have demostrated that aldosterone decreases insulin receptor gene expression at the RNA level in U-937 human promonocytic cells. In the present work we examine if such an effect could be mediated by specific aldosterone receptors. U-937 cells were grown in suspension in RPMI-1640 medium supplemented with fetal calf serum, glutamine and antibiotics. For experiments, 10^{-9} M aldosterone was directly applied to the cultures for 36 and 48 h. Binding studies were performed using cells previously washed with incomplete 1640 medium to remove endogenous steroids and serum proteins. Then, the cells (12 x 10^6 cells/ml) were incubated from 60 up to 180 min at 37°C with [^{3}H]- aldosterone (0.5 x 10^{-9} M) either in the absence or presence of unlabeled aldosterone (10^{-10} - 10^{-7} M). After that, U-937 cells were treated with 1% Triton X-100, and centrifuged at 1800 rpm for 10 min at 4°C. Finally, the supernatant cytosols were separated for scintillation counting. The results were expressed in terms of specific binding, subtracting the nonspecific binding in the presence of 5 x 10^{-7} M unlabeled aldosterone. The binding of aldosterone was found to be time-dependent. The maximal percentage of fraction bound (45%) was obtained at 120 min of incubation. Scatchard analysis revealed the presence of a single class of binding sites with high affinity (Kd: 1.1 x 10^{-9} M) and limited capacity (2400 sites per cell). Treatment with aldosterone for 48 h, clearly decreased aldosterone binding in U-937 cells. These findings represent the first demonstration of the presence of aldosterone receptors in U-937 cells, and also, the indication that aldosterone is involved in the homologous regulation of aldosterone receptors in these cells.

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METABOLIC EFFECTS OF IGF-I AND INSULIN IN THE PERFUSED RAT LIVER

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Human recombinant insulin-like growth factor-I (IGF-I) has been shown to exert antidiabetic effects mainly by increasing peripheral glucose uptake, glycogen synthesis and glycolysis in skeletal muscle and adipocytes. This study was designed to compare the effects of IGF-I with those of insulin on glucose metabolism, the release of cyclic AMP and cyclic GMP as well as changes in intracellular calcium concentration $[Ca^{2+}]_i$ (surface photometry using the Fura-2 fluorescence technique) in the perfused rat liver (Krebs-Henseleit buffer, flow-through, 3 ml/g liver.min). Basal incremental glucose-production (area under the curve, AUC within 30 min) was decreased by IGF-I (1nM) by 64% ($p<0.05$ Dunnett test) and by insulin (1nM) by 62% ($p<0.05$). Epinephrine (0.05 μM) - induced incremental glucose-production (control, CON: 42.2±4.5 μmol/g) was inhibited by IGF-I (1nM, 28.0±1.9 μmol/g, $p<0.05$ vs. CON) and insulin (1nM, 16.8±2.1 μmol/g, $p<0.05$ vs. CON). Basal and epinephrine-stimulated lactate release were not affected by insulin and IGF-I. Under basal conditions release of cyclic AMP was reduced by 27% ($p<0.05$, paired t-test) in response to IGF-I and by 40% ($p<0.001$) in response to insulin, while epinephrine elicited an increase by 75% ($p<0.05$ vs. basal). In contrast, cyclic GMP release was not affected by IGF-I, insulin and epinephrine. Insulin and IGF-I constantly increased (+15%) basal $[Ca^{2+}]_i$ ($p<0.05$, Wilcoxon test), but reduced the rapid epinephrine-dependent increase in $[Ca^{2+}]_i$ by 43%. These results show IGF-I to act similar to insulin on basal and epinephrine-stimulated hepatic glucose-metabolism and to counteract intracellular epinephrine mediated signal transduction.

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THE EFFECT OF PROGESTERONE ON 2-DEOXYGLUCOSE UPTAKE IS MEDIATED BY CERAMIDE

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Ceramide was reported to induce insulin resistance as a TNF-α mediated intracellular mechanism. The aim of this study was to assess the effect of ceramide on progesterone-induced insulin resistance *in vitro*. L-6 myocytes were treated with progesterone and membrane permeable ceramide analogs (C2) for 16 hours, and then the intracellular ceramide concentration and the 2-deoxyglucose uptake were measured. Progesterone increased dose-dependently the intracellular ceramide concentration. The maximum effect was observed at more than 0.1 μM of progesterone. Although progesterone augmented the basal 2-deoxyglucose uptake, it suppressed the insulin stimulated 2-deoxyglucose uptake. The maximum effect was observed at more than 0.1 μM. C2 (5 μM) mimicked the effects of progesterone on 2-deoxyglucose uptake. The additive effect of progesterone and C2 was not observed. In conclusion, progesterone was suggested to inhibit insulin-induced 2-deoxyglucose uptake by intracellular ceramide.

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NO DETECTION, BY REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION, OF GLUT 7 mRNA IN RAT LIVER.

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Glut 7 is the most recently described glucose transporter, identified in rat liver. We aimed to study the effect of diabetes and insulin-treatment on the abundance of Glut 7 mRNA. Because of the high homology with Glut 2 mRNA (75% between the two coding sequences) present in the same tissue, only a strategy based on RT-PCR in competition might be suitable. As a first approach, we tried to amplify Glut 7 mRNA specifically with regards to Glut 2 mRNA, involving two 18-mer oligonucleotide antisense primers exhibiting almost no homology (located in exon 10, based on Glut 2 sequence, the Glut 7 gene being not known) and two 18-mer non-homologous sense primers (located in exon 6). Temperatures for annealing were tested from 46 to 62°C and $MgCl_2$ concentrations from 1 to 5 mM. The expected 616 bp-long cDNA product was amplified in all cases, but one, from Glut 2 specific primers and in no cases from Glut 7 primers. Identical results were obtained using : 1) two other non-homologous sense primers located in exon 7 and an other common antisense primer located in a region of complete homology between the two sequences (exon 11) ; 2) other combinations of aforementioned sense and antisense primers ; 3) *Taq* polymerases from different origins ; 4) reverse transcription at high temperature (70°C) with thermostable reverse transcriptase ; 5) additional PCR runs from prior RT-PCR amplification mediums ; 6) total RNAs from other tissues which express Glut 2, eg. kidney, small intestine and pancreas. We conclude that Glut 2 mRNA may be very easily amplified by RT-PCR, whereas Glut 7 mRNA, if existing, may not be detected using the same approach, in spite of its high structural homology with the former.

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NON-ISOTOPIC TECHNIQUE FOR STUDYING MICROSATELLITE GENETIC MARKERS; APPLICATION TO BRITISH SIB-PAIRS WITH TYPE 2 DIABETES.

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A polymorphic microsatellite marker (D2S125) on chormosome 2 was recently reported to show significant linkage to NIDDM in a population of Mexican American affected sib pairs. We have used a simple non-isotopic screening technique employing the polymerase chain reaction with a biotinylated primer to study the genetic linkage and allele frequency distribution of the D2S125 marker in a population of 109 Europid subjects with NIDDM (62 possible affected sib pairs: 47 sibships with 2 affected and 5 with 3 affected members). Subjects were genotyped using a 5' biotinylated forward amplimer AFM112yd4a (GCAACAGAGTGAGACCCTGA) and the reverse primer AFM112yd4m (TTCTGAGAACCAGATTGTGATTG). After electrophoresis on 6% agarose gels, PCR products were blotted onto a nylon membrane by capillary action for 6 hours. The biotinylated PCR products were detected using the Tropix detection kit (Cambridge Bioscience).

Results;

Allele	size (bp)	frequency	Allele	size (bp)	frequency
1	102	0.013	5	94	0.243
2	100	0.116	6	92	0.158
3	98	0.046	7	90	0.095
4	96	0.134	8	88	0.196

Sib-pair analysis provided no evidence for linkage of the D2S125 marker in the limited number of sib-pairs we have currently available (MLS = 0.029, p>0.05). The PCR screening method proved to be a safe and reliable alternative to the radiolabelling of PCR products.

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MUTATION SCREENING OF THE HEPATOCYTE NUCLEAR FACTOR -1α AND -4α GENES IN MODY FAMILIES: SUGGESTION OF THE EXISTENCE OF AT LEAST A FOURTH MODY GENE.

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Maturity-onset diabetes of the young (MODY) is a genetically heterogeneous subtype of non-insulin-dependant diabetes mellitus (NIDDM) characterized by early onset, usually before 25 years of age, autosomal dominant inheritance and a primary defect in insulin secretion. At least 3 genes are responsible for MODY: the glucokinase gene (MODY2) on chromosome 7p, MODY1 and MODY3 genes on chromosomes 20q and 12q respectively. Recent studies have shown that mutations in the two functionally-related transcription factors, hepatocyte nuclear factor 4α (HNF-4α) and -1a (TCF1) are responsible for the MODY1 and MODY3 forms of diabetes.We previously described 8 MODY families with no mutation in the GCK gene and with no evidence of linkage to MODY1 or MODY3 markers. Therefore the aim of our study was to investigate that the MODY phenotype in families with non-conclusive Lod-score (-2 to 0.5) at the MODY loci is not caused by mutations in one of the two HNF genes. Probands from 4 and 5 of those families were screened for mutations in the HNF-1α gene (10 exons) and in the HNF-4α gene (11 exons) respectively by direct sequencing. We also scanned 15 probands from newly recruited MODY families. The exons and flanking intronic sequences of these genes were amplified by PCR using specific primers. After purification, PCR products were sequenced directly from both directions using an AmpliTaq FS dye terminator cycle sequencing kit and an ABI prism 377 automated DNA sequencer. A G to A transition in codon 161 of exon 2 was identified in one of these new families resulting in a Ala to Thr substitution. This new missense mutation localised in the DNA-binding domain of the HNF-1α protein co-segregated with diabetes in this family. Previously described polymorphisms in exon 4 and 7 were also found in other families. Thus we confirmed the existence of at least one other locus responsible for the MODY phenotype. We have identified 22 french families suitable for the search of other MODY genes.

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NO EVIDENCE FOR EXCESS ALLELE-SHARING ON CHROMOSOME 2q (REGION OF *NIDDM1*) IN A LARGE SIBSHIP COLLECTION

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Recent analysis in Mexican Americans families has suggested that a susceptibility locus for NIDDM lies on chromosome 2q (*NIDDM1*). To determine whether a 2q locus influences susceptibility to NIDDM in Europeans, we conducted an initial study of families from the British Diabetic Association (BDA) Warren 2 NIDDM repository. Ascertainment into this collection requires that all grandparents be of British/Irish origin and that affected sibs have NIDDM diagnosed between 35 and 70: the resource currently (Dec 1996) stands at ~700 collected pedigrees. To date, we have typed the first 224 pedigrees from the resource (471 affected sibs, 278 possible sibpairs) for markers in the *NIDDM1* region. Microsatellite markers from the telomeric 25cM of 2q (D2S125, D2S336, D2S338, D2S2285, AFMA064ZF9) were assayed by fluorescent genotyping methods: marker order was taken from the Whitehead Institute integrated map, release 11. Multipoint nonparametric analysis was performed using GENEHUNTER and allele frequencies were estimated from the family data. Overall genotyping accuracy was confirmed by close correspondence between interlocus distances inferred from the observed data and those published. Between 63% and 75% of inheritance information was extracted across the region. We found absolutely no evidence for excess allele-sharing across the terminal region of 2q - indeed, NPL scores were negative throughout (peak NPL -1.01, p=0.86). Though we plan to extend this analysis to include additional markers and families, these data indicate that any locus on 2q has little influence on NIDDM susceptibility in this European population. The data obtained are clearly consistent with significant ethnic heterogeneity in the genetic basis of NIDDM and emphasise the problems in replicating positive linkages obtained in diverse ethnic groups.

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MUTATIONS IN HEPATOCYTE NUCLEAR FACTOR 1α GENE IN MATURITY ONSET DIABETES OF THE YOUNG (MODY3)

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AIM: To characterize the genomic structure of human hepatocyte nuclear factor 1α (HNF-1α) and identify mutations in the HNF-1α gene in MODY3 patients. METHODS: Fragments of the PAC clone 254A7 which contain the HNF-1α gene were sequenced to determine exon-intron organization and promoter region sequence. This information was used to design PCR primers to amplify and screen each exon of HNF-1α in MODY3 patients for mutations by direct sequencing of both strands of the PCR product. RESULTS: The human HNF-1α gene is comprised of 10 exons that span about 23 kb, of which 6.2 kb were sequenced including 490 bp of the 5'-flanking promoter region. In eight unrelated MODY3 families, eight different mutations were found: three missense mutations (R131Q, C241G, P447L), two splice site mutations (IVS5nt-2A-G, IVS9nt+1G-A), and three frameshift mutations (P291fsinsC, P379fsdelCT, T547E548fsdelTG). Family members were screened for mutations and the mutations were found to cosegregate with affected status. Point mutations changed conserved amino acids and were not seen upon screening 50 unrelated nondiabetic subjects. CONCLUSION: Mutations in the HNF-1α gene are the cause of MODY3. Information on the sequence of the human HNF-1α gene and its promoter region will facilitate the search for mutations in other subjects as well as studies of its role in normal β-cell function.

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Genome scan for linkage to pre-diabetic traits in Pima Indians

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To investigate the genetic basis of selected risk factors for NIDDM, we performed a genome-wide scan in a group of healthy, non-diabetic Pima Indians who participated in a longitudinal study of the predictors of NIDDM. Glucose (G) and insulin (I) responses to a 75g oral glucose challenge (OGT), a mixed meal (MM) and a 25g intravenous glucose challenge (IVGTT) were measured as was insulin action (2-step hyperinsulinemic glucose clamp). 363 subjects (221M/142F) from 109 families (388 sib-pairs) were genotyped at 524 polymorphic DNA markers evenly distributed throughout the genome (average distance = 8 cM). Two-point linkage analyses (SIBPAL, SAGE) indicated that one marker, D3S1764, was significantly linked (LOD = 3.7) to glucose disposal during the low dose of the clamp. However, this linkage was not as significant (LOD = 1.6) by multipoint interval mapping (Fulker et al.). An additional 29 markers showed suggestive evidence of linkage to one or more sub-phenotypes in two-point analyses. Of these, 6 regions also showed suggestive linkage (LOD > 2.2) in multipoint analyses (Table).

Marker	Phenotype	2-point LOD	Multipoint LOD
D1S1646	OGT-G	3.1	2.9
D1S2125	IVGTT-G	2.7	3.3
D4S2382	Fasting-I	2.8	2.6
D7S798	IVGTT-G	3.2	2.3
GATA35	OGT-I	2.7	3.2
D18S535	MM-G	2.7	2.2

The results of this genome-wide scan suggest several regions which may be linked to aspects of glucose metabolism. Specific genes in these areas may contribute to the high prevalence of NIDDM in the Pimas.

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MUTATIONS IN THE HEPATIC NUCLEAR FACTOR-1α GENE ARE A COMMON CAUSE OF MODY IN DANISH CAUCASIANS

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One form of maturity-onset diabetes of the young (MODY3) results from mutations in the hepatic nuclear factor-1α (HNF-1α) gene, located on chromosome 12q24.2. The objective of the present study was to examine for genetic variability in the HNF-1α gene in 9 non-related Danish Caucasian subjects with MODY. Direct sequencing of the coding region and intron-exon boundaries of the HNF-1α gene revealed 2 novel ((I128N and H143Y) and 1 previously reported (P447L) missense mutations and 2 novel frameshift mutations (P379fsdelT and A559fsinsA) in 5 of 9 MODY subjects. These 5 mutations were neither found in 84 NIDDM patients nor in 84 control subjects. One glucose tolerant lean male, with a P447L missense mutation which in his relatives caused MODY, underwent an oral glucose tolerance test (OGTT), a tolbutamide modified frequently sampled intravenous glucose tolerance test and a glucagon test in order to examine for a possible early beta cell abnormality. He had a low insulin secretion during an OGTT, but a 2 fold increase in pancreatic beta cell response after intravenous glucose and a 2.5-4 fold increase in beta cell response after either intravenous tolbutamide or intravenous glucagon loads when compared to matched control subjects. In conclusion: 1) mutations in the HNF-1α gene are common in Danish Caucasian MODY patients, and 2) early stages in the pathogenesis of MODY3 caused by the P447L mutation may be characterized by a hyperexcitability of beta cells to intravenous secretagogues.

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MULTIPOINT LINKAGE ANALYSIS OF NIDDM IN 534 FINNISH FAMILIES IN THE FUSION STUDY.

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The FUSION study is a collaborative effort to map genes for NIDDM in the Finnish population. We are performing a genome scan with an initial 450 microsatellite markers in 734 NIDDM-affected sib pairs (ASPs) from 534 families. To minimize the number of families with late-onset IDDM we excluded families in which an affected individual had low C-peptide levels, high GAD autoantibody levels, and required insulin therapy $\leq$ 4 years after diagnosis. Our analysis strategy includes nonparametric linkage analysis using both single point and multipoint identity-by-descent methods, identity-by-state methods, and association analysis. To date no major susceptibility loci for NIDDM have been identified on chromosomes 1, 2, 7, 8, 11, 12, 15 and 16. In the course of the genome scan, we have investigated in particular two regions recently identified in linkage analyses of NIDDM in other studies: a region on chromosome 2q identified in Mexican-Americans (Hanis et al., Nat Genet 13:161-6) and a region on chromosome 12 near the MODY3 locus in families from the Botnia region of Finland (Mahtani et al., Nat Genet 14:90-4). Using multipoint linkage analysis on the entire set of ASPs we exclude (LOD< -2) both regions at low relative risk to sibs (λ): a 76 centiMorgan (cM) region including the chromosome 2q telomere and D2S125 at $\lambda = 1.32$; and a region at least 13 cM on either side of D12S76 at $\lambda = 1.25$. We are exploring linkage relationships on a subset of our ASPs and their offspring with several measures of glucose tolerance, including glucose effectiveness, insulin action, and impaired insulin secretion.

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MUTATIONS IN THE HEPATIC NUCLEAR FACTOR 1 ALPHA IN MATURITY-ONSET DIABETES OF THE YOUNG

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Mutations in the gene encoding the transcription factor hepatic nuclear factor 1α (HNF-1α) have recently been shown to cause maturity-onset diabetes of the young (MODY), a monogenic subgroup of non-insulin dependent diabetes mellitus (NIDDM). The incidence of HNF-1α mutations is unknown. We performed mutation analysis on fifteen probands from MODY families. All ten exons and flanking introns were amplified by PCR and alterations characterized by direct automated sequencing. We identified six novel mutations, one frameshift (A443fsdelCA) and five missense mutations (P129T, R131W, R159W, P519L, T620I) which involved conserved amino acids. Two previously described mutations (P379fsdelCT and P291fsinsC) were found in one and four families respectively. These mutations cosegregated with diabetes within pedigrees, were not seen in any non-diabetic family members with the exception of one individual who was not diabetic at age 42, and were not present in fifty normal chromosomes. We have demonstrated that mutations in the HNF-1α gene are the most common cause of MODY in UK families. Mutation detection based genetic counselling can now be offered to most patients with MODY.

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ASSOCIATION OF IRS-1 Asp^{972}->Gly VARIANT WITH INSULIN RECEPTOR MUTATIONS IN INSULIN RESISTANT DIABETES

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To understand how mutations in the insulin receptor (IR) gene may be pathogenically involved in severe as well as mild forms of insulin resistance we have investigated first-degree relatives of patients with Type A syndrome and *acanthosis nigricans*. The insulin resistance was analysed *in vivo* by minimal model of IVGTT and mutations in IR and IRS-1 genes were scanned by ABI 373A DNA sequencer. A genetic condition of compound heterozygocity for two mutations Asp^{59}->Gly and Leu^{62}->Pro in the insulin binding site (exon 2), reasonably explained the severity of insulin resistance (Si<0.15) and the deterioration of IR functions (insulin binding, tyrosine kinase, PtdIns 3' kinase activity) in the 24 years Type A proband. Paternal mutation Leu^{62}->Pro was associated in the father with obesity (BMI=34), hyperinsulinemia and Si value (3.39) in the range of nondiabetic obese controls (4.3±1.05). Maternal mutation Asp^{59}->Gly was associated with obesity (BMI=38), NIDDM and insulin resistance (Si=0.1) in the mother whereas in the 29 years proband's brother, carrying the same heterozygote mutation the phenotype was normal (Si=10.3), except overweight (BMI=28). Sequencing of IRS-1 gene revealed heterozygote Asp^{972}->Gly variant in the proband and mother while being absent in the brother and father. These data suggest that cumulative genetic defects in proteins involved in insulin signaling may represent a plausible explanation of variable expression of IR mutants and give new insights in the pathogenic mechanism of more common forms of insulin resistant diabetes.

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IDENTIFICATION OF A BALANCED TRANSLOCATION t (3 ; 20) (p21.2 ; q12) AT THE CHROMOSOMAL LOCATION (20q12) OF THE HNF-4α GENE IN A FAMILY WITH MATURITY-ONSET DIABETES OF THE YOUNG (MODY)

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Maturity-onset diabetes of the young (MODY) is a sub-group of non-insulin dependent diabetes mellitus (NIDDM) which is characterised by an early age of onset and autosomal dominant inheritance. Linkage studies have localised MODY genes on chromosomes 20q (*MODY1*), 7p (*MODY2*/ glucokinae) and 12q (*MODY3*). *MODY1* and *MODY3* have recently been shown to be genes encoding the transcription factors HNF-4α and HNF-1α respectively. MODY1 is a rare form of MODY, with an estimated prevalence of less than 5%. We have studied a MODY family where two members suffered recurrent miscarriages and were diagnosed with NIDDM at age 15 and 19 years. Karyotypic analysis revealed a balanced translocation t (3 ; 20) (p21.1 ; q12) in both family menbers. Fluorescent chromosome painting showed that MODY co-segregated with the translocation in five pedigree members. We propose that this balanced translocation has disrupted the HNF-4α gene on 20q12. To our knowledge this is the first balanced translocation reported to cause diabetes. Further studies are in progress to determine the precise position of the translocation breakpoint in relation to the HNF-4α gene.

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ANALYSIS OF TYPE-1 PROTEIN PHOSPHATASE GLYCOGEN-TARGETING SUBUNIT mRNA IN SKELETAL MUSCLE OF PIMA INDIANS

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It has recently been proposed that mRNA level of type-1 protein phosphatase glycogen-targeting subunit may contribute to mechanisms of insulin resistance in Pima Indians. Glycogen-bound type-1 protein phosphatase (PP1G) plays a key role in the insulin stimulation of glycogen systhesis, and a decrease of the enzymatic activity correlates with insulin resistance in the Pima Indians. PP1G in skeletal muscle is a heterodimer between an isoform of the catalytic subunits, and the muscle-specific glycogen-targeting subunit which is encoded by the *PPP1R3* gene. We have previously found an association of amino acid polymorphisms at codon 883 and 905 in *PPP1R3* with insulin resistance and non-insulin dependent diabetes mellitus (NIDDM) in Pima Indians. We have now quantitated *PPP1R3* mRNA by reverse transcription PCR in skeletal muscle biopsies from 20 non-diabetic Pimas, using β-actin as an internal standard. We found that the mRNA is positively correlated with the maximal insulin-mediated glucose uptake rate measured by the hyperinsulinemic-euglycemic clamp (r=0.51; p=0.02). We detected by PCR and sequencing of genomic DNA a T→A substitution approximately 2 kb 5' from the start of *PPP1R3* coding sequence, and a 5-base polymorphism involving an ATTTA motif in the 3' untranslated part of the gene. Both polymorphisms are in linkage disequilibrium (p<0.001), and they can be combined in two alternate haplotypes. We determined that the mRNA levels are significantly different between subjects homozygous for either combination (p=0.03). Measurements of PPP1R3 protein are currently in progress to determine its relationship with the mRNA levels. The ATTTA motif may affect mRNA stability, and we conclude that the polymorphism at this site in *PPP1R3* is a reasonable candidate that could cause the observed mRNA differences, and contribute to mechanisms of insulin resistance in the Pima Indians.

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50 YEARS OBSERVATION OF THE CLINICAL PHENOTYPE IN A GERMAN MODY3 FAMILY (MUTATION IN HEPATOCYTE NUCLEAR FACTOR-1α)

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We have shown that mutations in the gene encoding the transcription factor HNF-1α are the cause of one form of maturity-onset diabetes of the young (MODY3). One of the German families (G16) with a recently identified missense mutation (R272H in exon 4) has been in our care since 1947. The aim was to characterize the clinical phenotype, especially the long-term course of diabetes in the affected carriers of the mutation. Grandfather: Diabetes since 1925 (age 19 ys), treatment in Karlsburg-Garz 1947 (start of insulin therapy), 1949 and 1962 (severe proliferative retinopathy, begin of renal insufficiency, severe neuropathy, claudication). 1971 blindness, amputation, death due to renal failure. In 1996 we identified the mutation of the HNF-α1 gene in half of his 12 descendants. Years before, either IGT or diabetes had been diagnosed through careful family screening, in each case around the age of 6 (one exception: a premarital daughter had become ill with typical symptoms at 12), during the 2 to 25 years after diagnosis OGTT, IVGT or glucose-glucagon tests were regularly carried out. Glucose tolerance fluctuates (normal, impaired, diabetic), shifting towards low response of insulin secretion and towards diabetes at differing rates from individual to individual; three receive insulin. 15 and 25 years after diagnosis two have a retinopathy (grade II). HbA1c 6.7 ± 0,1 %. MODY3 caused by mutation in the HNF-1α gene can later be aggravated by serious diabetic complications despite the initially long-term mild course. Complications may possibly be avoided by early diagnosis in the identified carriers and by an extremely good control of the metabolic disorder, diabetes as well as IGT. It is unclear whether additional genetic defects contribute to the diverse clinical appearance of MODY3.

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ANALYSIS OF A MET->ILE SUBSTITUTION IN THE REGULATORY SUBUNIT OF PI3-K IN PIMA INDIANS

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Phosphatidylinositol 3-kinase (PI3-K) is one of the signaling molecules activated by IRS-1, and has been implicated in linking the insulin receptor to translocation of glucose transporters. We have begun structural analysis of the gene encoding the regulatory subunit of PI3-K, p85α, in Pima Indians to determine whether any genetic variants can be identified which could explain the high prevalence of diabetes and insulin resistance in this population. We have begun sequencing the entire coding region of p85α in cDNA derived from muscle cells from Pimas. To date, we have identified one missense polymorhism at nucleotide 1020 (G->A) which alters a Met to a Ile at codon 326. This polymorphism was initially reported in a Danish population, and was found to be significantly associated with glucose disappearance (Kg) and glucose effectiveness (Sg) in 380 unrelated healthy Danish Caucasians. In the Danish population, the allelic frequency of the Ile variant was 0.15 in NIDDM patients and 0.16 in matched glucose tolerant subjects; therefore, this substitution does not appear to confer an increased risk of diabetes in this population. In 331 Pima Indians we have found the allelic frequency of the Ile variant to be higher than that observed in the Danish (0.24). In addition, we have noted a difference in the allelic frequency of the Ile variant between Pimas with NIDDM (0.20) and non-diabetic Pimas (0.28), where the prevalence of NIDDM is higher (68%) in individuals homozygous for the more common Met, and lower (38%) in individuals homozygous for the variant Ile. The contradictory associations for this variant in p85α between the Danish and Pima studies could represent true population differences for this variant or could be biased due to the small number of individuals who are homozgous for the variant (2% of Danish population and 5% of Pimas). We are continuing to sequence additional Pimas to confirm our findings.

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MUTATIONS IN HEPATOCYTE NUCLEAR FACTOR 1 ALPHA GENE ASSOCIATED WITH NIDDM IN FRENCH MODY3 FAMILIES

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Maturity-onset diabetes of the young (MODY) is a genetically heterogeneous subtype of NIDDM characterised by early onset, autosomal dominant inheritance and a primary defect in insulin secretion. Recent studies have shown that mutations in the two functionally-related transcription factors, hepatocyte nuclear factor 4 alpha (HNF-4α) and hepatocyte nuclear factor 1 alpha (HNF-1α) are associated with the MODY1 and MODY3 forms of diabetes respectively, whereas mutations in the enzyme glucokinase are the cause of the MODY2 form. We have examined 10 unrelated Caucasian families in which MODY/NIDDM co-segregated with markers on chromosome 12q (*MODY3* region) for mutations in the HNF-1α gene (symbol - *TCF1*). Ten different heterozygote mutations were observed in these families, all of which co-segregated with diabetes. These mutations in HNF-1α are highly but not completely penetrant and result in a subtype of MODY characterised by a severe insulin secretory defect. There were no obvious relationship between the nature of the mutations observed (i.e. frameshift, nonsense, or missense) or their location in the gene with clinical features of diabetes (age at onset, severity) in these families. The mechanisms whereby these mutations cause diabetes are unknown, but might include abnormal foetal development of pancreatic islets, as well as impaired transcription of genes implicated in beta cell function. In conclusion, HNF-1α is a major gene for MODY with mutations in this transcription factor being present in 25-50% of French MODY subjects.

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CODON 972 POLYMORPHISM OF THE IRS-1 GENE AND INSULIN RESISTANCE IN THE JAPANESE POPULATION.

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To determine the relationship of polymorphism at the locus for insulin receptor substrate-1 (IRS-1) with insulin resistance and susceptibility to NIDDM in the Japanese population, we examined 130 patients with NIDDM and 143 control subjects with normal glucose tolerance for the most common GGG(Gly) to AGG(Arg) substitution at codon 972 of IRS-1 by PCR-RFLP. Six (4.6%) of the patients with NIDDM were heterozygous for the Gly972Arg mutation. Within the control subjects, six were heterozygous and only one was homozygous for the mutation (4.9%). There was no difference in the prevalence of this mutation between patients with NIDDM and control subjects. The age of onset of NIDDM was not different between subjects with and without the mutation (43:0$\pm$14.5 vs 43.8$\pm$11.8 years). Insulin sensitivity was evaluated by the hyperinsulinemic euglycemic clamp in patients with NIDDM. Glucose infusion rate (GIR) did not differ between subjects with and without the mutation (50.2$\pm$3.0 vs 51.3$\pm$12.1 μ mol/kg/min). Fasting plasma insulin concentration in the control subjects did not differ between subjects with and without the mutation (31.0$\pm$11.0 vs 27.9$\pm$16.4 μ mol/ml). A 25-year-old man with Gly972Arg homozygotes, whose parents were both heterozygous for the mutation, had no family history of NIDDM and showed normal glucose tolerance in the 75gOGTT and high insulin sensitivity by the hyperinsulinemic euglycemic clamp (GIR=67.2 μ mol/kg/min). Thus, it appears that the Gly972Arg mutation of the IRS-1 gene may not play an important role in the pathogenesis of NIDDM and insulin resistance in the Japanese population.

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Studies of the Genetic Variability in the Hepatic Nuclear Factor 1-α Gene in Patients with Late-Onset NIDDM

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NIDDM2, a gene for late-onset non-insulin dependent diabetes mellitus (NIDDM) associated with an insulin secretion defect was previously mapped to chromosome 12q in Finnish families. Recently it has been shown that the type 3 form of maturity-onset diabetes of the young (MODY3) is caused by mutations in the hepatic nuclear factor-1α (HNF-1α) gene which is located in the same chromosomal region. The objectives of this study were to examine for genetic variability in the HNF-1α gene in 84 Danish Caucasian subjects with NIDDM and in an association study to test whether genetic variants are associated with NIDDM. Single strand conformational polymorphism and heteroduplex analysis of the coding region and the intron-exon boundaries of the HNF-1α gene and subsequent sequencing revealed 3 missense polymorphisms (I27L, A98V and S487N), 1 missense mutation (R583Q) and several silent polymorphisms in the NIDDM population. An association study demonstrated that the R583Q mutation was present in 2 of 245 NIDDM patients and in none of 242 control subjects. The missense polymorphisms were equally prevalent in diabetic and control subjects (allelic frequencies: I27L: 31.5% (95% CI: 27.4-35.6%) vs. 26.3% (22.4-30.2%); A98V: 3.7% (2.0-5.4%) vs. 4.4% (2.6-6.2%) and S487N: 28.0% (24.0-32.0%) vs. 26.4% (22.5-30.3%)). In conclusion: Genetic variability in the HNF-1α gene is not a common cause of late-onset NIDDM in Caucasians of Danish ancestry.

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ASSOCIATION OF A VARIANT OF THE VITAMIN D BINDING PROTEIN WITH PLASMA GLUCOSE LEVELS IN NON-DIABETIC PIMA INDIANS

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To identify chromosomal regions containing susceptibility genes for NIDDM, we have undertaken an autosomal genome-scan for DNA markers linked to pre-diabetic metabolic phenotypes in non-diabetic Pimas Indians. 363 Pimas from 109 nuclear families (comprising 388 sib-pairs) were genotyped with 517 microsatellite DNA markers. Sib-pair linkage analysis identifed 4 markers (D4S3255-D4S3248-D4S1645-D4S2367) within a 10 cm region on chromosome 4 which are potentially linked to fasting plasma glucose concentrations ($p<0.005$), the glucose response to a 75g oral glucose tolerance test ($p<0.04$), the glucose response to a 25g intravenous glucose bolus ($p<0.006$), the fasting plasma insulin concentration ($p<0.02$), and maximal insulin action in vivo ($p<0.0003$), after adjusting for age, sex and obesity. The *Gc* locus, which encodes the vitamin D binding protein, maps to this region on 4q.12. Since this locus has previously been associated with NIDDM in a German and 7 southwest Pacific Island populations, and has been associated with fasting plasma insulin and glucose levels in 2 Native American populations, we analyzed this locus as a candidate gene for our linkage findings in Pimas. We sequenced all 12 exons of this gene and identified two polymorphisms. A GAG->GAT polymorphism at codon 416 (frequencies = 0.41 and 0.59) results in a Glu->Asp, while a ACG->AAG polymorphism at codon 420 (frequencies = 0.86 and 0.14) results in a Thr->Lys. Sequencing of these codons in 1200 Pimas identified an association between the polymorphism at 420 and measures of plasma glucose. Homozygotes for the ACG/ACG genotype had higher plasma glucose levels during a 75g OGTT ($p<0.04$), higher plasma glucose responses to a mixed meal ($p<0.002$) and higher glucose levels during an intravenous glucose tolerance test ($p<0.01$) when compared to AAG/AAG homozgotes combined with the heterozygotes.

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CLINICAL CHARACTERISTICS OF HNF1α (MODY3) AND GLUCOKINASE MUTATIONS

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Maturity Onset Diabetes of the Young (MODY) is characterised by young onset, non insulin dependent diabetes and autosomal dominant inheritance. We have shown that HNF1α mutations are the commonest cause of MODY in the UK (73%). The clinical characteristics of these patients have not been described. We compared the clinical characteristics of 41 patients with HNF1α mutations (34 diabetes or fpg > 6mmol/l) with 18 patients with a missense Glucokinase mutation (GCK) (17 diabetes or fpg > 6mmol/l). Both groups had an early diagnosis (22.6 ± 14 v 30.5 ± 17 years) (HNF1α GCK) and were non-obese (BMI 24.5 ± 4.6 v $25.5 \pm 5.5 kgm^{-2}$). HNF1α mutations results in more sever hyperglycaemia than GCK mutations as shown by osmotic symptoms at diagnosis (18/34 v 1/17, $p < 0.05$) and increased treatment requirements (7 diet, 13 OHA, 14 insulin v 15, 3, $p<0.001$) to achieve a similar fasting blood glucose (8.0 ± 3.5 v 7.3 ± 1.7 $mmol/l^{-1}$, $p>0.05$). Significant retinopathy (registered blind or laser therapy) was seen in 5/34 HNF1α patients (0/17 GCK). We have shown that patients with HNF1α mutations have different clinical characteristics to those with glucokinase mutations. HNF1α has a progressive clinical course with increasing treatment requirements, whereas glucokinase show stable mild hyperglycaemia throughout life. In MODY a molecular genetic diagnosis has important implications for clinical management.

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LINKAGE ANALYSIS AND MOLECULAR SCANNING OF *THE PC3 GENE IN NIDDM*

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Proinsulin is processed by the enzymes PC2 and PC3 to produce mature insulin.. Hyperproinsulinaemia observed in some NIDDM patients suggests an underlying impairment in proinsulin processing, which may be caused by mutations in the PC2 and/or PC3 genes.The PC3 gene, mapping to chromsome 5q14-21, was therefore examined as a candidate for susceptibility to NIDDM. Twenty-six large pedigrees of Europid, Afro-Caribbean and Asian ethnicities, segregating for NIDDM, were screened with five polymorphic markers for evidence of linkage. The markers D5S401, Afm205wg7, GATA48A11, D5S409 and D5S433 spanned a 15cM interval in the chromosome 5q14-21 region with genetic distances of 7cM, 4cM, 2cM and 2cM between subsequent markers. Standard two-point linkage analysis using several models, excluded linkage across the region under all models (LOD scores < -2). Multipoint nonparametric analysis with GENEHUNTER revealed no evidence for allele sharing in the region (max NPL scores in the interval containing gene=0.31, P=0.36). In addition, all 14 exons of the PC3 gene were screened for novel and known mutations (exon 2 and exon 14 variants described in Japanese) amongst affecteds (n=80), unaffecteds with high proinsulin levels (n=10) and unaffected spouses (n=25) from the pedigrees, using heteroduplex and PCR-RFLP analysis. No new variants were evident. The exon 2 variant (Arg^{53}-Asn^{53}) was not observed and the exon 14 variant (Gln^{638}-Glu^{638}) occurred at a similar frequency in diabetic and non-diabetic subjects. These results indicate that genetic variation in the PC3 gene is not likely to contribute to NIDDM in these pedigrees.

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Phenotype of Human Hepatocyte Nuclear Factor 1α Mutations: Diabetes, Late Complications, and Glucosuria.

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As we have recently shown, a form of early-onset type 2 diabetes (MODY3) is caused by mutations in the Hepatocyte Nuclear Factor 1α (HNF-1α). We also demonstrated that HNF-1α mutations occur frequently in families with overt early onset type 2 diabetes in a Caucasian (German) population, i.e. probably in more than 30% of these families. Interesting is the comparison of the human phenotype and a mouse model of a homozygous disruption (knockout) of the HNF-1α gene. The knockout mice develop hepatic failure, phenylketonuria (PKU) and Fanconi syndrome with massive glucosuria. The aim of the present study is to evaluate initial data on clinical phenotype and progression of diabetes in 20 patients carrying a mutation in the HNF-1α gene originating from seven families with inheritance of early-onset type 2 diabetes/MODY. Though initially the diabetes seems to be mild, eight of the patients required insulin treatment within 10 years after diagnosis of the diabetes. Complications (retinopathy, nephropathy, neuropathy) developed in 3 out of 6 patients with a known diabetes duration of more than 15 years. A lowered descending kidney threshold for glucose could be demonstrated in at least one of the families studied, and in a different family PKU is present in a twin pair carrying a HNF-1α nonsense mutation. This suggests that a partial overlap of phenotypic features with the mouse model might be present in selected families.

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A MUTATION OF MUSCLE GLYCOGEN SYNTHASE GENE IS RELATED WITH INSULIN RESISTANCE IN JAPANESE NIDDM

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Muscle glycogen synthase (GYS) is a key enzyme of non-oxidative pathway of glucose metabolism which has been reported to be related with insulin resistance in NIDDM patients. It has also been considered that impaired GYS activity in the skeletal muscle is an inherited trait in patients with NIDDM. We therefore scanned the GYS gene for mutation in non-obese and late-onset Japanese NIDDM patients (n=244) by polymerase chain reaction/single strand conformational polymorphism analysis and direct DNA sequencing. We detected two types of missense mutation ; Met at position 416 to Ala (M416V mutation) in the exon 10 and Pro at position 442 to Ala (P442A mutation) in the exon 11. The P442A mutation (heterozygote) was found only one NIDDM patient treated with sulfonylureas. She had hypertension and ischemic heart disease. The M416V mutation was also found in non-diabetic subjects (non-DM : n=181, >60 years, non-obese, no family history of diabetes). The M416V mutant allele frequency was slightly high in NIDDM patients (13.7 %) but not statistically significant from non-DM subjects (9.7 %). However, the insulin sensitivity index [SI:10^{-4}/min • (μ U/ml)] estimated by the Minimal Model analysis in the NIDDM patients carrying the mutation was significantly lower than that in NIDDM patients without the mutation (1.16 ± 0.28, n=21 Vs 2.20 ± 0.20, n=60, Mean$\pm$SE, $p<0.01$). The glucose effectiveness (SG), age, body mass index and levels of HbA1c and serum lipids were not significantly different between the two groups. The same trend was observed in non-DM group. These findings suggest that the M416V mutation of GYS gene is one of the factors contributing to reduced insulin sensitivity in Japanese NIDDM patients.

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DIMINISHED INSULIN AND GLUCAGON SECRETORY RESPONSES TO ARGININE IN MODY1 SUBJECTS

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Nondiabetic (ND) subjects who carry the MODY1 gene have impairment of glucose-induced insulin secretion. The purpose of this study was to ascertain the effects of the nonglucose secretagogue arginine on plasma concentrations of insulin, C-peptide and glucagon and on glucose potentiation of arginine-stimulated insulin secretion in 14 ND subjects of the RW pedigree, 7 of whom are MODY1 gene marker positive (+) and 7 negative (-), and in 4 mildly diabetic (DM+) MODY1 subjects. Arginine (A) was given as a 5 gram pulse followed by a 25 minute infusion of 2.9 mM/kg before and after a pulse plus infusion of glucose (G) to clamp plasma glucose at 11 mM. The acute insulin responses (AIR) to secretagogue pulses and the 10-60 minutes areas under the curves (AUC) were compared for the 3 groups. Results: (mean±SEM), insulin in pmol/l, glucagon in ng/l

Group	AIR_{Arg}	AIR_{A+G}	AUC_{Arg}	AUC_{A+G}
ND(-)	288± 60	1626±198	12252±1194	97890±10710
ND(+)	438±114	972±216	3990± 858	23256± 6300
p<	NS	.05	.0001	.0001
DM(+)	162± 42	300± 60	582± 120	3888± 1086
vsND(-)p<	NS	.001	.0001	.0001

Similar differences were obtained when C-peptide levels were compared. The acute glucagon responses were similar for the three groups. AUC glucagon (10-60 minutes) was greatest for ND(-), intermediate for ND(+) and lowest for DM p<.04 (7549 vs 5772 vs 4778). Conclusions: The decreased insulin and C-peptide areas in ND(+) compared to ND(-) subjects in response to constant arginine infusion, magnified by glucose potentiation, indicate that the MODY1 gene defect affects the signalling pathway for arginine-induced insulin secretion. The decrease in glucagon area in response to arginine infusion suggests that mutations in the gene for HNF4, the MODY1 gene, leads to alpha, as well as beta cell defects or reduction in the mass of pancreatic islets.

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EXPRESSION OF TWO MUTATIONS IN THE GLYCOGEN SYNTHASE (GS) GENE

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Although association between the GS gene and NIDDM has been found in several populations, only two aminoacid polymorphisms, M416V (in Japan) and G464S (in Finland) have been identified in the gene. Our aim was to study the frequency of these aminoacid variants in different populations and the biological consequences of these mutations by expressing them in COS cells. M416V was determined by RFLP in 369 unrelated NIDDM patients and 285 controls from Finland and in 95 unrelated NIDDM patients and 91 controls from Sweden. Glucose disposal rate was studied during an euglycemic hyperinsulinemic clamp in a subset of 137 nondiabetic subjects. G464S was determined by RFLP in 518 unrelated NIDDM patients and 274 controls from Finland and in 231 unrelated NIDDM patients and 122 controls from Sweden. The mutated cDNAs (GS-M416V, GS-G464S) were created by PCR, cloned into pcDNA3 vector and expressed in COS7 cells. GS activity was analysed at 0.1 and 10 mM G-6-P with 0.3 and 7.1 mM UDPG. K_M was studied with 0, 0.1, 0.3, 0.6, 1.2 and 7.1 mM UDPG at 0.1 mM G-6-P. The allele frequency of M416V polymorphism did not differ between NIDDM patients and controls (3.3 vs. 4.2%) from Finland and it was detected only in one Swedish subject. Glucose disposal rate did not differ between the nondiabetic subjects with the MM or MV genotypes (6.5±2.6 vs. 5.9±2.1mg/kg.min). The frequency of G464S polymorphism was low in Finnish NIDDM patients and controls (0.6% and 0.7%) and was not detected at all in Swedish subjects. No significant differences were observed in the GS fractional velocities of the wild type GS, GS-M416V or GS-G464S at 0.3 mM UDPG (22.0±3.5, 20.3±2.1 and 19.4±2.1%) or at 7.1 mM UDPG (18.3±2.3, 16.5±0.7 and 16.1±0.8%). Also the K_M (1.1±0.1, 1.2±0.1, 1.1±0.1 mmol/l) and Vmax ($V_{(0.1)}$: 47.8±4.7, 46.7±3.5 and 43.9±1.7 or $V_{(10.0)}$: 264±28, 283±25 and 273±2 nmol/min.mg prot.) did not differ significantly between the expressed enzymes. Conclusions: The observed aminoacid variants had no significant effect on the function of the glycogen synthase enzyme, which is consistent with the normal insulin sensitivity observed in the nondiabetic sujects with the M416V mutation.

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HEPATIC NUCLEAR FACTOR 1α MUTATIONS RESULT IN BETA-CELL DYSFUNCTION

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We have demonstrated that mutations of HNF1α are the major cause of Maturity Onset Diabetes of the Young (MODY) in the UK. The underlying pathophysiology is unknown. We used specific insulin and proinsulin assays to study 21 patients with HNF1α mutations and 20 age matched, family members without mutations. The hyperglycaemia seen in subjects with mutations (7.2±2.8 v 4.9+0.6 mmol/l [mean ± SD)] $p<0.005$) resulted from severe beta cell impairment as shown by HOMA analysis of fasting insulin and glucose values(65±48 v 121±79%B $p<0.05$). Proinsulin levels were not significantly increased (PI/I ratio 0.32±0.26 v 0.21±0.18). There was no difference in insulin sensitivity (HOMA 89±51 v 94±105). Not all patients with HNF1α mutations had fasting hyperglycaemia. To determine the determinants of FPG we compared 10 subjects with an fpg< 6.0 mmol/l (9.3±2.0) with 11 subjects fpg>6.0 mmol/l. (4.8±0.8). The hyperglycaemic group had marked beta cell dysfunction (30±24 v 100±41) and were more obese (BMI 24.8±3.4 v 1.6±3.2kg/m^2). These results indicate that the primary determinant of hyperglycaemia in patients with HNF1α mutations is beta-cell dysfunction but obesity may modify the phenotype. Weight loss should be considered as a strategy for delaying or treating non-insulin dependent diabetes in patients who have inherited mutations of HNF1α.

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Diabetic complications in patients with MODY3 diabetes

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MODY3 diabetes, which is caused by a mutation in the HNF1α gene on chromosome 12 represents a relatively common monogenic form of diabetes in Finland. Age at onset of diabetes can vary from 10 to 60 years. Therefore, previously most cases have been misdiagnosed as IDDM or NIDDM. The possibility of an etiological diagnosis allows for the first time a description of the natural course of the disease. In order to examine the prevalence of chronic diabetic complications in this form of diabetes, we examined 42 carriers of the MODY3 mutation (38 with diabetes and 6 with IGT) for the presence of retinopathy (Wisconsin retinopathy scale through fundus photography), neuropathy (clinical score and vibration threshold with Biothesiometer), microalbuminuria (AER > 20 µg/min in ovbernight urine) and hypertension (lood pressure > 160/95). Of 38 MODY3 patients 21 (55%) were free of retinopathy, while 12 (32%) had mild non-proliferative retinopathy (NPDR) while 5(13%) had severe NPDR or proliferative retinopathy. The prevalence of retinopathy did not differ from a group of 76 NIDDM patients matched for age, duration of diabetes and HbA1c. Distal sensory neuropathy was found in 8 patients (21%), whereas 9 patients (24%) had AER > 20 µg/min (4 of them had macroalbuminuria), which is similar to the prevalence in a group of 138 NIDDM patients with diabetes duration of 8 years (22%). Eight patients had hypertension (21% vs 75% in NIDDM patients). As well retinopathy (r= 0.66;p=0.001) as neuropathy (r=0.38; p=0.19) correlated with HbA1c. In a logistic multiple regression analysis both HbA1c (RR =2.5, 95% CI 0-6.1) and duration of diabetes (RR 1.11, 95% CI 1.0-2.0) were associated with an increased relative risk of retinopathy. In conclusion, microangiopathic complications are observed with the same frequency in MODY3 as in NIDDM and strongly related to glycemic control. In contrast, hypertension is 3 times less commmon than in NIDDM.

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NEW VARIANTS IN THE GLYCOGEN SYNTHASE GENE (Gln71His, Met416Val) IN PATIENTS WITH NON-INSULIN-DEPENDENT DIABETES

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Impaired glycogen synthesis after insulin stimulation accounts for most of the insulin resistance in patients with non-insulin dependent diabetes (NIDDM). The glycogen synthase gene (GS), which encodes the rate-limiting enzyme for glycogen synthesis, is a promising candidate gene for NIDDM. Therefore, we screened all 16 exons of this gene by single-strand conformation polymorphism analysis in 40 patients with NIDDM (age 67±2 years, body mass index 28.2±0.6 kg/m^2) from Taipalsaari, eastern Finland. The Gly464Ser mutation (exon 11) and a silent polymorphism TTC342TTT (exon 7) have been reported previously. In addition, we found a new mutation Gln71His (exon 2) and a new amino acid polymorphism Met416Val (exon 10). An additional sample of 65 patients with NIDDM and 82 normoglycemic men (age 54±1 years, body mass index 26.3±1.4 kg/m^2) were screened. The allele frequency of the TTC342TTT silent polymorphism was 0.29 in both NIDDM and normoglycemic subjects. The Gln71His mutation was found in 1 (1%) of the 105 NIDDM patients and in none of the 82 normoglycemic men. Correspondingly, the Gly464Ser mutation was present in 3 (3%) of the 105 NIDDM patients and in none of the 82 normoglycemic men. The Met416Val polymorphism was found in 16 (15%) of the 105 NIDDM patients and in 14 (17%) of the 82 control subjects (all heterozygous). None of the allele frequencies of the variants differed significantly between NIDDM and control groups. In addition, the Met416Val polymorphism was not associated with insulin resistance in two groups of normoglycemic subjects. In conclusion, the new Gln71His and Met416Val substitutions and other variants of the glycogen synthase gene are unlikely to make a major contribution to insulin resistance and NIDDM in diabetic patients from eastern Finland.

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Association of glucokinase gene with non-insulindependent diabetes mellitus in Chinese

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Abstract Using polymerase chain reaction, the microsatellite repeat polymorphisms in the 3'-flanking region (GCK1) of the human glucokinase gene from 60 non-diabetic, 61 NIDDM and 22 IGT subjects were typed. The differences in allelic frequencies among the three groups were compared. The Z+4 allele was found more frequently in NIDDM patients than in nondiabetic subjects (p<0.005). The frequency of Z+4 allele was 9% in IGT group, between NIDDM (16%) and control group (4%), but it didn't reach statistical significance. The presence of the Z+4 allele was estimated to increase the risk of NIDDM by 4.5 times. The frequency of the Z+2/Z+4 genotype was greater in the NIDDM group (p<0.001) and the frequency of the Z/Z+2 genotype less (p<0.005) compared with those in the nondiabetic group. The results indicate that GCK1 is associated with NIDDM in Chinese.

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MOLECULAR SCANNING OF p85α REGULATORY SUBUNIT OF PI3-KINASE IN HUMAN SYNDROMES OF SEVERE INSULIN RESISTANCE

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Insulin receptor mutations are the only known genetic cause of severe insulin resistance, but account for only 10% of subjects with Type A insulin resistance syndrome. Phosphatidyl inositol 3-kinase (PI3-kinase) activation appears necessary for insulin-stimulated glucose uptake. Using mRNA from skin fibroblasts or Epstein-Barr virus transformed lymphocytes from 21 subjects with features of the Type A syndrome, we have examined the gene encoding the regulatory subunit of PI3-kinase (p85α) by RT-PCR-SSCP. Four subjects were heterozygous for the common amino acid polymorphism Met 326 Ile. Two subjects had a common silent polymorphism at nucleotide 663. One subject with typical Type A insulin resistance was heterozygous for a novel amino acid change Arg 409 Gln. This was shared with her only sibling, who also had acanthosis nigricans. Within the family, median fasting plasma insulin in those with the mutation (n=4) was 218 pmol^{-1} and in those with the wild-type sequence (n = 2) was 69.5 pmol^{-1} (reference range <60 pmol^{-1}). The position of this mutation within the N-terminal SH2 domain provides further support for the notion that it may be contributing to insulin resistance in this family. This is the first scanning study of PI3-kinase in severe insulin resistance and the first description of a naturally-occurring mutation in a known functional domain.

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Identification of Glucokinase Mutations in Subjects with Post-Renal Transplantation Diabetes Mellitus

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Background: Mutations in the glucokinase(GK) gene are considered as possible cause of maturity-onset diabetes of young. The purpose of this study was to evaluate the contribution of this gene to the development of post-renal transplantation diabetes mellitus(PTDM) patients.

Method: Identification of GK mutation was attempted on 58 selected renal allograft recipients with PTDM and 45 normal controls. The exons in the glucokinase gene were examined by the polymerase chain reaction(PCR), and by analysis of the single-stranded DNA conformational polymorphism(SSCP). DNA sequences were also performed by direct sequencing analysis. The exons of affected family members were also investigated for the mutations of GK gene.

Results: Two of 58 PTDM patients(3.2%) were found to have mutations on exon 5, and one patient on intron 7 whereas one control subjects on intron 9. The mutation of exon 5 was identified substitution of CCT(proline) for CTT(leucine) at codon 164 which has not ever reported. The family members of PTDM with mutation of exon 5 were analyzed by PCR followed by SSCP, and two of the family had same mutation. The abnormal band on the SSCP analysis of exon 7 was identified the insertion of base C/T at 39th nucleotide in intron 7. Two of the family had same band on SSCP. The 45 normal controls were also analyzed by PCR-SSCP and had one mutation of G→C located 9th nucleotide in intron 9.

Conclusion: We found the glucokinase mutations in subjects with the PTDM and we speculate that glucokinase mutation may be one of the contributing cause of PTDM.

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COMPOUND HETEROZYGOSITY OF NOVEL INSULIN RECEPTOR MUTATIONS IN A PATIENT WITH LEPRECHAUNISM.

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A patient with leprechaunism, the most severe form of insulin resistance, was identified as a novel compound heterozygote of the insulin receptor gene, Arg1174Trp & Cys274Tyr, by PCR-SSCP and direct sequencing. In order to characterise these mutant receptors we have reconstructed the 1174 and 274 mutants in the expression vector pRc.CMV and performed transient transfections into CHO cells. The 1174 mutant is defective in tyrosine kinase activity, (p=0.0286, compared with wild-type receptor) as determined by *in vitro* tyrosine kinase assays and the absence of receptor autophosphorylation in anti-phosphotyrosine blots. The 274 mutant exhibits significantly decreased pro-receptor processing, insulin binding and cell surface expression compared to wild-type receptor (all $p<0.03$) as determined by Western blotting, *in vitro* binding studies and cell surface biotinylation respectively. These data indicate that it is the combination of the 2 receptor mutations that are responsible for the patient's most severe form of insulin resistance and represent the first case of such a combination being identified.

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NO RELATION BETWEEN INSULIN SECRETION AND β-CELL SPECIFIC GLUCOKINASE PROMOTER VARIATION IN GESTATIONAL DIABETES.

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There is a large body of evidence that many women with gestational diabetes mellitus(GDM) have abnormal pancreatic β-cell function. Very little is known about processes that underlie the defects in insulin secretion . Therefore we investigated whether the G/A variation at position -30 of the β-cell glucokinase gene promoter is related to insulin secretion in patients with GDM. Twenty six patients with GDM were selected according to NDDG recommendation. The variation of the β-cell glucokinase gene promoter was determined by single-strand confirmation polymorphism and direct sequencing. Pancreatic β-cell function was evaluated using the ratio of the incremental response in immunoreactive insulin(IRI) to that of glucose during the first 30 min of the 75 g oral glucose tolerance test(ΔIRI[30 min - 0 min/Δglucose[30 min - 0min]] performed at postpartum 8 week. Fourteen patients were G/G homozygote for this variant(56 %). G/A heterozygote was determined in 9 patients(36 %), A/A homozygote in 2 patients(8 %). Their BMIs before pregnancy did not differ between patients with G/G and patients with G/A or A/A(22.5± 2.3 *vs* 23.8± 2.9 $P = 0.283$). Fasting IRI(67.6 pmol/l *vs* 61.9 pmol/l as median, $P = 0.775$) and ΔIRI[30 min - 0 min/Δglucose[30 min - 0min](40.1x10^{-9} *vs* 62.8x10^{-9} as median, $P = 0.224$) did not differ between groups. These results suggest that the variation at -30 of the β-cell glucokinase gene promoter is not associated to reduced insulin secretion in Korean patients with GDM.

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ANTIBODIES TO ICA512 AND GAD65 IN PATIENTS WITH NON-INSULIN-DEPENDENT DIABETES (NIDDM) FROM EASTERN INDIA.

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Antibodies to tyrosine pyrophosphatase (ICA512) and glutamate decarboxylase 65 (GAD65) are markers for IDDM. The aim of our study was to determine the prevalence of ICA512 and GAD65 antibodies (Ab) in NIDDM patients (n=218) and 123 healthy controls from Cuttack in Eastern India.. Early onset (EO) NIDDM (onset <35 years) were 128 and late onset (LO) NIDDM (onset >35 years) were 90. ICA512Ab and GAD65Ab were evaluated by RIA using *in vitro* translated recombinant human 35S-ICA512 and 35S-GAD65 respectively. In controls, ICA512Ab were present in 3/123 (2%) and GAD65Ab in 8/123(7%). ICA512Ab were present in 85/218(39%) of all NIDDM patients ($p<0.001$) and 51/128 (40%) ($p<0.001$ vs controls) EO-NIDDM with a male preponderance and 34/90 (38%) ($p<0.001$ vs controls) LO-NIDDM with a female preponderance. GAD65Ab were present in 15/218 (7%) of all NIDDM (p=ns) and 12/128(9%) (p=ns vs controls) of EO-NIDDM and 3/90(3%) of LO-NIDDM (p=ns vs controls). When ICA512Ab and GAD65Ab positivity was analyzed in relation to age, 0/3 in <20; 30/41(73%) in 20-30; 25/97 (26%) in 31-40; 12/39(31%) in 41-50; 14/23(60%) in 51-60 and 4/15 (26%) in >60 years were ICA512Ab positive showing a distinct peak in 20-30 and 51-60 years. GAD65Ab were found in 0/3 in <20; 7/41(17%) in 20-30; 5/97 (5%) in 31-40; 2/39(5%) in 41-50; 0/23 in 51-60 and 1/15 (7%) in >60 years. Both ICA512Ab and GAD65Ab were negative in 66/128(52%) EO-NIDDM and 53/90(59%) LO-NIDDM; both Abs were positive in 3/128(2%) EO-NIDDM and 0/90 LO-NIDDM; either ICA512 Ab or GAD65Ab were positive in 60/128(47%) EO-NIDDM and 37/90 (41%) LO-NIDDM; ICA512Ab+/GAD65Ab- in 48/128(38%) in EO-NIDDM and 34/90(38%) in LO-NIDDM; and ICA512Ab-/GAD65Ab+ in 9/128(7%) EO-NIDDM and 3/90(3%) in LO-NIDDM. We conclude that patients from Cuttack in Eastern India, have GAD65Ab at a low and ICA512Ab at a significant high frequency in NIDDM compared to controls. ICA512Ab and GAD65Ab account for 47% of NIDDM patients indicating the presence of autoimmune process in the etiology of NIDDM.

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SCREENING OF SULPHONYLUREA RECEPTOR 1 (SUR1) FOR MUTATIONS IN DIFFERENT TYPES OF NIDDM

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Sulphonylurea receptor 1 (SUR1) is a subunit of the pancreas islet β-cell K-ATP channel that regulates glucose-induced insulin secretion. As mutations in a nuclear binding fold (NBF-2) cause neonatal hyperinsulinism, other mutations could impair insulin secretion. Two population association studies have shown that polymorphisms in SUR1, at exon 22 (T761T) and intron 24 splice acceptor site -3 tag GCC to -3cag GCC were significantly more prevalent in patients with NIDDM than normal control subjects, the combined polymorphisms having an odds ratio of 21 (95% CI 2.9 - 160). These polymorphisms thus could be in linkage disequilibrium with a pathogenic mutation in SUR1. We have screened SUR1 for mutations by SSCP in different types of white Caucasian patients with NIDDM who have neither ICA nor GADA: (i) 20 non-obese (BMI <27 $kg.m^{-2}$) who presented age <50 yrs with fpg <8 mmol/l (i.e. similar to glucokinase deficient, MODY2 patients) (ii) 20 non-obese subjects who presented with fpg >12 mmol/l who had marked β-cell deficiency and a family history of diabetes (iii) 20 NIDDM subjects with both the exon 22 and intron 24 mutations (iv) 20 randomly selected patients with NIDDM. No mutations were identified in the exons of any of these subjects. This indicates that mutations in SUR1 are unlikely to be a common cause of diabetes in NIDDM in white Caucasian subjects, but does not exclude mutations in the promoter region of SUR1 or in neighbouring genes on chromosome 11 p15.1.

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The phenotypes, genotypes and antibodies to glutamic acid decarboxylase (GAD) in young Chinese diabetic patients

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The prevalence of diabetes amongst Hong Kong Chinese aged less than 40 years old is 1.3%. The incidence of childhood IDDM is 1.8/100,000/year. In a clinic-based population, 16% of adult patients had onset of disease before 35 years old. In these young patients, 10% had IDDM compared to 2% in patients with disease onset after 35 years. The prevalence of obesity (36% vs 28%), positive family history (42% vs 17%, $p<0.01$) and insulin treatment (50% vs 20%, $p<0.01$) also tended to be higher in these young patients. We examined the phenotypes, auto-immune status and genotypes in a cohort of 140 Chinese diabetic patients with disease onset before 35 years and aged less than 40 years. IDDM was defined as presentation with diabetic ketoacidosis or ketonuria (>3+) or continuous insulin treatment within one year of diagnosis. Insulin deficiency was defined as a post-glucagon plasma C peptide concentration <0.6 nmol/l. The prevalence of insulin deficiency was 50% but anti-GAD (>18 units) was 12% only. The prevalence of anti-GAD was 23% in IDDM and 7.2% in NIDDM [sensitivity: 23% (10/43); specificity: 93% (9097)]; 18.5% in insulin deficient and 6.7% in non-insulin deficient patients [sensitivity: 18.5% (12/65); specificity: 93% (70/75)]. The prevalence of anti-GAD was 29% in patients who had both insulin deficiency and IDDM presentation compared to 6% in those who were non insulin deficient and had NIDDM. The anti-GAD negative patients had higher BMI (26 vs 23 kg/m^2, $p=0.05$), systolic BP (114 vs 107 mmHg, $p=0.03$), triglyceride (1.26 vs 0.7 mmol/l, $p<0.01$), and lower HDL-C (1.23 vs 1.48 mmol/l, $p<0.01$), HbA_{1c} (7.7% vs 9.3%, $p=0.04$) and prevalence of insulin deficiency (43% vs 71%, $p<0.05$). Over 50% of these patients had evidence of microangiopathic complications. In the anti-GAD negative group, 4 patients had mitochondrial DNA mutation. None of the patients had glucagon receptor gene mutation. These findings confirm the rarity of classical IDDM and heterogeneity of both phenotypes and genotypes in young Chinese diabetic patients.

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IDENTIFICATION AND FUNCTIONAL ANALYSIS OF VARIANT SUR1 IN JAPANESE WITH NON-INSULIN-DEPENDENT DIABETES MELLITUS

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Mutations of the sulfonylurea receptor 1 (SUR1) gene were examined by means of single strand conformation polymorphism analysis in 100 Japanese subjects with non-insulin-dependent diabetes mellitus (NIDDM). After examining all 39 exons of this gene, we identified 5 amino acid substitutions and 19 silent mutations. A Ser to Ala substitution, located in the second nucleotide binding fold (NBF-2), was common (allelic frequency 0.41) and was also found at an equal frequency in non-diabetic control subjects. Four other missense mutations were rare: an Arg to Gln substitution in exon 6 (6RQ), a Val to Met substitution in exon 12 (12VM), an Asp to Asn substitution in exon 20 (20EN) and an Arg to Cys substitution in exon 21 (21RC) were identified in one patient each, all in the heterozygous state. The 6RQ and 20EN were not found in 67 control subjects. The 21RC and 12VM were present in one and three control subjects, respectively. The 6RQ, 12VM and 21RC did not appear to cosegregate with NIDDM in the probands' families. To analyze possible functional alterations, we introduced 4 rare mutations at the corresponding positions of mouse SUR1 cDNA. We then co-expressed the SUR1 mutants with mouse BIR, a channel subunit of the ß-cell ATP sensitive K channel (I_{KATP}), in HEK293T and COS-7 cells, and examined the channel activities by the patch clamp technique and $^{86}Rb^+$ efflux measurements. The 20EN and 21RC, both located in the NBF-1, did not alter Ki for the ATP of I_{KATP} regardless of the presence or absence of Mg^{2+}. Inhibition of $^{86}Rb^+$ efflux by glibenclamide and activation by metabolic inhibition or diazoxide did not differ between the cells expressing the wild type SUR1 and either of the two mutants. Functional studies of the other two mutations are currently underway. In conclusion, SUR1 mutations impairing I_{KATP} function are rare in Japanese NIDDM patients, and do not appear to be major determinants of the susceptibility to NIDDM in the population.

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GENETIC INTERACTION BETWEEN IDDM AND NIDDM

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An overrepresentation of IDDM in the offspring of NIDDM patients supports a genetic interaction between IDDM and NIDDM. To investigate whether sharing an HLA haplotype with an IDDM proband influences insulin secretion in other family members, we studied 26 mixed IDDM/NIDDM Finnish families. HLA DRB1-DQA1-DQB1 haplotypes were typed by PCR and dot-blot hybridisation using oligonucleotide probes. The 173 GAD-antibody negative 2nd or more distant degree relatives (105 NGT, 24 IGT, 44 NIDDM) of 23 unrelated IDDM probands were assigned into 2 groups according to whether they shared one or zero HLA-haplotype with the IDDM proband. The shared haplotype was 0401/4-0301-0302 or 17-0501-0201 in 75% of the families. 80% of the relatives sharing an HLA haplotype with the IDDM proband had an IDDM susceptibility haplotype. Relatives with NIDDM sharing IDDM HLA haplotypes had a lower insulin-response to OGTT (Incremental area 3489.4±1615.2 vs. 8287.6±6919.5 mU/L, $p=0.034$), and a higher fasting glucose level (8.9±2.9 vs. 7.5±3.2 mmol/l, $p=0.048$) compared with those not sharing. In response to OGTT, nondiabetic relatives sharing IDDM HLA haplotypes had higher glucose values than those not sharing (glucose area 243.7±141.5 vs. 180.6±134.7, $p=0.044$). To examine whether the reduced insulin response was due to the presence of the IDDM susceptibility haplotype in general or whether it had to be shared with the IDDM proband, we also studied 101 unrelated NIDDM patients (66±11 yr, 44M/57F) and 205 nondiabetic subjects (104 NGT/101 IGT, 54±15 yr, 93M/112F) without family history of IDDM. The insulin and glucose responses did not differ between subjects with or without IDDM susceptibility HLA haplotypes.

Conclusions: Sharing an HLA haplotype with an IDDM proband is correlated with impaired insulin secretion and elevated glucose response in NIDDM patients and nondiabetic subjects. The findings indicate the presence of a genetic interaction between IDDM and NIDDM, which cannot be explained solely by the presence of IDDM susceptibility HLA haplotypes in the NIDDM population.

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ASSOCIATION OF SULFONYLUREA RECEPTOR (SUR) GENE VARIANTS WITH JAPANESE NIDDM.
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Sulfonylurea receptor(SUR) has been suggested to play a crucial role in the regulation of insulin secretion. SUR gene abnormalities may result in impaired insulin secretion,leading to glucose intolerance. A recent observation indicates that SUR gene variants are associated with NIDDM in Caucasian populations. We investigated whether three variants of the SUR gene are associated with Japanese patients with glucose intolerance by using PCR-RFLP method. We screened 599 Japanese subjects (NIDDM, n=383,non- diabetic controls,n=216). The c/c mutant genotype in intron 24 splice acceptor site was significantly increased in NIDDM patients compared with that in non-diabetic control subjects (chi square=6.01, p=0.014). A silent variant (Thr761Thr) in exon 22 was not detected in 373 NIDDM patients but was present in only one control subjects, unlike the association of it with Caucasian NIDDM patients. An association of a SUR gene variant with Japanese NIDDM patients,as well as an association of another SUR gene variant with Caucasian counter- part, suggest an important role of the SUR gene in the pathogenesis of NIDDM in Japanese as well as in Caucasian subjects.

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INSERTION / DELETION POLYMORPHISM IN ANGIOTENSIN CONVERTING ENZYME GENE IN HEALTHY THAIS AND PATIENTS WITH NIDDM
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Insertion/deletion (I/D) polymorphism in the angiotensin converting enzyme (ACE) gene has been shown to associate with various cardiovascular disorders in diabetic and non-diabetic patients. Its association to the development of NIDDM has been raised. This study was aimed to examine prevalence of I/D ACE genotypes in healthy Thai subjects and patients with NIDDM. The I/D ACE genotypes were determined by polymerase chain reaction technique. Healthy unrelated subjects were hospital staffs and blood bank donors who had no known medical illness at the time of study. They were 151 males and 147 females, aged 17-70 years old (mean±SD = 37.5±10.4). The unrelated diabetic patients were 42 males and 66 females, aged 20-79 years old. (mean±SD = 54.7±12.0). The results were shown in the Table below. There were no differences in the prevalence of I/D ACE genotypes between healthy males and females (p = 0.86), diabetic males and females (p = 0.98), as well as healthy and diabetic subjects (p = 0.81). The frequency of I and D alleles in diabetic patients were 0.69 and 0.31, respectively which were similar to those of healthy subjects, 0.70 and 0.30, respectively (p = 0.69). The frequency of I and D alleles in healthy Thai subjects were similar to those of Japanese (0.66 and 0.34) but different from those of Caucasians (0.44-0.46 and 0.56-0.54). In conclusion, the frequency of I and D alleles may possess a racial difference. The similar frequency of both alleles in diabetic patients and healthy subjects suggests that there is no association between I/D polymorphism of ACE gene and diabetes mellitus in Thai individuals.

Genotypes	Healthy Subjects (%)			Diabetic patients (%)		
	males	females	all	males	females	all
DD	9.3	10.9	10.1	9.5	10.6	10.2
ID	40.4	38.1	39.2	42.9	42.4	42.6
II	50.3	51.0	50.7	47.6	47.0	47.2

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A NOVEL MUTATION OF AMYLIN GENE (SER20GLY) IDENTIFIED IN JAPANESE NON-INSULIN-DEPENDENT DIABETES MELLITUS.

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Non-insulin-dependent diabetes mellitus (NIDDM) is a multifactorial disease, and the amyloid deposit in pancreatic islets is one of the characteristic finding in NIDDM patients. Amylin, a 37 amino acid peptide which is co-secreted with insulin from islet β cells, is a major constituent of islet amyloid in NIDDM patients. Although its true action(s) is still unclear, recent studies suggest that amylin may have the possible pathogenesis of NIDDM. We therefore scanned the amylin gene for mutations in 294 unrelated Japanese NIDDM patients using PCR/SSCP analysis, and found a single heterozygous missense mutation at the position 20 of amylin molecule (AGC^{Ser} to GGC^{Gly}: Ser20Gly mutation) in 12 NIDDM patients. None of 187 non-diabetic subjects (over 60 years old and negative family history of diabetes) and 59 patients with insulin-dependent diabetes mellitus had the mutation. Eight out of the 12 patients carrying the mutation were diagnosed as having NIDDM before 35 years old, and they had treated by insulin and each of them had a parent having late-onset NIDDM. On the other hand, the remaining 4 patients were diagnosed as having NIDDM after 51 years old. They had mild diabetes without family history of diabetes. Family studies disclosed the mutation was transferred from the parent having impaired glucose tolerance (IGT), not from the parent having NIDDM. The frequency of the mutation among whole NIDDM was 4.1 % and it reached to 10.0 % (8/80) in relatively early onset (≦35 years) NIDDM patients. Amylin immunoreactivities extracted from affected patient's plasma were appeared at the position corresponding not only to the normal amylin (16.0 %) but also to the synthesized Gly20-amylin (84.0 %) in HPLC analysis. These data suggest that Ser20Gly mutation can cause mild diabetes on its own; however, when it is combined with unknown susceptibility genes for late onset NIDDM, it contributes to the earlier onset of the NIDDM and makes it more severe. We present the first report referring to a missense mutation of amylin gene in Japanese NIDDM patients. This Ser20Gly mutation may play a important role in the pathogenesis of early onset NIDDM in Japanese population, and may provide a good model to investigate and interpret the true physiological action(s) of amylin.

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I/D POLYMORPHISM OF THE ACE GENE IN PATIENTS WITH HYPERTENSION, NIDDM AND CORONARY HEART DISEASE.

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An insertion/deletion (I/D) polymorphism of the angiotensin converting enzyme (ACE) gene has been identified which determined most of the plasma ACE activity genetically. Association of the D-allele with insulin sensitivity, and D/D genotype with coronary heart disease (CHD) have been reported in various ethnic populations. To study the role of this genetic polymorphism in patients with hypertension, non-insulin-dependent diabetes mellitus (NIDDM), and NIDDM with CHD in Taiwanese population, we employed a polymerase-chain-reaction-based genotyping technique with an insertion-specific primer for confirmation of the I-allele. One hundred and ninety-seven unrelated normal controls, 67 subjects with hypertension, 107 subjects with NIDDM, and 70 NIDDM with CHD were recruited for this study. All of them were Han Chinese. Subjects without a history of diabetes were studied by a standard 75 g oral glucose tolerance test. Hypertension was diagnosed according to the 5th JNC criteria and CHD was confirmed by the history of acute myocardial infarction and coronary angiographic intervention. The frequency of I-allele of the ACE gene in the normal population was 64.2% which was higher than those reported in the Caucasian populations. The prevalence of the I-allele of the ACE gene was not significantly increased in subjects with hypertension (73.1%), NIDDM (62.1%), and NIDDM with CHD (65%) as compared to that of healthy controls. I-allele of the ACE gene did not correlate with demographic and metabolic variables. The I/D polymorphism of the ACE gene is not a marker for hypertension, NIDDM or CHD in Taiwanese population.

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ABSENCE OF THE S20G MUTATION OF THE AMYLIN GENE IN PATIENTS WITH GESTATIONAL DIABETES MELLITUS (GDM).

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A missense mutation in the amylin/IAPP gene has recently been reported in 4.1% of Japanese patients with NIDDM (Ser to Gly at position 20). When this mutation is not combined with other hereditary factors, it is associated with mild glucose intolerance. The aim of the present study was to investigate whether the S20G mutation of the amylin gene could be one of the factors responsible for the development of GDM, which represents a mild form of glucose intolerance, unmasked because of the pregnant state. So far, we have studied 115 patients with GDM (age range 20-46 yrs), who were diagnosed according to the NDDG criteria. All patients were reevaluated 2-12 months after delivery by a standard 75g OGTT; none of them had overt diabetes. 62% had a positive family history for NIDDM (either paternal or maternal side or both). In parallel, we have studied 15 patients with NIDDM with early (<35yrs) onset of the disease, a subgroup in which the original paper from Japan reported a higher (10%) frequency of the mutation. DNA was extracted from peripheral lymphocytes using standard methods. The third exon of the IAPP gene was amplified by PCR and tested for the presence of the S20G mutation by RFLP using the enzyme Msp1, which recognises the mutated sequence. In the sample we have studied so far, no S20G mutation of the amylin gene has been observed in neither the GDM nor the NIDDM group. One explanation is that this mutation may not be present in the Greek population at all, although the NIDDM group is still very small. From the results in the GDM group one may conclude that this mutation is not associated with a temporary disturbance of glucose metabolism. It is therefore possible that the S20G mutation, if present in caucasians at all, should be looked for in patients with glucose intolerance persisting outside pregnancy.

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THREE CANDIDATE GENES FOR DIABETIC ANGIOPATHY IN GENERAL MOSCOW POPULATION AND NIDDM PATIENTS

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Vascular tone abnormalities, polyol pathway activation, and oxidative stress are known to involve in diabetic angiopathy (DA) development. We have begun to study genetic susceptibility/resistance to DA using a set of polymorphic markers attributed to such candidate genes as angiotensin I-converting enzyme (ACE), aldose reductase (ALR2) and catalase (CAT). Polymerase chain reaction (PCR) was used to detect insertion/deletion (I/D) polymorphism of ACE gene and polymorphic microsatellite at 5'-region of ALR2 gene (7 alleles; 132-144 bp). To identify a polymorphic marker nearby CAT gene we used STS (sequence-tagged site) developed for CAT gene. This STS was used for identification of individual YAC clones in a YAC library developed in CEPH (Paris) in collaboration with Dr. I.M.Chumakov. It was shown that YAC clone AFM109ya1 contained both CAT gene STS and D11S907 polymorphic microsatellite (7 alleles; 161-173 bp). The first aim of this work was to study distribution of alleles and genotypes of these markers in control donors from general population and in a group of subjects with NIDDM. The latter composes vast majority of diabetic patients highly predisposed to DA. Distribution of ID/ACE alleles and genotypes in general population (n=168) and NIDDM patients (n=48) was very similar. We observed significant difference in allele distribution for ALR2 and CAT genes between general population and NIDDM patients: ALR2 allele Z+4 frequency was 0.246 vs. 0.152, respectively ($p < 0.05$); and CAT 169 bp allele frequency was 0.321 in general population vs. 0.118 in NIDDM patients ($p < 0.0002$). Differences in distribution of another alleles and genotypes for ALR2 and CAT loci did not reach significance level. In conclusion, the observed differences allow us to suggest that either ALR2 and CAT genes or some another genes located in the same chromosome regions might be implicated in NIDDM and/or its complication development.

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ANALYSIS OF THE RELATIONSHIP BETWEEN A COMMON CYS282TYR VARIANT OF THE HEREDITARY HAEMOCHROMATOSIS GENE AND NIDDM

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Hereditary haemochromatosis (HH) is an autosomally inherited recessive disorder causing excessive iron uptake and deposition in various organs including liver and pancreas, ultimately leading to organ failure in untreated cases. Recently, the HH causing gene, HLA-H was cloned, and a Cys282Tyr variant was shown to be responsible for most cases of HH in a US population. Since HH is often associated with decreased glucose tolerance or overt diabetes most likely due to the iron deposition in the pancreas we examined the hypotheses that the Cys282Tyr variant of the HLA-H gene was associated 1) with an increased prevalence of NIDDM, 2) impaired insulin secretion or 3) impaired insulin sensitivity.

We screened for the codon 282 variant by RFLP using *RsaI* restriction enzyme in 2 Danish cohorts: I) In a cohort of 246 NIDDM patients (mean age 55 years) and in 242 age matched glucose tolerant control subjects; the allele frequency of the 282 variant was 3.1 % and 4.8 % respectively ($p > 0.1$). Measurements of fasting insulin, and results of OGTT from all 242 control subjects showed no correlation between specific phenotypes and the Cys282Tyr variant and II) In a cohort of 377 young healthy Caucasians, the allele frequency of the 282 variant was 5.2 %. All participants in this study were tested with an intravenous glucose tolerance test with addition of tolbutamide (Minimal model). Analyses of insulin secretion, insulin sensitivity and glucose effectiveness with or without gender stratification failed to show any significant relationship between the presence of the Cys282Tyr variant and the measured variables. The distribution of genotypes in both cohorts were in Hardy-Weinberg equilibrium and only one homozygous carrier was identified in each cohort.

In conclusion: 1) The codon 282 variant of the HLA-H gene is common in the Danish population with an allele frequency of about 5 %. 2) However, this common variant is not associated with an increased prevalence of NIDDM or alteration in insulin sensitivity or insulin secretion.

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RELATIONSHIP BETWEEN ACE GENE POLYMORPHISM AND OBESITY IN JAPANESE NIDDM PATIENTS

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Since Cambien reported angiotensin-I converting enzyme (ACE) gene polymorphism was associated with ischemic heart disease (1992), many investigations were carried out about ACE gene D/I polymorphism related to diabetic nephropathy. However, there is no confirmative agreement. We examined age, sex, BMI, blood pressure, plasma glucose, lipid profiles (TC, TG, HDL-C, LDL-C. FFA), UA, duration of diabetes, nephropathy (plasma creatinine, and proteinuria) ,and retinopathy in 104 NIDDM patients (aged 57.4± 10.8, BMI 24.2± 4.5, HbA1c 7.5± 1.7% (SD)). D/I polymorphism was analysed by the PCR method. There was increase in BMI in DD type (II 24.1±3.9, ID 23.6±3.4, DD 26.6±6.9, P=0.049) by ANOVA. Plasma leptin concentration (n=37) was also significantly higher in DD type (II 4.4±2.0, ID 6.1±5.4, DD 9.1±6.4 ng/ml). There was no correlation between ACE gene polymorphism and blood pressure, glucose, lipid profiles, UA, duration of diabetes, nephropathy or retinopathy. In conclusion, the data suggest that ACE gene polymorphism is associated with body mass index and plasma leptin concentration in Japanese NIDDM patients. We found no correlation with blood pressure, diabetic control or severity of complications in this study.

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AMINO ACID VARIANTS IN HUMAN GIP RECEPTOR AND THEIR IMPACT ON THE PANCREATIC B-CELL RESPONSE

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The two incretins, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), are insulinotropic factors released from the small intestines to the blood stream in response to oral glucose ingestion. The insulinotropic effect of GLP-1 is maintained in patients with NIDDM, whereas, for unknown reasons, the effect of GIP is diminished or lacking in NIDDM patients. We performed a mutational analysis of the GIP receptor to test the hypothesis that genetic variability of the GIP receptor: 1) affects the pancreatic β-cell responses in the fasting state or after oral glucose and 2) is associated with an increased prevalence of NIDDM. The primary screening of 61 random Caucasian NIDDM patients revealed two amino acid polymorphisms, A207V and E354Q, in the GIP receptor gene. In an association study of 227 Caucasian NIDDM patients and 224 matched glucose tolerant control subjects, the allelic frequency of the A207V polymorphism was 1.1% in NIDDM patients and 0.7% in control subjects ($p>0.2$), whereas the allelic frequency of the codon 354 polymorphism was 24.9% in NIDDM patients versus 23.2% in control subjects. The phenotype of the glucose tolerant heterozygous carriers of the E354Q polymorphism was indistinguishable from that of the wildtype carriers. Interestingly, however, compared to subjects with the wildtype GIP receptor the 6% of the glucose tolerant control subjects who were homozygous for the codon 354 variant had a 14% decrease in fasting serum C-peptide level ($p=0.014$) and an 11% decrease in the same variable 30 min after an oral glucose load ($p=0.034$). The 3 control subjects carrying the heterozygous A207V polymorphism also tended to have lower serum C-peptide responses during an OGTT. In conclusion: the reduced fasting serum C-peptide level in homozygous carriers of the common codon 354 amino acid polymorphism in the GIP receptor may suggest, that GIP even in the fasting state exerts a regulatory impact on the β-cell secretory response.

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POLYMORPHISM OF THE ALDOSE REDUCTASE GENE IS ASSOCIATED WITH ALDOSE REDUCTASE PROTEIN LEVELS AND DIABETIC MICROANGIOPATHY.

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AIM: Glycation affects the development of diabetic complications such as retinopathy, nephropathy and neuropathy. Aldose reductase (AR) is a key enzyme which plays an essential role in the polyol pathway. We have measured erythrocyte aldose reductase (AR) protein levels and genotyped AR gene polymorphism in 140 NIDDM patients without overt renal dysfunction or a history of taking AR inhibitors. METHODS: Erythrocyte AR protein levels were measured by highly sensitive two-site ELISA using recombinant human AR. Among the NIDDM patients, 53 were without retinopathy, 51 had simple retinopathy, and 36 had preproliferative or proliferative retinopathy (PDR). Microsatellite polymorphisms in the 5'-flanking region of the AR gene were typed using the polymerase chain reaction. RESULT: The average AR protein level in these patients was 9.7±3.2 (mean±SD) ng/mgHb, which is nearly equal to the reported levels of the normal controls. Tandem C-A repeats of the AR gene were highly polymorphilic, 9 alleles were observed in the NIDDM group and 10 alleles were seen in 74 normal control subjects. Patients who had alleles 5 and 10 (the latter one is not observed in the Chinese population) showed higher AR levels. The relationship between AR levels and rate of retinopathy were estimated using the Cox's proportional hazard model. Proliferative retinopathy were significantly associated with AR levels. The frequency of PDR was 4.85 fold higher in patients whose AR levels were over 12.0 ng/mgHb compared to whose levels were lower than 7.4ng/mgHb. This result suggested that high levels of AR may play a role in the development of proliferative retinopathy. Patients with allele 5 of 136 bp in size were the most common in the group with PDR, which corresponded to the size previously reported to be associated with retinopathy in a Chinese population. Allele 10 was found to be significantly associated with PDR in patients with NIDDM ($p<0.03$). CONCLUSION: These observations show that AR protein levels are regulated by genetic factors and are also associated with the development of retinopathy (and nephropathy) in NIDDM.

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Is abnormal expression of mitochondrial genes in skeletal muscle genetically determined ?

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Decreased VO_{2max} associated with insulin resistance in muscle of NIDDM patients suggests the existence of defects in mitochondrial respiration. We have isolated four mitochondrial genes (NADH dehydrogenase subunit 1, cytochrome c oxidase subunit 1, $tRNA^{leu}$, and D-Loop region) with abnormal expression in muscle by cDNA differential display. It is, however, not known whether these defects are acquired or inherited. To address this question, we examined the expression of these genes in muscle biopsies from 15 NIDDM patients (age: 65 ± 1 yrs, BMI: 28.2 ± 0.9 kg/m^2 , FBG: 10.8 ± 1.0 mmol/l), 15 IGT patients (age: 66 ± 1 yrs , BMI : 27.1 ± 1.0 kg/m^2 , FBG: 5.4 ± 0.2 mmol/l), and 14 control subjects (age: 65 ± 1 yrs , BMI: 27.6 ± 0.8 kg/m^2 , FBG: 4.7 ± 0.1 mmol/l) and 12 monozygotic twin pairs discordant for NIDDM but concordant for insulin resistance. Muscle biopsies were taken at the end of an euglycaemic insulin clamp combined with indirect calorimetry. As expected glucose disposal (control 6.29 ± 0.64; IGT 4.35 ± 0.45; NIDDM 2.82 ± 0.42 mg/kgmin; $p < 0.01$), glucose oxidation (control 2.12 ± 0.15; IGT 1.95 ± 0.14; NIDDM 1.47 ± 0.17 mg/kgmin; $p = 0.01$) and VO_{2max} (control 31.00 ± 2.06; IGT 28.54 ± 1.44; NIDDM 23.55 ± 1.89 ml/kg; $p = 0.02$) were decreased in NIDDM and IGT vs controls. NADH dehydrogenase subunit 1 (NADH DH 1) mRNA expressed relative to cytoplasmic β-actin was decreased by 54 % in NIDDM and by 14 % in IGT vs controls ($p < 0.05$), while the expression of the other genes was not different between the groups. NADH DH 1 mRNA content correlated positively with VO_{2max} ($r = 0.44$, $p < 0.01$). However, there was no difference in expression of NADH DH 1 between the twins. In conclusion, decreased expression of genes encoding for mitochondrial respiratory chain enzymes may contribute to decreased aerobic capacity in NIDDM. This is an acquired rather than an inherited defect, possibly an adaption to the reduced amount of glucose taken up by the cell.

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THIS ABSTRACT HAS BEEN WITHDRAWN BY THE AUTHOR.

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MUTATION OF THE CALCIUM-BINDING DOMAIN OF THE MITOCHONDRIAL GLYCEROPHOSPHATE DEHYDROGENASE GENE IN A FAMILY OF DIABETIC SUBJECTS

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The Ca^{2+}-sensitive and mitochondrial enzyme FAD-linked glycerophosphate dehydrogenase (mGDH) represents an essential component of the pancreatic B-cell glucose-sensing device. This report deals with the first identified case of mutation in the calcium-binding domain of the mGDH gene in a family of diabetic subjects. The diabetic proband was a normal weighed 62 year old male recently found to display an abnormally low activity of mGDH in CD3+ T lymphocyte homogenates. He was examined together with his 60 year old non-diabetic wife, a 65 year old non-diabetic sister, a 30 year old non-diabetic daughter, his 68 year old diabetic brother and a 57 year old glucose-intolerant half sister (same mother). Single-strand conformation polymorphism analysis of the cDNA sequence of interest, namely that coding for the Ca^{2+}-binding domain of mGDH, revealed an abnormal mobility pattern of the ^{32}P-labelled PCR product in the diabetic proband and his glucose-intolerant half sister, such being not the case for the four other members of the same family. In the two affected subjects, base pair mutations were observed at positions 2018, 2069 and 2136, corresponding to $Phe^{635}Ser$ and $Gln^{649}Pro$ amino acid changes. In CD3+ T lymphocyte homogenates incubated in the presence of 0.1 mM L-[2-^{3}H]glycerol-3-phosphate and 0.05 mM FAD, the relative extent of enzymatic activation by Ca^{2+} (10^{-7} to 10^{-4} M) was lower in the diabetic proband than in his diabetic brother with a normal mGDH gene sequence. This anomaly in the intrinsic properties of the enzyme could conceivably represent a contributing factor in the perturbation of islet B-cell responsiveness to D-glucose.

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ABNORMAL VITAMIN D BINDING PROTEIN FRAGMENT IN BLOOD OF FIRST DEGREE RELATIVES OF PATIENTS WITH TYPE 2 DIABETES MELLITUS

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We examined the whole blood protein pattern to identify the existence of specific marker proteins which may relate to genetic or metabolic abnormalities in type 2 diabetic individuals of Mexican American descent. Whole blood was obtained from 29 first degree relatives (including individuals with normal GT, IGT, and overt NIDDM) of patients with type 2 diabetes mellitus (age: 42±15 y; BMI: 30±6 kg/m^2) and from 21 control subjects (age: 29±10 y; BMI: 25±10 kg/m^2) without family history of diabetes mellitus. Blood samples were drawn into a tube containing protease inhibitors and were analyzed first by isoelectric point focusing, followed by molecular weight using high resolutional 2D-gel electrophoresis. Several spots were found to exist more frequently in relatives of NIDDM patients. One of these spots (MW = 20 kDa, pI = 4.9), which was found in 80% of relatives and 32% of controls ($p<0.05$ by Chi-square test), was targeted for further analysis by electrophoretic blotting onto 20 PVDF membranes. The amino acid sequence of the target spot was determined and identified to have a sequence similar to that of vitamin D binding protein. Since the mol wt of this spot was smaller than that of vitamin D binding protein, this spot was considered to represent a fragment of vitamin D binding protein. This finding is consistent with a recent genetic analysis in Pima Indians and suggests that increased production of the vitamin D binding protein may play a role in the pathogenesis of NIDDM in certain ethnic populations.

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PREVALENCE OF BLOOD AND MUSCLE MT DNA ANOMALY IN A HIGHLY SELECTED DIABETIC POPULATION AT RISK OF MITOCHONDRIAL CYTOPATHY

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Mitochondrial diabetes (MIDD syndrome) has been recently described. The aim of the study is to evaluate the prevalence of anomaly of mt DNA in a highly selected population of 13 diabetic patients presenting at least 2 parameters of MIDD syndrome : maternal inheritance (observed in 10), neurosensorial deafness (in 12), macular pattern dystrophy (in 11) and clinical myoencephalopathy (in 4). In blood cells deletions, 3243 and 14709 mutations of mt DNA were searched in every patient. The 3243 mutation was found in 3, the 14709 in 1, deletion in none. In 12 subjects a deltoïd muscle biopsy was performed and in 8 cases anomalies suggesting a mitochondrial cytopathy were observed. The measurement of activity of the complexes of the respiratory chain was abnormal in 5. The molecular analysis in muscle sample confirmed the presence of the 4 mutations and showed the presence of 2 additional deletions (by large fragment PCR). In 9 patients a 31-P MR spectroscopy was performed, showing oxidative defect in 6 (4 mutations and 1 deletion). In conclusion, diagnosis of mitochondrial diabetes is difficult as long as all the diabetes-prone anomalies are not extensively identified . Study of muscle by invasive (biopsy) and non invasive (31- PMR spectroscopy) can be useful for this diagnosis.

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FAMILIAL ASSOCIATION AND λ_s FOR DIABETES, OBESITY, HIGH TRIGLYCERIDES & LOW HDL-CHOLESTEROL IN SIBS OF NIDDM PATIENTS

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The Diabetes in Families Study (DIF), a population-based collection of sibships of NIDDM patients ('siblings') together with a age and sex-matched randomly selected sample from the same general population ('controls') provides a resource for the study of the heritability and genetics of Type 2 diabetes. To date, 111 sibships and 78 controls have been recruited. The frequency of diabetes (measured fasting plasma glucose >7.8 mmol.l^{-1} plus known diabetes) was 21.4% and 6.4% in the siblings and controls, respectively: relative risk (λ_s) = 3.3 ($p=0.001$). The siblings were more obese than the controls: BMI 28.2 (SD 4.9) and 25.4 (4.0) kg.m^{-2}, respectively ($p=0.0002$). 37% of probands were obese (BMI > 30 kg.m^{-2}). The frequency of obesity amongst siblings of obese type 2 diabetic probands and among controls was 46.8% and 7.3%, respectively: relative risk (λ_s) = 6.4% ($p<0.00001$). The siblings had higher triglycerides than the controls: 1.59 (1SD range: 1.39-1.82) and 1.15 (1.04-1.27) mmol/l ($p<0.0001$). 25% of probands had plasma triglycerides greater than 2.15 ('HighTrig': 90th centile of controls). The frequency of HighTrig amongst siblings of diabetic probands with HighTrig was 40%: relative risk (λ_s) = 4.0 vs controls ($p<0.00001$). The siblings had lower HDL-C than the controls: 1.19 (1SD range: 1.15-1.24) and 1.39 (1.32-1.46) respectively ($p=0.0002$). 25 % of probands had HDL-C less than 0.94 mmol/l ('LowHDL': 10th centile of controls). The frequency of LowHDL amongst siblings of diabetic probands with LowHDL was 35%: relative risk (λ_s) = 3.5 vs controls ($p<0.00006$). BMI, HDL-C (corrected for sex and BMI), triglycerides (corrected for sex, glucose and HDL) showed significant familial association by ANOVA, with 48% ($p<0.0001$), 59% ($P<0.0001$) and 47% ($p<0.0003$) of the variance accounted for by family membership. Conclusion: In addition to the higher risk of diabetes, siblings of NIDDM patients have a higher risk of obesity, high triglycerides and low HDL-C, with a significant family association of these factors.

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A central European population can be characterized by certain mtDNA haplogroups: one is associated with syndromic diabetes (DIDMOAD)

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Wolfram (or DIDMOAD) syndrome is a neurodegenerative disorder characterized by juvenile-onset diabetes mellitus and optic atrophy as the essential features. The phenotypic characteristics of the syndrome and the systemic involvement suggest an underlying mitochondrial dysfunction. Although Wolfram syndrome has recently been linked to chromosome 4p, there is evidence for heterogeneity (1). On the other side, sporadic cases with DIDMOAD have been ascribed to a distinct defect of the mitochondrial (mt) DNA. In a systematic approach, we screened the mtDNA of eight DIDMOAD patients for known and unknown mutations in all ND, tRNA and cyt b genes by RFLP and SSCP/direct sequencing, respectively. We could not detect any causative mutation, however, we found the DIDMOAD patients being frequently associated with a distinct mtDNA haplotype consisting of the transition mutations at nucleotide positions (nps) 4216, 11251, 4917, 10463, 13368, 14233, 14905, 15607, and 15928 (5/8 patients). As a reference we used the haplotype distribution in a healthy German control group (n=67). The bulk of the controls could be described by a few defined mtDNA haplogroups. The „DIDMOAD-associated haplogroup", presumptively consistent with the European-specific haplogroup T in the nomenclature of Torroni et al. (2), represents 9% of our healthy German controls, but 63% of our DIDMOAD patients (Fisher's exact test: p=0.009). Besides Leber hereditary optic neuropathy (LHON), which has been shown to be frequently associated with a distinct European haplogroup (nps 4216+13708) (3), this is the first report linking another neurodegenerative disorder with a specific mtDNA haplogroup. Since certain haplotype backgrounds might act as susceptibility factors in hereditary disorders, we initiated a respective analysis in a group of selected type II diabetic patients (with a maternal background, n=50). This approach should reveal whether distinct mtDNA haplotypes contribute to the reported predominance of maternal transmission in type II diabetes. The results will be discussed.

(1) Collier et al. (1996) Am. J. Hum. Genet. 59: 855-863; (2) Torroni et al. (1996) Genetics 144, in press; (3) Brown et al. (1995) Hum. Mut. 6: 311-325

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INSULIN SECRETION AND INSULIN SENSITIVITY IN MONOZYGOTIC AND DIZYGOTIC TWIN PAIRS

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There is plenty of evidence that NIDDM is an inherited disease. Both impaired insulin secretion and impaired insulin action are considered to contribute to the development of the disease, but there are conflicting data on which defect is under stronger genetic control. To address this question, we quantitated insulin secretion and insulin sensitivity in 22 monozygotic (MZ) (7 female, 15 male, age 55-73 yrs, BMI 26.6±0.56 kgm^2) and 21 dizygotic (DZ) (8 female, 13 male, age 61-68 yrs, BMI 26.8±0.53 kgm^2) non-diabetic twin pairs of same gender with an intravenous glucose tolerance test (IVGTT, serum insulin response 0-60 min after an i.v. bolus of 0.5g/kg of 50% glucose) and a euglycemic hyperinsulinemic clamp (45mU/m^{2}min).

Results: Intrapair correlations

	MZ	p	DZ	p
Incr. insulin area (0-60min)	0.84	<0.00001	0.32	n.s.
Glucose disposal	0.53	<0.05	-0.03	n.s.
BMI	0.61	<0.005	0.12	n.s.
Waist-hip -ratio	0.87	<0.00001	0.53	<0.05
Height	0.95	<0.00001	0.74	0.0001
Weight	0.73	0.0001	0.51	<0.05

Conclusion: As well measures of insulin secretion as of insulin sensitivity show significant intrapair correlation in MZ but not in DZ twins, indicating that they are under genetic control, which seems to be stronger for insulin secretion than for insulin action.

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THE MITOCHONDRIAL DNA (tRNA Leu(UUR)) MUTATION IN ITALIAN NIDDM PATIENTS.

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A point mutation of the mitochondrial tRNA Leu(UUR) gene,consisting in an A to G transition at nucleotide pairs (np) 3243,has been observed in some diabetic patients,especially in those with maternally inherited diabetes.No data,however,concerning the prevalence of this mutation in Italy are actually available.In this study 231 consecutive non-insulin-dependent (NIDDM) diabetic patients,attending an outpatient clinic,were recruited.They were born and lived in Sicily or Calabria regions (South Italy);their mean age was 59±8 years and known diabetes duration was 13±7 year.Of them 87 were treated with insulin and 118 had at least one diabetic parent:72 had the mather,15 had the father and 31 had both parents.Mitochondrial DNA was extracted from peripheral blood and the region encompassing nucleotides from 2770 to 3456 was amplified by means of Polimerase Chain Reaction (PCR).PCR products were then digested with ApaI enzyme,which cleaves the mutant but not the wild-type sequence at np 3243.A positive digestion control with a standard lambda DNA was also included.With this method the tRNA Leu (UUR) mutation was never detected,neither in those patients with maternally inherited diabetes.These results indicate that the 3243 mitochondrial DNA mutation is at least extremely rare,or insufficiently expressed on peripheral blood cells,in Southern Italian diabetic patients and do not suggest a major role for this mutation in our NIDDM population.

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FAMILIAL DIABETES MELLITUS IN LAGOS NIGERIANS.

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Several studies have shown that relatives of diabetic patients have an increased risk of developing diabetes mellitus (DM). Familial DM in Black Africans appears to have received little attention. We determined the frequency of a family history of DM (FHDM) among Nigerian subjects with and without DM. About 5% of non-diabetic subjects had a positive FHDM in the first degree relatives (FDRs) vs about 25% occurrence in the diabetic subjects (p<0.001). Parents of diabetic probands were more likely to have DM than the children of the same probands. Of all FDRs, the siblings had the highest a history of DM. The more educated the proband, the more likely was the occurrence of a positive FHDM ; this might suggest that among the uneducated, the frequency of positive FHDM might have been underestimated. Probands diagnosed at age 40 yrs, or less showed a higher rate of FHDM than patients diagnosed at older ages. In Nigerians, a positive FHDM is significantly more common in diabetic than in non-diabetics. The positivity rate of FHDM appears similar to reports from other cultures but may be an underestimation due to a low degree of diabetes awareness. Detailed family studies of DM are needed to provide a comprehensive insight into familial DM in Nigerian and other parts of Black Africa.

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VARIATIONS OF GLUCAGON-LIKE PEPTIDE-1 RECEPTOR IN JAPANESE PATIENTS WITH NON-INSULIN-DEPENDENT DIABETES MELLITUS

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Glucagon-like peptide-1(GLP-1) stimulates glucose-induced insulin secretion and may regulate feeding behavior. GLP-1 binds to a specific receptor (GLP-1R), which belongs to a Gs protein coupled receptor family, and activates adenylyl cyclase system. GLP-1R$^{-/-}$ mice created by the targeted disruption of the gene, showed increased levels of blood glucose following oral glucose challenge test in association with diminished levels of circulating insulin. Therefore, to assess the possible role of mutations of the gene in the defective insulin secretion in humans, we examined human GLP-1R gene in Japanese patients with non-insulin-dependent diabetes mellitus (NIDDM) at a single nucleotide level. We first determined the nucleotide sequences of exon-intron boundaries of 13 exons of the human gene by analyzing genomic clones we isolated previously. Based on the sequences, oligonucleotide primers were synthesized and polymerase chain reaction-single strand conformation polymorphism analysis was conducted for the entire coding region of the gene in 100 Japanese NIDDM patients. We found three missense mutations (Pro^{7}→Leu, Arg^{44}→His, Phe^{266}→Leu^{266}), and seven silent mutations including polymorphisms in introns. Among the three missense mutations, Leu^{7} and His^{44} are present in the corresponding positions of the rat GLP-1R, suggesting that these substitutions are functionally silent. Since Phe^{266} was conserved between human and rat GLP1R, we compared the frequency of the Phe^{266}→Leu^{266} mutation between NIDDM patients (n=100) and non-diabetic controls (n=96). The allelic frequencies of Phe^{266} did not differ between the two groups (0.50 vs 0.50). We conclude that mutations in the GLP-1R gene are not major contributor to the inherited basis of NIDDM in Japanese, confirming the previous linkage and association studies in Caucasians and African Americans.

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INSULIN RESISTANCE AND BLOOD PRESSURE IN OFFSPRING OF TYPE II DIABETIC PATIENTS WITH AND WITHOUT NEPHROPATHY

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Family studies point to an important genetic element in the genesis both to diabetes and diabetic nephropathy. The aim of the study was to assess insulin resistance and blood pressure of healthy offspring of type 2 diabetic patients with and without nephropathy. We examined 425 consecutive type II diabetic patients and examined all available normotensive, normoglycemic, non smoking offspring (n=56; 30 of whom without parental history of nephropathy- nDN, and 26 with- DN) and compared them to 30 healthy offspring of non diabetic parents (controls). All groups had similar age and BMI. Measurements in offspring and controls included: tissue glucose uptake-TGU (euglycemic hyperinsulinemic clamp), 24-h ambulatory blood pressure-ABP and polymorphism I/D of ACE gene (PCR technique). TGU was significantly lower in both DN and nDN groups when compared to controls (6.3±2.5, 5.6±2.2 and 9.5±2.2 mg/kg/min respectively; $p<0.005$). No difference between DN and nDN groups was found. 24h systolic ABP was significantly higher in DN group when compared to nDN and controls (124.5±16.7, 117.2±12.9 and 114.1±8.5 respectively; $p<0.05$). When estimated all examined subjects (n=86) significantly higher risk of systolic BP above 95 percentile was found in DD homozygotes of ACE gene (OR-3.15; CI- 0.68-14.7). No significant relation of ACE ID polymorphism was found in relation to insulin resistance. We conclude: (i) predisposition to type 2 diabetes is manifested by insulin resistance in offspring, (ii) higher blood pressure may be the clinical sign of predisposition to nephropathy, (iii) insulin resistance is not related to nephropathy and ACE I/D gene polymorphism.

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NO ASSOCIATION BETWEEN THE GLY40SER MUTATION (GCG-R GENE) AND JAPANESE NIDDM.

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Significance of a glucagon receptor gene variant in the pathogenesis of NIDDM has been implicated in Sardinian and French populations with NIDDM. The mutation was present with significantly higher prevalence in subjects with NIDDM compare with non-diabetic controls. We investigated 242 randomly selected Japanese NIDDM patients whether a G123A mutation causing Gly40Ser substitution is associated with Japanese NIDDM. We also searched for unknown mutations or deletions in GCG-R gene by SSCP method. We could not find any abnormalities in 30 patients with glucose intolerance and with a family history of NIDDM. By using PCR-RFLP method,no one carried the Gly40Ser mutation in with Japanese NIDDM patients. The C-to-G substitution in exon 5 proved to be very rare in Japanese,unlike Sardinians and French populations. These observations indicate that marked ethnic differences exist between Caucasians and Japanese.

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NON INSULIN DEPENDENT DIABETES AND PARENTAL HISTORY.

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Some authors have noticed that diabetic mothers generate as many as twice diabetic sons than diabetic fathers.With the aim of determine the frequency of the parental antecedent and its sexual distribution in a diabetic sample, we analized data from 286 NIDDM patients assisted by one of us (to avoid potential biases) between 1970 and 1996. The studied sample came from a Buenos Aires city suburb. For each patient was registered the familial history,BMI, age,diabetes duration,complications and other relevant clinical data.Satistical analysis:Fleiss quadratic 95% confidence interval(FQ95%CI) was obtained.$X^2$was performed to determine differences between groups.Soft:CSS/Statistica,StatSoft,Tulsa,1993.Results:parental antecedents were distributed as follows:

	Male Patients(n=142)		Female Patients(n=144)	
ANTECEDENT	Proportion	FQ95%CI	Proportion	FQ95%CI
None	40.1%	32.1-48.7	43.0%	34.9-51.5
Mother	26.7%	19.8-34.9	28.4%	21.4-36.7
Father	16.9%	11.3-24.1	7.6%	4.0-13.5
Both	8.4%	4.6-14.6	5.5%	2.6-11.0
Others	7.7%	4.1-13.7	15.2%	10.0-22.4

Between sexes difference:p=0.04.The main contribution to between sexes significance was the difference in the paternal antecedent.Conclusion: although maternal antecedent was more frequent than the paternal one in both sexes,paternal history was significantly more frequent among men.

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INHERITANCE OF DIABETES IN A NEW MOUSE MODEL OF NIDDM, THE NSY MOUSE

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The NSY (Nagoya-Shibata-Yasuda) mouse is an inbred strain of mice with spontaneous development of diabetes mellitus with moderate obesity (Ueda H et al. Diabetologia 1995). NSY mice spontaneously develop diabetes mellitus in an age-dependent manner. The cumulative incidence of diabetes is 98% in males and 31% in females at 48 weeks of age. Both insulin resistance and impaired insulin secretion contribute to the development of diabetes in NSY mice. To determine the mode of inheritance of diabetes in NSY mice, two reciprocal crosses, female C3H x male NSY F1 (C3NF1) and female NSY x male C3H F1 (NC3F1) mice, were performed. The cumulative incidence of diabetes reached 100% (25/25) in male C3NF1 mice and 97% (29/30) in male NC3F1 mice at 48 weeks of age, indicating that diabetes in NSY mice was transmitted to F1 hybrids in an autosomal dominant manner with high penetrance. Insulin resistance also showed an autosomal dominant mode of inheritance. In contrast, impaired insulin secretion in response to glucose in NSY mice showed an autosomal recessive mode of inheritance. BMI and fat accumulation showed a co-dominant mode of inheritance. These data suggested that NSY mice were polygenic animal model of diabetes and were expected to be a useful animal model of NIDDM to clarify genetic susceptibility to NIDDM.

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PATHOGENESIS OF NON-INSULIN-DEPENDENT DIABETES MELLITUS IN THE URBAN BLACK COMMUNITIES OF SOUTHERN AFRICA

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Urban black South Africans show an escalating incidence of non-insulin-dependent diabetes mellitus (NIDDM) (age-adjusted prevalence approaching 7%). The aim of this study was to examine underlying pathogenic mechanisms. We reviewed data from black and white subjects with normal glucose tolerance, obesity, impaired glucose tolerance (IGT) and NIDDM. Stimuli to insulin secretion included oral glucose and maximal beta-cell stimulation. The homeostasis model assessment (HOMA) was used to calculate insulin resistance. Molecular scanning of the insulin receptor (INSR) and insulin-receptor-substrate 1 (IRS-1) genes was undertaken with single-stranded conformation polymorphism and direct DNA sequencing. Insulin secretion was significantly reduced in black subjects compared to white after all stimuli. Calculated insulin resistance in black subjects increased from a low mean value of 1.24 in nonobese to 2.48 in obese nondiabetic, 2.42 in IGT and 6.55 in NIDDM ($p<0.001$). Molecular scanning of INSR and IRS-1 genes revealed polymorphisms with similar frequencies in nondiabetic and diabetic black and white subjects. We conclude that NIDDM in urban black Africans is due to progressive insulin deficiency, with acquired insulin resistance developing as a late contributory factor.

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COENZYME Q_{10} TREATMENT PREVENTS PROGRESSION OF INSULIN SECRETORY DEFECTS IN MATERNAL-INHERITED DIABETES MELLITUS WITH MITOCHONDRIAL DNA MUTATIONS.

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Progressive insulin secretory defect is a clinical feature of diabetes mellitus with mitochondrial DNA (mtDNA) mutations. This study investigated the effects of coenzyme Q_{10} (CoQ) treatment on insulin secretory capacity and clinical symptoms of the Japanese diabetic patients with mtDNA mutations. 44 diabetic patients, 12 impaired glucose tolerance (IGT) , and 20 normal glucose tolerance (NGT) subjects with the 3243 (A-G) mutation, 7 diabetic patients with the 8344 (A-G) mutation, and 7 diabetic patients with multiple deletions, were treated for 3 years with daily oral administration of 150 mg of CoQ. After the CoQ therapy, insulin secretory response assessed by 24 hour urinary C-peptide excretion and glucagon-induced C-peptide secretion were significantly improved in the diabetic patients with multiple deletion and 3243bp mutation, but not changed in the IGT and NGT subjects with the 3243bp mutation and diabetics with 8344bp mutation. Blood lactate concentrations after exercise were significantly reduced in the diabetic patients with multiple deletion, but not changed in the subjects with the 3243bp and 8344bp mutations. CoQ treatment did not improve diabetic complications nor neurosensory deafness. These data demonstrates therapeutic usefulness of CoQ on insulin secretory defects in diabetic patients with mitochondrial DNA multiple deletions and 3243bp mutation.

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CASE REPORT
WERNER'S SYNDROME

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Werner's syndrome is a rare disorder resembling premature aging. This autosomal recessive disorder is frequently associated with endocrinological problems, particularly diabetes mellitus and hypogonadism. Although the diagnosis is not difficult when it is kept in mind it may be so because of its rarity. In this report we present a case of Werner's syndrome who has been hospitalized in our clinic because of diabetic foot. Detailed physical and laboratory examinations revealed that this 53 years old male patient also had hypergonadotropic hypogonadism, gynecomastia and mild mental deficiency. Details of the syndrome with regard to clinical characteristics, diagnosis and therapeutic modalities are also discussed.

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INCREASED INSULIN STIMULATED GLUCOSE DISPOSAL RATE IN 1161 CAUCASIAN MALES: INTERACTION BETWEEN OBESITY AND THE ASP905TYR VARIANT OF THE GLYCOGEN ASSOCIATED SUBUNIT OF TYPE 1 PROTEIN PHOSPHATASE

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The allelic frequency of the E905Y variant of the glycogen associated subunit of type 1 protein phosphatase (PP1G) is 43% in the Pima Indians and it has been reported that the E905Y polymorphism in this ethnic group is associated with NIDDM and in the non-diabetic subjects with increased insulin mediated glucose disposal during a euglycaemic and hyperinsulinaemic clamp. The aim of the present study was therefore to look for an interaction between the E905Y polymorphism and obesity (defined as body mass index (BMI) ≥ 80% percentile) on insulin sensitivity in 1161 Caucasian men born 1920-24 and participating in the Uppsala health survey study and characterized with a euglycaemic hyperinsulinaemic clamp. At the time of examination 174 subjects had developed NIDDM and 147 impaired glucose tolerance (IGT). All participants were genotyped for the E905Y polymorphism of PP1G by PCR-RFLP. The allelic frequency of the E905Y variant of the PP1G was 11%. Subsequent test for interaction showed an interaction of obesity and E905Y on insulin sensitivity index ($p < 0.021$). Furthermore, when obese carriers ($n = 50$) of the E905Y polymorphism were compared to obese non-carriers ($n = 173$) they had a 24% increase in insulin sensitivity index ($p < 0.009$). However, the E905Y variant was not associated with an altered prevalence of NIDDM or IGT. We conclude that the E905Y variant of PP1G in obese males of Caucasian origin is associated with an increased insulin sensitivity.

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PPARγ EXPRESSION IS DOWN-REGULATED BY VITAMINS A AND D DURING ADIPOCYTE DIFFERENTIATION

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Recently, it was leveled that the characteristics of trans-acting proteins and their presumptive roles in the program of transcriptional activation of genes expressed during adipocyte differentiation. We and other investivators reported that active forms of vitamins A (retinoic acid; RA) and D (1,25-dihydroxyvitamin D3; VD) inhibit adipocyte differentiation. At the early stage of adipocyte differentiation, ligand-dependent transcriptional factors, especially peroxisome proliferator-activated receptor (PPAR), regulate positively the expression of target differentiation genes.

So we investigated the effect of RA and VD on the expression of master regulator, PPARγ, during adipocyte differentiation by Western and Northern blot analyses in 3T3-L1 cells. We revealed that the receptor members of retionoid (RAR,RXR) / vitamin D (VDR) / PPAR subfamily genes expressed during adipocyte differentiation. These cells were strongly inhibited differentiation by the addition of RA or VD3 even in the presence of PPARγ specific ligans, thiozolidinediones (a new class of antidiabetic agents). These agents strongly stimulate adipocyte differentiation *via* PPAR. The expression of PPARγ2 was increased during adipocyte differentiation with or without thiazolidinedione. At that time, the presence of RA or VD led to strongly inhibition of the expression of PPARγ2. Concomitantly, at this stage the presence of RA or VD3 causes the increase of RAR or VDR. These findings suggest that the signal of these ligands may be cross-talked at the level of nuclear receptors (RAR/VDR/PPAR) and then regulate positively and negatively the signal transduction of adipocyte differentiation at the level of gene transcrition.

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NO ASSOCIATION OF INTESTINAL FATTY ACID BINDING PROTEIN POLYMORPHISM WITH INSULIN RESISTANCE IN JAPANESE

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A mutation from alanine (Ala54) to threonine (Thr54) at codon 54 of intestinal fatty acid binding protein (FABP2) was identified. FABP2 has been reported as a possible genetic factor in determining insulin resistance in Pima Indians, a population known to have higher prevalence of diabetes. We investigated the association of polymorphism at FABP2 codon 54 mutation with insulin resistance in Japanese subjects. 75g oral glucose tolerance test (75gOGTT) was performed and serum lipids, blood pressure and percentage of body fat (%BF) were measured in 133 unrelated individuals who underwent regular medical examination (male/female =118/15, mean age 50.5 years old, mean BMI 24.0kg/m^2). Of these 133 subjects, 13 were diabetic and 120 were nondiabetic. DNAs were isolated from white blood cells and typed as FABP2 codon 54 restriction fragment length polymorphism (RFLP) by HhaI after PCR proliferation. Allele frequencies of Ala54 and Thr54 were 0.68 and 0.32, respectively. The frequencies did not differ from those of Pimas and Caucasians. RFLP of FABP2 did not show any association with BMI, %BF, plasma glucose, plasma fasting and 2-h insulin concentrations, HbA_1c, total cholesterol, HDL-cholesterol, or category of glucose tolerance. Subjects with Thr54 allele showed higher triglyceride levels (164±73mg/dl) than those with Ala54 (142 ± 58mg/dl), but the difference was not significant (p=0.07). We conclude that FABP2 codon 54 polymorphism may not be significantly associated with insulin resistance in Japanese subjects.

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MELANIN CONCENTRATING HORMONE STIMULATES LEPTIN SECRETION FROM ADIPOCYTES

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The mouse obesity gene (ob) product (leptin), which is expressed exclusively in white adipose tissue (WAT) inhibits food intake and stimulates energy metabolism. Leptin acts at sites in hypothalamus, but non-neuronal tissues are also targeted. Thus, we have previously shown that administration of leptin inhibits insulin secretion from perfused pancreas and isolated pancreatic islets of mice and suggest that high leptin production, associated with adiposity, could lead to development of overt diabetes. Another molecule associated with hypothalamic regulation of food intake is melanin concentrating hormone (MCH). In contrast to leptin, it has been shown to stimulate food intake in mice. Furthermore, the hypothalamic concentration of MCH is drastically increased in ob/ob mice, which are deficient in functional leptin. We have studied the effects of MCH on the secretion of leptin in freshly isolated adipocytes from Sprague Dawley rats. Adipocytes were incubated for 6h in Hepes pH 7.5 (10mM), bovine serum albumin (5mg/ml), FCS (5mg/ml) and penicillin/streptomycin with or without 100nM MCH. MCH significantly increased leptin release into the medium from 0.06 ± 0.02 to 0.17 ± 0.02ng/10^{-6} cells, $P < 0.001$. These results demonstrate a novel interaction between MCH and leptin that could help in understanding how body weight homeostasis is maintained. It is suggested that MCH and leptin might participate in a feedback loop between the hypothalamus and WAT to regulate body weight in mammals.

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THE SIGNIFICANCE OF THE TRP64ARG MUTATION OF THE β3-ADRENERGIC RECEPTOR GENE IN INSULIN RESISTANCE.

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It has been shown that the Trp 64 Arg mutation of the human β3-adrenergic receptor (β3-AR) gene is related to an earlier age of onset of non-insulin-dependent diabetes mellitus (NIDDM), features of insulin resistance and weight gain in morbidly obese patients. However, such findings have not been consistent in different ethnic populations.

In the present study, we investigated the frequency of the Trp 64 Arg mutation in Japanese control subjects (n=253), NIDDM (n=314) and impaired glucose tolerance (IGT) patients (n=100). We compared the frequency of the mutation with the body mass index (BMI) in these groups,and with the metabolic clearance rate (MCR) of glucose in the NIDDM patients. A Trp 64 Arg mutation was observed in 36.7%, 31.6% and 37.0% of the control, NIDDM and IGT subjects, respectively. The frequency of the homozygotes for the mutation was 4.3%, 4.8% and 3.0%, respectively. Neither the genotype frequency (Trp/Arg, Arg/Arg) nor the frequency of the mutated allele was significantly different among the three groups. The BMI of the subjects with the mutation was not significantly higher than that of the subjects without the mutation in each group. Futhermore, the allele frequency (A) was not different among the subjects with different BMI (BMI < 22.0, 22.0 ≦BMI ≦26.4, BMI > 26.4) in each group. In a separate group of NIDDM patients, the MCR of the subjects with intermediate BMI (22.0 ≦BMI ≦ 26.4) with the mutation tended to be lower than that of those without the mutation. In addition, the MCR of the subjects with the mutation in this group was significantly lower compared with that of those with a BMI less than 22.These results indicate that the Trp 64 Arg mutation of the β3-AR gene may not contribute to the development of NIDDM or be a determinant of obesity in the Japanese population. However, the mutation may contribute to insulin resistance in NIDDM patients with intermediate BMI.

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INSULIN ANTAGONIZES THE STIMULATION OF OB GENE EXPRESSION BY DEXAMETHASONE IN CUTURED EXPLANTS OF HUMAN VISCERAL ADIPOSE TISSUE.

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The ob gene, specifically expressed in fat cells, encodes leptin, a hormone which induces satiety and increases energy expenditure. Visceral fat plays a determinant role in obesity-linked disorders. To assess the direct role of hormones on the expression of fat genes, cultured explants of adipose tissue have decisive advantages over clonal cell lines or stromal cells differentiated by long-term exposure to pharmacological doses of "adipogenic cocktails" : the approach is more physiological, the model highly responsive to insulin and interactions between cells are preserved. We thus investigated the hormonal control of ob gene expression in explants of human visceral adipose tissue cultured in MEM supplemented with FCS for up to 48h. We more particularly focussed on the interactions between insulin and glucocorticoids. Adipose tissue (15-100g) was obtained from patients undergoing elective abdominal surgery (age: 51 ± 9 yr; BMI: 25.6 ± 2.5 kg/m2). In response to dexamethasone, ob mRNA levels rose progressively to a maximum of ~ 10-fold starting values after 48h. This increase was concentration-dependent : the glucocorticoid was effective at concentrations above 1nM and, if the effect of 100 nM dexamethasone was maximal, half-maximal stimulation was produced by 10 nM. Unlike dexamethasone, insulin added to the medium at 100 nM, for up to 48h, did not stimulate ob gene expression. Unexpectedly, insulin prevented the dexamethasone-induced accumulation of ob mRNA. Thus, after 24 and 48 h of culture, the effect of dexamethasone was respectively 55% and 50% inhibited by 100 nM insulin. In conclusion, unlike dexamethasone, insulin had no direct stimulatory effect on ob gene expression in cultured explants of human visceral adipose tissue. On the contrary, insulin even inhibited dexamethasone-induced accumulation of ob mRNA. This suggests that the in vivo stimulatory effect of insulin on ob gene expression is indirecty mediated.

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POLYMORPHISMS IN THE UNCOUPLING PROTEIN (UCP) AND β3 ADRENERGIC RECEPTOR (β3-AR) IN NONDIABETIC (ND) AND NIDDM SUBJECTS.

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The mechanisms linking obesity and insulin resistance are not known. Genes influencing basal metabolic rate and weight gain, as the UCP and β3-AR, could be putative candidate genes for NIDDM. To test this hypothesis, using PCR-RFLP we studied whether the A->G (-3826) polymorphism in the UCP gene, previously claimed to be associated with weight gain, or the Trp64→Arg64 mutation in the β3-AR, could influence prevalence of obesity and NIDDM in Swedish subjects. UCP gene polymorphism was determined in 1097 subjects from 257 families (652 NIDDM, 364 ND first degree relatives and 81 unrelated healthy controls). The frequency of the G allele was increased in the NIDDM subjects compared to the controls (25% vs 20% p= 0.04) In ND relatives and controls, carriers of the G allele, had higher incremental glucose area during OGTT compared to the AA genotype carriers (267 vs 231 mmol/l; p= 0.02). No differences were seen in insulinarea, BMI or lipids between the carriers of G allele, compared 'A allele carriers. β3-AR mutation was determined in 1063 subjects from 253 families (643 NIDDM, 360 ND relatives and 60 controls). No differences in allele frequences of the Arg mutation were seen between NIDDM subjects (7%), ND relatives (7%) and controls (6%). Sibpairs discordant for the Arg mutation (n=47) were also discordant for BMI (27.0 vs 26.0 p=0.05) and waist hip ratio (males n=18, 0.99 vs 0.96 p=0.04; females n=22, 0.89 vs 0.85 p=0.02). Five subjects homozygous for the Arg allele were found (1 NIDDM, 3 relatives and 1 control). No subjects homozygous for both UCP G allele and β3-AR Arg allel were found but 5 of the NIDDM patients vs none of the ND subjects had the genotype combination GG (UCP)/ ArgTrp (β3-AR). In conclusion the study proposes a role for the UCP gene in the pathogenesis of glucose intolerance and NIDDM and confirms in a sibpair study the association of the β3-AR Trp64→Arg64 mutation with abdominal obesity.

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PERIPHERAL ACTIVITY OF LEPTIN: DESENSITIZATION OF ADIPOCYTES *IN VITRO* FOR INSULIN ACTION

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Leptin, an adipocyte hormone, reduces food intake and increases metabolic rate in rodents by a central mode of action. Aim of this study was the search for leptin actions on metabolically relevant cells. Isolated rat adipocytes were kept for 15 h in primary culture in the presence or absence of recombinant murine leptin and then key metabolic pathways were tested. Leptin *per se* had no significant effect on glucose uptake, glycogen synthase activity, lipogenesis, lipolysis, and protein synthesis but impaired insulin effects on these pathways. Leptin preincubation caused an increase of insulin EC_{50} from 0.2 nM (without leptin) to 0.45 and 1.6 nM (at 0.5 and 1 nM leptin, resp.) for glucose transport. At 10 nM leptin, the stimulation of glucose uptake by 10 nM insulin was reduced from 13.5-fold to 3.6-fold. Insulin at higher concentrations could not overcome this decrease of responsiveness. The antilipolytic effect of insulin on isoproterenol-induced lipolysis was also antagonized by leptin. The IC_{50} of leptin for the inhibition of insulin effects was around 3 nM in all these assays. Time-course for onset of leptin-induced desensitization of adipocytes for insulin action is slow, reaching its maximum only after 15 h at 2 nM leptin. Leptin effects are reversible and regain of insulin sensitivity is more rapid than desensitization. Stimulation of glucose uptake or lipogenesis by vanadate can not be inhibited by leptin preincubation. We conclude, that leptin at physiological concentrations reduces sensitivity of adipocytes for insulin action after long-term incubation in primary culture. At higher concentrations, leptin also reduces insulin responsiveness. Leptin might be involved in the development of insulin resistance in obese people.

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ASSOCIATION IN THE β 3-ADRENERGIC-RECEPTOR GENE WITH CLINICAL FINDINGS IN JAPANESE NIDDM SUBJECTS.

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〈Purpose〉 There are reports that the Trp64Arg mutation of the β 3-Adrenergic-Receptor (β 3AR) is associated with obesity and the onset of diabetes mellitus.In the present study, we examined association in the β 3AR gene with diabetic complications in Japanese NIDDM subjects. **〈Methods〉** We studied the frequency of the Trp64Arg mutation of the β 3AR and investigated diabetic complications in 182 Japanese NIDDM subjects,84 male patients (the mean duration of diabetes was 10.6 years),98 female patients(the mean duration of diabetes was 10.1 years) .Genotic DNA was extracted from leukocytes,we determined genotype using method of E.Widen et al(1995). **〈Results〉** A Trp64Arg mutation was detected with allelic frequencies of 0.16 in Japanese NIDDM subjects. When we compared patients with normal homozygotes and Trp64Arg homo- or heterozygotes,there was no significant difference in the Body Mass Index, the mean age at the onset of NIDDM and chemical data. There was no difference in diabetic retinopahy,neuropathy or nephropathy. **〈Conclusion〉** Environment and life history are more important role than the Trp64Arg mutation of the β 3AR for diabetic complications.

	with mutation (n=51)	without mutation (n=131)	p value
Age at onset(yr)	50.9	50.0	0.8452
BMI	22.61	23.43	0.0992
Retinopathy (+/-)	16/35	51/80	0.3260
Neuropathy (+/-)	20/31	46/85	0.6053
Nephropathy (+/-)	10/41	32/99	0.4883

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FAILURE OF LEPTIN TO COUNTERACT THE EFFECTS OF GLUCOSE ON INSULIN AND GLUCAGON RELEASE BY THE PERFUSED RAT PANCREAS

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It was recently postulated that leptin may inhibit insulin release, as part of an adipoinsular feedback loop. This proposal was based on the finding that leptin receptor mRNA is present in rat islets and that unlabelled leptin decreases the binding of ^{125}I-leptin to insulinoma cells. In subsequent work, however, we documented that the prevailing leptin receptor in rat islets corresponds to the ubiquitous short isoform, for which no signalling activity has yet been assigned. Moreover, when tested at a 1.0 nM concentration, human leptin failed to affect glucose-stimulated insulin release from the isolated perfused rat pancreas. The concentration of leptin used in these experiments was not higher, however, than that found in the plasma of obese human subjects. Therefore, we have now examined the effect of a ten times higher concentration of leptin upon both insulin and glucagon release from the isolated perfused pancreas of fed female Wistar rats. The glucose concentration of the perfusate was raised from 3.3 to 8.3 mM between min 25 and 70 and leptin (10 nM) administered from min 40 to 55. The rise in glucose concentration provoked a biphasic stimulation of insulin release and rapid and sustained suppression of glucagon output. Leptin failed to affect the release of the two hormones. Whether in the absence or presence of leptin, the rate of insulin release progressively increased during stimulation by 8.3 mM glucose. The paired ratio in secretory rate at min 55/min 40 averaged 243 ± 36 % (n = 4) in the presence of leptin, as compared ($P > 0.1$) to 180 ± 6 % (n = 3) in its absence. These findings reinforce the view that leptin does not exert any direct and immediate effect upon the secretory activity of the endocrine pancreas.

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GENETIC VARIATION IN β3-ADRENERGIC RECEPTOR AND FATTY ACID BINDING PROTEIN IN JAPANESE.

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A mutation of β3-adrenergic receptor gene (Trp64Arg) has been reported to be associated with obesity, insulin resistance and earlier onset of NIDDM. It has been reported that a polymorphism at codon 54 of the intestinal fatty acid binding protein locus (FABP2) was associated with insulin resistance in Pima Indians. We therefore studied the frequency of these mutation and polymorphism in Japanese, and investigated the association of genetic variation with obesity, dyslipidemia and hypertention. 114 Japanese subjects were recruited from the general health check-up center (92 male, 22 female, mean age 53.2 years, NGT 58 IGT 49 NIDDM 7). The allele having Trp64Arg mutation or Ala54Thr polymorphism was detected by PCR-RFLP method using restriction enzyme BstNI and HhaI. The frequencies of Trp64Arg and Ala54Thr were 0.27 and 0.38, respectively. Both frequencies were similar among NGT, IGT and NIDDM. There were no differences in BMI, systolic and diastolic blood pressure, fasting insulin levels and dyslipidemia between subjects with and without these mutation and polymorphism.

These data suggest that β3-adrenergic receptor gene mutation and polymorphism of FABP2 may not be associated with obesity and dyslipidemia in Japanese.

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GLUCOCORTICOID AND GENDER REGULATE PLASMA LEPTIN LEVELS IN HUMANS

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Using the radioimmunoassay (RIA) specific for human leptin, which we have developed, we showed that a significant amount of leptin was present in the circulation, and that the plasma leptin levels were positively correlated with adiposity. In order to assess the mechanisms regulating leptin secretion in humans other than adiposity, we have studied plasma leptin levels in subjects without metabolic or endocrine diseases. At the same percentage of body fat, plasma leptin levels in premenopausal females were doubled and significantly elevated as compared with those in age-matched males by analysis of covariance ($p<0.05$), and the levels in premenopausal females were almost equivalent to those in age-matched postmenopausal females at a given percentage of body fat. Furthermore, suppression of estradiol by LH-RH analogue did not at all decrease the plasma leptin levels. These results indicate that there is evident sexual dimorphism in the plasma leptin levels, although estrogen is unlikely, at least directly, to affect the gender difference. Moreover, in the patients with Cushing's syndrome of various causes including adrenal adenoma, pituitary adenoma and iatrogenic Cushing's syndrome with collagen diseases, plasma leptin levels were approximately doubled and significantly higher than those in control subjects at a given percentage of body fat ($p<0.05$). The resection of adrenal and pituitary adenoma caused remarkable decrease in plasma leptin levels with concurrent attenuation of plasma cortisol levels. In the organ culture of human adipose tissue, dexamethasone potently induced the leptin gene expression and subsequent secretion of leptin from adipose tissue. The results presented here demonstrate that glucocorticoid and gender are involved in the regulation of plasma leptin levels in humans.

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GENETIC VARIATION IN THE ß$_3$-ADRENERGIC RECEPTOR GENE AND NIDDM

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Introduction: Obesity is the most powerful risk factor for Non-Insulin-Dependent Diabetes Mellitus (NIDDM) and, like NIDDM, has clear genetic determinants. Recent works have evidenced that molecular abnormalities in the ß$_3$-adrenergic receptor may lead to obesity and NIDDM; however, some studies have failed in finding such associations. We have studied the possible association between the Trp64Arg mutation in the ß$_3$-adrenergic receptor gene and either NIDDM and its long-term complications in a Mediterranean population. **Material and methods**: We have used the polymerase chain reaction to amplify a region of the gene for the ß$_3$-adrenergic receptor to analyze the frequency of a C→T mutation that results in a replacement of Trp by Arg at position 64 (Trp64Arg) in 187 NIDDM patients and 87 healthy controls. A plasmatic lipidic profile, glycated haemoglobin and microalbuminuria were quantified. **Results**: The frequency of the Trp64Arg allele was similar in the controls and in the NIDDM patients (0.096 and 0.075, respectively). Neither was the mutation associated with a greater body mass index nor with an earlier age at onset of NIDDM. There were not any differences in the NIDDM group when studying the presence of either nephropathy or retinopathy. **Conclusions**: The Trp64Arg mutation of the ß$_3$-adrenergic receptor is not associated with NIDDM and with any of its long-term complications in our population.

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LEPTIN INHIBITS INSULIN BINDING IN ISOLATED RAT ADIPOCYTES.

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Leptin is thought to be an important regulator of energy balance and body weight homeostasis. It is secreted from adipose tissue and circulating levels correlated with body weight, BMI and plasma insulin concentration in humans. Leptin is thought to act centrally to reduce food intake and increase energy expenditure. However, leptin has also been shown to affect fertility and neuroendocrine function, and leptin receptors have been found in various tissues outside the hypothalamus, including liver, lung and adipose tissue. These findings suggest that leptin may have significant effects on peripheral tissues, and several studies have investigated a possible link between leptin and insulin in the regulation of metabolism. In this study we investigated the effects of leptin on insulin binding by isolated rat adipocytes. Sprague-Dawley rat adipocytes were isolated by collagenase digestion and incubated with varying amounts of unlabelled insulin (0, 2, 10 & 40 ng/ml, 10 µg/ml) and ^{125}I-labelled insulin, with or without 50 nM murine leptin (kindly supplied by Amgen Inc, California, USA). Leptin reduced total insulin binding (no unlabelled insulin present) by 25%. In addition, 50 nM leptin reduced insulin binding at each insulin concentration by between 15 and 25%. Analysis of the binding data suggested that leptin affected the affinity of insulin for its binding site. In summary, leptin directly inhibited insulin binding by adipocytes, and therefore may impair insulin action in fat cells. These results raise the possibility that the hyperleptinemia found in obese states could contribute significantly to adipose tissue insulin resistance which is characteristic to obesity. The precise effects of leptin on adipocytes, and on other peripheral tissues, requires further investigation.

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SIGNIFICANCE OF β3-ADRENERGIC RECEPTOR GENE POLYMORPHISM IN THE PATHOGENESIS OF NIDDM IN KOREANS.

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The β3 adrenergic receptor (β3-AR) may play an important role in the regulation of energy expenditure and lipolysis. A mutation of the β3-AR gene(Trp64Arg) has been reported to be associated with early onset of non-insulin dependent diabetes mellitus (NIDDM), obesity and syndrome X which are related with insulin resistance. It is well known that Korean NIDDM patients, in contrast to Caucasians, are mainly non-obese and have experienced severe weight loss during the course of disease. We studied the frequency of the mutation in Korean NIDDM patients and non-diabetics control and evaluated the clinical characteristics of Korean obese NIDDM patients.We investigated the frequency of the mutation in NIDDM patients and clinical characteristics of the patients with the mutation in order to elucidate the significance of the mutation in the pathogenesis of NIDDM in Koreans. We studied 401 NIDDM patients and 99 controls. The NIDDM patients were divided into two groups, non-obese group and obese group, according to their body mass index at diagnosis of the disease. The Trp64Arg mutation was detected by the PCR/RFLP method using restriction enzyme Mva I. The Trp64Arg allele frequency (16%) of NIDDM did not differ from that (16%) of controls. Although the mutant allele frequency was not different between non-obese and obese group both in NIDDM patients and controls, the frequency of patient with the mutant allele was significantly higher in obese NIDDM patients than in non-obese NIDDM patients (38.5% vs. 26.9%, p=0.04). However, no significant difference was found in clinical and laboratory findings between the NIDDM patients with the mutant allele and those without the mutant allele.These data suggest that β3-AR mutation might be associated with Korean obese NIDDM, and other factors might also be associated with the development of obesity and insulin resistance in NIDDM patients.

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LEPTIN DIRECTLY STIMULATES BASAL INSULIN RELEASE FROM PANCREATIC ß CELLS.

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Leptin, a 16-kDa peptide released from adipocytes, plays an important role in regulating body weight via its effect on the satiety center in the brain. There are strong positive correlations among the percentage of body fat, plasma leptin levels and fasting plasma insulin levels. We hypothesized that the increased leptin levels contribute to fasting hyperinsulinemia in obese subjects, and thus examined the possibility of direct leptin effects on insulin secretion from pancreatic ß cells. The leptin receptor isoforms, a and b, are present in the pancreatic ß cell line MIN6 and in rat pancreatic islets, based on RT-PCR. Incubation with 1 nM recombinant mouse leptin, the concentration observed in obese subjects, stimulated basal (at 5 mM glucose) insulin secretion by approximately 40 % in both MIN6 and rat islets. Stimulatory effects of leptin were dose-dependent, and not observed without glucose, while 1 nM leptin did not induce an increase in insulin mRNA or tyrosine-phosphorylation of STAT3 in the presence of 5 mM glucose. In conclusion, leptin acts directly on pancreatic ß cells, and this stimulatory mechanism may account in part for the fasting hyperinsulinemia observed in obese subjects.

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ASSOCIATION BETWEEN POLYMORPHISM OF THE ß3-ADRENERGIC RECEPTOR GENE AND BODY WEIGHT GAIN IN PREGNANT DIABETICS

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AIM: Obesity is a risk factor for toxemia and hyperglycemia during pregnancy in diabetic women, and also affects fetal growth. However, the pathogenesis of obesity during pregnancy has not been elucidated. The ß3-adrenergic receptor (ß3-AR) plays an essential role in fat metabolism and thermogenesis. Polymorphism of the ß3-AR gene has been reported to be associated with body weight gain in NIDDM subjects. The aim of this study was to clarify the relationship between the ß3-AR gene and body weight gain during pregnancy. MATERIALS AND METHODS: The 127 subjects consisted of 81 NIDDM patients and 46 IDDM patients treated in the pregnancy clinic of the Diabetes Center. Their mean age at the onset of diabetes was 22.1±7.7 years (mean±SD), and their average age at delivery was 29.5±4.4years. Genomic DNA was prepared from peripheral blood leukocytes. Polymorphism of the ß3-AR gene was detected by restriction fragment length polymorphism after polymerase chain reaction using Bst OI, which recognizes Trp64Arg substitution. RESULTS: The allele frequency of the Trp64Arg allele was 0.19 in NIDDM and 0.16 in IDDM. The prevalence of heterozygotes and homozygotes was 25% (20) and 6% (5), respectively, in the NIDDM subjects, and 24% (11) and 4% (2), in the IDDM subjects. The basal BMI before pregnancy was 21.5±3.2 in the wild type, 21.1 ±3.4 in heterozygotes and 23.0±3.2 in homozygotes (NS). Among the NIDDM subjects, excess weight gain during pregnancy, judged by maximum BMI minus basal BMI exceeding 5, was observed in 8.9% (5) in the wild type, 25.0% (5) in the heterpzygotes and 40.0% (2) in the homozygotes. The homozygotes were significantly associated with excess weight gain during pregnancy (Odd's ratio: 6.80; 95% confidence interval: 1.10-41.71). None of the IDDM homozygotes showed excess weight gain. CONCLUSION: These results provide evidence that the ß3-AR gene is a genetic factor in the pathogenesis of body weight gain during pregnancy in NIDDM in Japanese.

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HUMAN SKELETAL MUSCLE NITRIC OXIDE SYNTHSE CHARACTERIZATION AND ITS ACTIVITY IN OBESE SUBJECTS.

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Recent observations have identified nitric oxide (NO) as a physiologic modulator of skeletal muscle metabolism. The enzyme responsible for NO production is nitric oxide synthase (NOS). Three isoforms of NOS are known to exist. The constitutive enzymes, neuronal and endothelial NOS, are calcium-calmodulin dependent, while the inducible NOS is independent of calcium levels. The main aim of the present study was to characterize the NOS activity in human skeletal muscle and its maxmial activity in obese subjects muscle. Tissue was homogenized in 50 mM Tris-HCl, pH 7.4 containing protein inhibitors and centrifuged at 20000 x g (10 min, 4°C). The pellet was washed with 1M KCl and centrifuged for 20 min. Both supernatants were combined. The NOS activity was determined in both pellet and supernatant by conversion of [^{14} C]-L-arginine to [^{14}C]-L- citrulline. Results are expressed as pmol of citrulline per min per mg protein. We demonstrated that (a) the pellet contains most of the NOS activity (90%); (b) human skeletal muscle NOS is calcium and calmodulin dependent, reaching maximum activity with 1.25 mM and 10 U, respectively; (c) NOS activity was inhibited by EGTA (2mM) and by the calmodullin antagonist (100µM); (d) the apparent Km Values for arginine is 25 µM. Muscle strips from hyperinsulinemic (23.8±3.1 µU/ml) obese subjects with BMI 0f 40.6±3.4 % , demonstrated a dramatic decrease in insulin sensitivity as expressed by glucose transport and glucose oxidation. NOS activity in obese subjects (n=9) was significantly lower (30%) than in healthy subjects . In conclusion, human skeletal muscle contains a constitutive NOS regulated by Ca^{++} and calmodulin. The reduced NOS activity demonstrated in skeletal muscle from insulin resistant obese subjects may lead to decreased NO availability. It is suggested that this alteration may play a role in skeletal muscle insulin resistance associated with obesity.

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ASSOCIATION OF THE GLN223ARG POLYMORPHISM IN THE LEPTIN RECEPTOR GENE WITH PLASMA LEPTIN LEVELS, INSULIN SECRETION AND NIDDM IN PIMA INDIANS.

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The human leptin receptor gene (LEPR) lies on chromosome 1, just distal to the microsatellite marker, D1S198, where we previously mapped a locus that contributes to variation in insulin secretion in the Pima Indians. The Gln223Arg polymorphism of LEPR was typed in 1244 Pima Indians, allele frequencies Gln=0.33 and Arg=0.67, compared to 54 Caucasians, Gln=.46 and Arg =.54. This polymorphism was not associated with body mass index or percentage body fat as determined by underwater weighing. In a subset of non-diabetic individuals, after adjusting for obesity, sex and family membership, the Gln homozygotes (N=22) had lower fasting plasma leptin concentrations than the heterozygotes (N=137) or the Arg homozygotes (N=135) - geometric means 13, 18 and 23 ng/ml, respectively ($p<0.002$). Also, the mean fold increase in plasma insulin concentration 2 hours after ingesting 75g glucose was 24% lower in the Gln homozygotes (N=28) compared to the heterozygotes and Arg homozygotes combined (N=311), ($p<0.03$), after adjusting for obesity, sex, insulin action and family membership. Among full-blooded Pimas, the Gln homozygotes (N=100) had a higher prevalence of NIDDM (72%) compared to the heterozygotes and Arg homozygotes (N=896) (58%) ($P=0.007$). Whether these associations are causally related to the Gln223Arg polymorphisms per se, or due to another polymorphism in LEPR, or another gene in linkage disequilibrium with LEPR, remains to be determined.

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LIPOLYTIC CATECHOLAMINE RESISTANCE IN NON-OBESE WOMEN WITH POLYCYSTIC OVARY SYNDROME

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The polycystic ovary syndrome (PCOS) is characterized by a high rate of metabolic disturbances and cardiovascular mortality. Lipolysis regulation was investigated in isolated abdominal subcutaneous adipocytes from 10 non-obese women with PCOS and 11 age and BMI matched healthy women. Eight PCOS women were re-investigated after 3 months treatment with combined oral contraceptives (OC). PCOS women showed a marked resistance to the lipolytic effect of noradrenaline due to defect at two different levels in the lipolytic cascade. First, the seven-fold reduction in sensitivity of the beta$_2$-selective agonist terbutaline ($p<0.005$) which could be ascribed to a 50% lower beta$_2$-adrenoceptor density ($p<0.02$). Second, the maximum lipolytic response was also 40% lower ($p<0.02$) in the PCOS women compared to the healthy women regardless if lipolysis was stimulated with beta-adrenergic agonists or post-receptor acting agents as forskolin and dibutyryl-cyclic AMP. There was no difference as regards beta$_1$ or alpha$_2$-adrenoceptor functions or densities. Neither of the lipolytic defects or receptor density reduction was restored by 3 months of OC treatment. The results indicate the existance of marked impairment of catecholamine induced lipolysis in non-obese PCOS women displaying an early feature of the insulin resistance syndrome due to multiple lipolysis defects as a lower beta$_2$ adrenoceptor density and reduced function of the protein kinase-hormone sensitive lipase complex.

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AMINO ACID POLYMORPHISMS IN THE LONG ISOFORM OF THE LEPTIN RECEPTOR IN JUVENILE ONSET OBESITY.

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The recently uncovered putative lipostat system controlled by leptin and its hypothalamic receptor provides good candidate genes for the molecular basis of inherited obesity in humans based upon the profound obesity observed in *obese*, *diabetes*, and *fatty* rodents in which leptin or its receptor are mutated. Numerous studies have shown that obesity prevails in spite of increased circulating leptin levels and the concept of leptin resistance as a basis of obesity has evolved. In this study we tested the hypothesis that juvenile onset obesity in humans may be caused by leptin resistance mediated through genetic variations in the hypothalamic leptin receptor. One hundred and fifty-eight obese subjects were selected at the draft board examination having a BMI > 31 kg/m^2 and for having a history of juvenile onset obesity. From the same geographical area, a matched cohort of 200 lean subjects were selected. Single strand conformational polymorphism (SSCP) scanning of the entire long isoform of the leptin receptor in 60 subjects with juvenile onset obesity on genomic DNA revealed a total of three frequent amino acid variants located in coding exons 2, (Lys109Arg), 4 (Gln223Arg) and 12 (Lys721Asn), respectively. The frequencies of all variants were determined in the cohort of 158 subjects with juvenile onset obesity and 200 lean subjects, shown in the table.

Exon	Position base #	Variant	Allele frequency (%) Obese	Lean	Carrier frequency (%) Obese (ho/he)	Lean (ho/he)
2	326	K109R	24,5	19,5	7,0 / 35,0	7,0 / 25,0
4	668	Q223R	44,0	44,7	19,5 / 49,2	17,2 / 51,8
12	1968	K721N	17,0	15,0	3,6 / 26,6	2,7 / 25,0

The prevalence of the three mutations was not significantly different between the obese and the lean cohorts both in regards to allele- and carrier frequency ($p > 0.1$ in each case). The distribution of the alleles was in Hardy-Weinberg equilibrium (not shown). In conclusion: It is unlikely that mutations in the coding region of the long isoform of the leptin receptor are a common cause of juvenile onset obesity.

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ADIPOSE CELL APOPTOSIS IS REGULATED BY TUMOR NECROSIS FACTOR -α AND INSULIN-LIKE GROWTH FACTOR 1

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Adipose tissue mass reflects the balance of factors affecting average adipose cell volume (triglyceride content) and adipose cell number. Adipose cell number is determined by relative rates of preadipocyte proliferation/differentiation and adipose cell apoptosis. We have investigated the roles of serum deprivation, TNF-α and IGF-1 in the induction and modulation of adipose cell apoptosis *in vitro*. Apoptosis was assessed using morphological (light and electron microscopy, acridine orange staining) and biochemical (DNA fragmentation and annexin V staining) criteria, and apoptotic indices were determined by cell counting. Cell types studied were cultured human adipocytes (in explants), human preadipocytes and murine 3T3-L1 preadipocytes. Under control conditions in serum-containing medium, apoptotic indices were low (<2%) in all cell types. Human adipocytes cultured in 50-500 pM TNF-α for 12-24 hours had apoptotic indices of 16-24 % ($p < 0.01$), whilst serum deprivation of 4-12 days induced apoptotic indices of between 2-14 % ($p < 0.05$). Human preadipocytes showed maximum apoptosis of 5.6 % ($p<0.05$) after 4 hours culture in TNF-α, but the cells showed resistance to serum deprivation of up to 96 hours. Apoptosis of 3T3-L1 cells was induced by serum deprivation or addition of TNF-α (5 nM), reaching levels of 8.6% ($p <0.01$) and 12.6 % ($p<0.01$) respectively after 24 hours. The combined effects of serum deprivation and TNF-α were additive. IGF-1 (100 ng/ml) inhibited apoptosis induced by serum deprivation (by 50%, $p <0.05$) but not TNF-α, indicating that distinct apoptotic biochemical pathways may be utilised by these stimuli. We conclude that apoptosis of adipose cells is modulated by endocrine/paracrine factors, and suggest that this process may be an important determinant of adipose tissue mass.

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THE PUTATIVE ROLE OF THE LEPTIN RECEPTOR GENE IN THE PATHOGENESIS OF LEPTIN RESISTANCE

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Mutations in the leptin-receptor gene cause leptin-resistance, obesity and diabetes in several rodent strains. To evaluate the role of leptin receptor gene in the pathogenesis of leptin resistance, obesity and NIDDM we screened 30 obese NIDDM patients and 30 control subjects for mutations in this gene by heteroduplex analysis. We also measured serum leptin levels (S-LEP), BMI, fatmass (FM), and glucose and insulin response to oral glucose tolerance test in 404 unrelated NIDDM patients (186M, 218F) and 233 control subjects (115M, 118 F) from a population-based NIDDM family study in Western Finland (Botnia Study). We identified two polymorphisms; in exon 2 (E2; A→G; Lys to Arg) and in exon 4 (E4; A→G; Gln to Arg). These polymorphisms were typed by PCR-RFLP in 404 unrelated NIDDM patients and 233 controls. The main determinators of S-LEP in both NIDDM patients and controls were sex, FM and fasting insulin; in NIDDM patients also fasting glucose ($p<0.00001$). After adjustment for these confounders, NIDDM men, but not women, had higher S-LEP levels than controls (mean ± SEM 5.75±1.0 vs. 4.81±1.0, $p=0.018$). The E2 and E4 polymorphisms, in marked linkage-disequilibrium with each other, did not significantly affect the S-LEP levels or measures of obesity. However, the E4 1/1 genotype was significantly associated with higher fasting and 2-hour glucose levels in NIDDM patients (median [interquartile range]: 1/1 vs. 2/2 homozygotes: 8.2 [3.5] vs. 7.4 [3.1], $p=0.008$; and 13.8 [7.4] vs. 12.3 [6.4], $p=0.005$) and with 2h-glucose in control women (5.4 [1.3] vs. 4.7 [1.4], $p=0.021$). We conclude that the aminoacid variants in the leptin receptor gene do not explain leptin resistance in obesity, but could contribute to the development of glucose intolerance. Sex, fat mass and insulin levels are the main determinants of serum leptin levels.

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TUMOR NECROSIS FACTOR α AS A LINK FACTOR BETWEEN OBESITY AND NON-INSULIN-DEPENDENT DIABETES MELLITUS

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In animal models of obesity and NIDDM the overexpression of tumor necrosis factor alfa (TNFα) in adipocytes led to insulin resistance by decreasing the signal transduction of insulin receptors. The aim of the study was the investigation of the role of TNFα in the human obesity-diabetes link. In a follow up study of 9 months (samples taken in every 6 weeks) plasma TNF bioactivity was measured by using the L929 cell cytotoxicity assay in the following groups: 59 patients with NIDDM (group 1), 16 patients with IDDM (group 2), 28 patients with obesity (BMI>30, group 3) and 30 matched healthy controls (BMI<24, group 4). TNF concentrations were compared to the patients' basal C-peptide and glucagon levels. Recombinant human TNFα (Sigma) was used as a standard. Monoclonal neutralizing anti-TNFα immunoglobulins (Boehringer) were applied to detect the TNFα cytotoxicity in the samples. A significant elevation of the TNFα concentration was observed in group 1 (x±SE pg/mL:90±10) and group 3 (78±12) as compared to group 2 (40±11) and group 4 (22±8). TNFα levels positively correlated with the BMI values in the NIDDM and obesity groups A positive correlation between TNFα and the increased C-peptide concentration in these groups was also found. These correlations were not observed in group 2 and 4. The increased plasma TNFα levels in NIDDM and obesity can contribute in humans to hyperinsulinemia and insulin resistance.

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Identification of variants in the genomic leptin receptor sequence, and a comparison of frequencies in African American NIDDM and control probands

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Obese, non-insulin dependent diabetes mellitus (NIDDM) phenotypes are manifest in animals with mutant leptin receptor, suggesting that leptin feedback may play an important role in an adipocyte-hypothalamus-beta cell axis of metabolic regulation. We have tested the hypothesis that genetic variations in the leptin receptor may be associated with human obesity and NIDDM, by screening a population of 15 morbidly obese hyperleptinemic French probands (diabetic and non-diabetic) for variants in the 5 leptin receptor isoforms, using single stranded conformation polymorphism (SSCP) analysis. Six variants were found, including 3 missense, 1 silent and 2 intronic variants; SSCP analysis of 15 African American obese (NIDDM and non-diabetic) probands revealed seventh variant, a 5 bp insertion in the leptin receptor b isoform 3'UTR. A population of 94 diabetic and non-diabetic African Americans (controlled for age and sex) were genotyped for the missense and 3'UTR variants, using PCR-RFLP and length polymorphism assays. Although allele frequencies in the lean and obese or NIDDM and non-diabetic phenotypes were not significantly different, evaluation using age of diabetes onset ($\leq$ 45 vs. > 45) yielded a p=0.026 for the K109R (26% vs. 12%) variant and p=0.026 for the Q223R variant (67% vs. 51%). Stratification of the diabetic age groups using BMI did not a show significant difference for either variant. Evaluation of the other 5 variants revealed no significant difference within the diabetic group (age of onset or BMI) or between the diabetic and control groups (diabetic status or BMI). These results suggest that an association may exist between early onset NIDDM and leptin receptor sequence variants.

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ASSOCIATION BETWEEN A POLYMORPHISM IN THE HORMONE-SENSITIVE LIPASE GENE AND THE METABOLIC SYNDROME

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Clustering of glucose intolerance, abdominal obesity and hypertriglyceridemia has been described as the metabolic syndrome (MS) and considered to have strong genetic background. Hormone-sensitive lipase (HSL; chrom 19q13.2) is the key enzyme in adipose tissue lipolysis. Genetic alterations in the HSL gene could therefore be involved in the pathogenesis of MS. To address this question we have studied whether a polymorphism in intron 7 of the HSL gene (15 alleles, amplified with radioactive PCR) is associated with MS in 101 unrelated patients with MS (53M/48F, age 60±13yrs, BMI 30.0±3.9 kg/m^2, WH 1.00±0.07 (men) and 0.89±0.05 (women), plasma triglycerides (TG) 4.8±2.6 mmol/l, 75% with NIDDM and $HbA1_c$ 7.6±2.1%), 204 unrelated patients with NIDDM but without signs of MS (99M/105F, age 63±15yrs, age at diagnosis 53±17yrs, BMI 23.9±2.0 kg/m^2, WH 0.92±0.05 (men) and 0.82±0.04 (women), TG 1.2±0.4 mmol/l and $HbA1_c$ 7.5±1.8%) and 197 unrelated healthy controls with no family history of NIDDM (100M/97F, age 56±11yrs, BMI 23.6±2.2 kg/m^2, WH 0.91±0.05 (men) and 0.79±0.05 (women), TG 1.0±0.4 mmol/l and $HbA1_c$ 5.2±0.5%). A significant difference in the allele frequency distribution (DF:7, p<0.005) was found between patients and controls but not between NIDDM and controls (cells of <5 alleles were combined). We therefore performed a linkage study in 39 Scandinavian families including 100 affected subjects (55M/45F, age 59±14yrs, BMI 30.0±4.0 kg/m^2, WH 1.00±0.05 (men) and 0.90±0.06 (women), TG 4.0±2.2 mmol/l, 60% with NIDDM and $HbA1_c$ 7.6±2.4%) and 87 unaffected subjects (49M/38F, age 53±19yrs, BMI 26.6±4.8 kg/m^2, WH 0.96±0.05 (men) and 0.85±0.06 (women), TG 1.7±0.7 mmol/l and $HbA1_c$ 5.4±0.9%). Using an IBD-APM approach, no linkage between the HSL gene and MS could be shown (Z-score -0.51). Finally, we screened the 9 coding exons of the HSL gene for mutations using SSCP in 30 subjects with MS. No variant forms of the gene were found. In conclusion, the association between a polymorphic site in the HSL gene and MS suggests a role for HSL in the pathogenesis of MS. Absence of linkage points at a polygenic etiology of MS, in which HSL together with other genes may increase the susceptibility to MS.

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AUTOSOMAL GENOME-SCAN FOR GENETIC LINKAGES TO ENERGY METABOLISM IN PIMA INDIANS

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Since both a low "relative" metabolic rate and a low fat/carbohydrate oxidation ratio are risk factors for weight gain and are familial traits, an autosomal search for linkages of energy metabolism to DNA markers was completed in Pima Indians, a population with a high prevalence of obesity. 24-hour energy expenditure (24EE), sleeping metabolic rate (SMR) and 24-hour respiratory quotient (24RQ) were measured in a respiratory chamber. 24EE and SMR were adjusted for fat-free mass, fat mass, age and sex; whereas, 24RQ, a measure of average daily fat vs carbohydrate oxidation, was adjusted for the effects of energy balance and percent body fat. Trait values were selected from multiple measures at the lowest body weight and when subjects were non-diabetic. Genetic linkage (236 sib-pairs from 82 families) was tested by single-marker sib-pair linkage (SM) and by multipoint interval mapping (MI) to quantitative traits. Two regions showed evidence of possible linkage to metabolic rate: a region near D20S103 to 24EE (LOD=2.7 by SM) and a region near D4S1629 to SMR (LOD=2.2 by SM). For 24RQ, possible linkages were found in in three regions: near D1S1631 (LOD=2.7 by SM), near D10S189 (LOD=1.5 by MI) and near D3S2387 (LOD=2.2 by MI). Searching for candidate genes underlying the variability in energy metabolism seems warranted in these regions of the genome.

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GENETIC POLYMORPHISM OF LIPOPROTEIN LIPASE GEN IN AN ABORIGINAL GROUP FROM TEMUCO, CHILE.

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Lipoprotein lipase (LPL) plays a crucial role in the lipoprotein metabolism. Several LPL gene polymorphisms have been found associated with lipid disturbances, premature atherosclerosis and cardiovascular disease. We have been investigating for the first time, the genotype distribution of LPL polymorphism in the Mapuche population, an aboriginal group from Chile, and their relation with lipids levels and obesity. We have determined the Hind III and Pvu II polymorphism by means of PCR and restriction fragment lenght polymorphism in 50 mapuche indians upper 20 years old without non insulin dependent diabetes mellitus (NIDDM). The homozigous genotype for Hind III with cut (+/+) was more frequent in this group (+/+=0.56, +/-=0.36 and -/-=0.08, p<0.05) for the Pvu II genotype the frequency between homozigous and heterozigous was similar (+/+= 0.46, +/-=0.54) .We analyzed the impact of both genotypes on plasma lipid levels and we found that the Hind III (+/+) genotype was associated with high levels of total cholesterol (TC) (54% of the indians with TC>200 mg/dl) and with high levels of Triglycerides (TG) (78% of the indians with TG>150 mg/dl were Hind III +/+). We did not find this relationship for the Pvu II polymorphism. Finally, we determine the association of these genotypes according to body mass index (BMI) and waist/hip ratio (WHR) respect to obesity in this population. Hind III (+/+) polymorphism was effectively associated with increase in BMI and high WHR (56% of the indians with BMI>30 Kg/m^2 and 60% of the indians with WHR>0.9, were +/+ for Hind III). Pvu II polymorphism was also related with high WHR in this group. In conclusion, we confirmed the association of genetic variation of LPL locus (Hind III polymorphism) with high lipid levels and with possible predisposition to obesity in this aboriginal population.

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AUTOSOMAL GENOME-WIDE SCAN FOR OBESITY SUSCEPTIBILITY GENES IN PIMA INDIANS

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An autosomal search for linkages of obesity to DNA markers was completed in Pima Indians, a population with a very high prevalence of obesity and NIDDM. Data collected on non-diabetic Pima Indians from 127 nuclear families provided 451 sib-pairs for linkage analysis. Fat distribution was assessed by waist/thigh circumference ratio and body composition by hydrostatic weighing. Trait values were selected from multiple measures at the highest body weight and adjusted for the effects of age and sex by linear regression. Genetic linkage for quantitative traits was tested by single-marker and multipoint sib-pair linkage. Regions containing markers or intervals showing evidence of linkage (LOD>1.2) were further analyzed by the multipoint methods of Mapmaker/Sibs. For waist/thigh ratio, four markers in intervals) — D1S2127, D3S1307-D13S218 and D13S887 — showed single marker linkage (1.2<LOD<2.6). Multipoint analyses did not result in improved evidence for linkage. For percent fat , eight markers (seven intervals)— D2S1363, D2S427, D5S1716, D11S2366, D14S1280-D14S617, D17S785 and D18S877 — showed single marker linkage (1.2<LOD<2.4). Multipoint analyses did not result in notably improved LOD scores for any region except the region near D11S2366. Here, the LOD score increase from 1.8 to 2.5 when analyzed by the variance estimation method. This linkage at 11q21-q22 is the best evidence for a gene influencing body composition in Pima Indians.

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The novel lipoprotein lipase(LPL) activating compound NO-1886 prevents weight gain in high-fat fed NIDDM model rats

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[Aim] The accumulation of fat in the internal organs is an important risk factor of diabetes. We examined wether the novel LPL activating compound NO-1886 prevents the accumulation of fat in the internal organs or not.

[Method] SD rats(9 months, n=6) were fed a high fat diet containing NO-1886(50mg/kg) for 3 months. After 3 months we measured respiratory quotient (R.Q.) and took blood sample, heart and fat in the internal organs and subcutaneous tissue. We also measured the LPL activity and the total amount of fat in the organs and subcutaneous tissue.

[Result] After high-fat feeding, the total amount of fat in the internal organs increased 3.3 times of normal rats. High-fat fed rats with NO-1886 increased 1.5 times of normal rats. At the same time NO-1886 prevented the accumulation of subcutaneous fat. NO-1886 increased the activity of LPL in heart, but did not increase the activity of LPL in fat tissue. R.Q. decreased significantly in NO-1886 administrated gruop (by Dunnett's-test).

[Conclusion] The novel LPL activating compound NO-1886 prevented the accumulation of fat in the internal organs and subcutaneous tissue. This phenomenon is thought to result from the increased combustion of fat as evidenced by the decreased rate of R.Q.

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CANDIDATE GENES FOR HUMAN OBESITY: ASSOCIATION AND SIB-PAIR STUDY

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The aim of our study is to identify major genes predisposing to human obesity and to investigate their metabolic effects. We studied the possible association between microsatellite markers near the *ob* gene and morbid obesity in 252 morbidly obese patients (mean BMI 43 ± 7 kg/m^2) and 151 lean controls (mean BMI 22 ± 2 kg/m^2). The genomic regions homologous to murine obesity genes *ob, db, tubby, agouti and RIIβ* were analyzed in 102 affected sib-pairs (BMI ≥ 32 kg/m^2). Highly polymorphic microsatellite markers flanking the areas of interest were analyzed on polyacrylamide gels using radioactive PCR products. Serum leptin levels of the probands were determined by radioimmunoassay. Markers of the human *ob* gene region failed to show association with morbid obesity, whether tested in the case-control study or the affected-sib-pair analysis. There was a strong positive correlation between serum leptin levels and BMI in morbidly obese women ($r = 0.41$, $p = 0.0001$) and men ($r = 0.56$, $p = 0.0001$). A carrier status for either of the two most prevalent alleles of the microsatellite marker D7S530 in the vicinity of the *ob* gene were associated with serum leptin levels in the obese subjects ($p = 0.04$). One allele of the *ob* gene marker D7S649 showed a statistically significant association with the amount of weight lost ($p = 0.006$) on a 16-week weight-loss program. The affected-sib-pair study of the murine homologous regions failed to show linkage. In conclusion, in contrast to some earlier studies we were not able to find any association between the *ob* gene area and obesity although two different approaches were used. Serum leptin levels were statistically significantly associated with BMI even in morbidly obese subjects, and allelic variation near the *ob* gene is associated with minor variations in leptin levels and response to treatment for obesity. A candidate gene approach based on murine obesity genes could not provide evidence favoring the role of equivalent genes in human obesity.

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LIPOPROTEIN LIPASE (Asn291→ Ser) MUTATION IN CZECH NON–INSULIN DEPENDENT DIABETES MELLITUS PATIENTS

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Lipoprotein lipase (LPL) is the key protein in the clearance of plasma triglycerides. Recently seven genetic variants of the lipoprotein lipase gene has been found, which may affect the individual´s lipaemia in obesity and NIDDM and other hyperlipidaemias. The LPL (Asn291→Ser) mutation was found in 9,3% of Dutch patients with familial combined hyperlipidaemia and those carriers had signif. higher plasma and VLDL–triglycerides, VLDL– and HDL–cholesterol. Therefore we searched for the occurence of this mutation in Czech NIDDM patients (n=95). The LPL (Asn291→Ser) mutation was detected by PCR and Rsa I restriction. Six of NIDDM patients were heterozygous for this mutation (6,3%). Our carriers and non–carriers did not differ in levels of total serum cholesterol (6,40 ± 2,00 vs. 6,64 ±1,42 mmol/l), HDL cholesterol (1,22 ± 0,32 vs. 1,32 ± 0,38 mmol/l) and triglycerides (2,61 ± 1,91 vs. 2,42 ± 2,15 mmol/l). The only difference between those two groups was found in systolic blood pressure (130 ± 14 vs. 141 ± 24 mmHg, p=0,04, Student´s t test). It is obvious that the LPL (Asn291→Ser) mutation could contribute to elevated plasma lipids, but this polymorphism is the only one out of several causes giving rise to this pathological state.

Supported by grants IGA MH CR 3414–3 and COST B.5.

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SCREENING OF CANDIDATE GENES INVOLVED IN REGULATION OF THERMOGENESIS AMONGST GESTATIONAL DIABETIC SUBJECTS.

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Women with previous gestational diabetes mellitus (GDM) have a substantially-increased risk of overt NIDDM in later life.GDM is associated with a reduction in postprandial thermogenesis (PPT) compared to pregnant control women: this reduction continues in the non-pregnant state. This defect in PPT may reflect variation in genes regulating thermogenesis and contribute to progression to NIDDM. We are screening the genes *B3AR* (β_3 adrenergic receptor) and *UCP* (uncoupling protein) to document the total extent of candidate genetic variation in (i) 40 women with GDM (mixed ethnicity) and (ii) a cohort of Bengali women, 21 with GDM and 50 with normal glucose tolerance during pregnancy. Single-stranded conformational analysis has not identified any novel significant variations in the coding regions of the *B3AR* gene; four variants found within exons of the *UCP* gene are under evaluation. In the small cohorts used for mutation screening, we see no increase in prevalence of the previously-reported variants in *B3AR* (Trp64→Arg) and *UCP* (A→G;-3826bp) in GDM subjects. However, within the Bengali control women, we find significant associations between weight at 30 weeks' gestation (the only weight measure available for these women) and presence of each of the variants (*B3AR* : 58.0 (12.7)kg [Trp/Trp] vs 63.8 (20.9)kg [Trp/Arg], p=0.035; *UCP*: 55.0 (9.6)kg [A/A] vs 59.5 (13.0)kg [G/-], p=0.034) (data as median (IQR): analysis by Mann-Whitney U). To the extent of the survey completed and the sensitivity of the methods employed, we have identified no novel variants in either the *B3AR* or *UCP* genes thus far. The associations with weight seen with the known mutations offer confirmation of previous findings in a new ethnic group: this supports a direct pathogenic role for these variants. Further studies of additional aspects of these genes in larger cohorts are in progress.

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LDL PARTICLE SIZE AND APOLIPOPROTEIN E POLYMORPHISM IN NONDIABETIC JAPANESE-AMERICANS

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Small dense LDL is associated with insulin resistance syndrome and is considered a risk factor of coronary heart disease. A recent study suggested that LDL particle size is associated with apolipoprotein E (apoE) polymorphism. We examined the LDL particle size, apoE phenotype and lipid metabolism in nondiabetic Japanese-Americans. LDL particle size was determined on plasma samples by the method of Krauss and Burke. The apoE phenotype was determined on plasma samples by using isoelectric focusing followed by immunoblotting. Phenotypes were subdivided into three groups : E2 phenotype (E2.2+E3.2), E3 phenotype (E3.3), and E4 phenotype (E4.3+E4.4). The mean LDL particle size was similar in men being 254.4, 259.7, and 258.2 Å for phenotypes E2, E3, and E4, respectively, but the women with the E4 phenotype showed significantly smaller LDL particle size (259.6Å) than those with the E3 phenotype (264.2 Å)($p<0.05$). LDL particle size did not show any correlation with fasting insulin (FIRI), triglyceride (TG), or HDL-cholesterol (HDL-C) in either men or women with the E2 phenotype. On the other hand, LDL particle size was significantly associated with HDL-C positively and with TG and FIRI negatively in both men and women with the E3 and E4 phenotype ($p<0.0005$). Furthermore, LDL particle size was significantly correlated with FIRI in women ($p<0.05$) but not in men with E4 phenotype after adjustment of TG, HDL-C and body mass index (BMI). Thus, FIRI, TG and HDL-C might be associated with LDL particle size in both men and women with the E3 and E4 phenotypes. Insulin resistance might play an important role for determining LDL particle size in women with the E4 phenotype.

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THIS ABSTRACT HAS BEEN WITHDRAWN BY THE AUTHOR.

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THIAZOLIDINEDIONES PROMOTE THE DIFFERENTIATION OF HUMAN BROWN PREADIPOCYTES *IN VITRO*.

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Thiazolidinediones are insulin-sensitizing compounds effective in the treatment of NIDDM. They are ligands for the nuclear hormone receptor PPARγ, a transcription factor expressed at high levels in white and brown adipocytes. Thiazolidinediones are adipogenic *in vitro* but animal and human studies do not indicate significant increases in total fat mass. One possible explanation for this could be that the compounds stimulate brown adipose tissue, as demonstrated in animal studies, and hence increase overall energy consumption. Using UCP as a specific marker, we have investigated the distribution of brown adipocytes in adult humans. Using RT-PCR, UCP mRNA was measured in total RNA from isolated adipocytes prepared from perirenal, omental and subcutaneous adipose tissue biopsies. UCP mRNA was detected in 7 out of 10 omental, 3 out of 3 perirenal and 5 out of 12 subcutaneous samples. We have also investigated the effect of thiazolidinediones on differentiation of brown adipocyte precurser cells obtained from the same depots. UCP mRNA abundance was compared by semi-quantitative RT-PCR in total RNA isolated from cells differentiated for 10-20 days in the presence of thiazolidinedione compound BRL 49653 or vehicle. Compared to the vehicle-treated cultures thiazolidinedione treatment induced an increase in UCP gene expression in 4 out of 5 omental samples, 7 out of 8 subcutaneous and 3 out of 3 perirenal samples. In addition, immunocytochemistry demonstrated UCP protein in perirenal preadipocytes cultured for 5 days in the presence of BRL 49653. The detection of UCP mRNA in total RNA from cells isolated from adult human 'white' adipose tissue depots demonstrates that brown preadipocytes and brown mature adipocytes are present within these sites. This is the first demonstration of UCP in human subcutaneous adipose tissue. Furthermore, these data suggest that thiazolidinediones promote the *in vitro* differentiation of human brown preadipocytes derived from different adipose depots.

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Epidemiology of NIDDM and IGT

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TRIVANDRUM DISTRICT DIABETES SURVEY:PREVALENCE OF KNOWN DIABETES IN DIFFERENT POPULATION

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This study for the prevalence of known diabetes was carried out to get an insight in to the depth of diabetes problem in Trivandrum District of Kerala State in South India. A house to house survey for patients with known diabetes was made at all residents with in the defined areas of urban Trivandrum City, Semiurban Nedumangad Muncipal Town and the rural Karode Panchayath of Trivandrum District. 15250 urban, 31349 Semiurban and 17128 rural people were surveyed and the number of known diabetic patients among them were 610,504 and 103 respectively. The prevalence of known diabetes was 9.8% for urban, 3.4% for semiurban and 0.9% for rural in all subjects aged 40 or over and the prevalence rose to 19.2%, 8.1% and 1.4% respectively in those aged 60-69. The overall crude prevalence of known diabetes was 4% for urban, 1.6% for semiurban and 0.6% for the rural population. The findings reveal the high magnitude of diabetes problem in the urban population when compared to the semiurban and rural population.

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ANALYSIS OF MORTALITY IN DIABETIC PATIENTS COMPARED TO THE GENERAL POPULATION

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We calculated the standardised mortality ratio (SMR) in a sample of diabetic patients based on the annual death rates of the general population from England and Wales. Using a computerised diabetic clinical records system which has been recently linked to the National Health Services Central Register in U.K., we have identified all patients who died between 1980 and 1995. 802 out of 4633 patients (17.5%) attending the clinic died over this 15 year period. The cause of death was retrieved from death certificates. The overall SMR was 1.47 [95% Confidence interval 1.31~1.62] for women and 1.14 [95% Confidence interval 1.04~1.25] for men. The SMR was 2.11 [95% CI 1.67~2.55] for ischaemic heart disease and 2.77 [95% CI 2.24~4.96] for cerebrovascular disease in patients <65 years. The SMR was 0.89 [95% CI 0.65~1.25] for cancer related deaths. We conclude that excess mortality in diabetic patients is higher mainly due to increased mortality in diabetic women compared to the general population. As expected vascular events contributed to the high mortality seen in diabetic patients, but cancer related mortality was not significantly different from that in the general population.

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THE PREVALENCE OF DIABETES AMONG ABORIGINAL POPULATION OF CHUKOTKA PENINSULA

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The main task of the study was to reveal the prevalence of diabetes among Chukotka Natives according to data of official statistics and screening. The first diabetic native person was registered in 1983. At this moment the prevalence of diabetes among people older than 15 years does not exceed 2 cases per 1000 inhabitants. The survey, with the use of standard (75 g) glucose load, was conducted at 1991 year. Overall 55% of native male and female costal population of Chukotka, 25-64 years old, in four villages, were randomly selected and invited to participate. The response rate was 65,5%. We examined 170 men and 192 women. The average fasting glucose level among women-5,05 mmol/l(95% CI 4,92-5,18) was higher than among men-4,77(4,65-4,89), as was true of the average postloading level and of the fasting insulin level. We confirmed just one case of NIDDM concerning eskimo woman during the screening. The prevalence of IGT was 1.2% in men and 4.2% in women. These data indicate the lowest diabetes prevalence among Natives of Chukotka in comparison with the other circumpolar native populations.

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CAUSES OF DEATH AMONG DIABETICS IN THE STATE OF SÃO PAULO, BRAZIL: AN ANALYSIS OF MULTIPLE CAUSES OF DEATH.

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Death certificates from the State of São Paulo, an industrialized area in the Southeast of Brazil with a population of 30 million inhabitants, were analysed through the system ACME - Automated Classification of Medical Entities, for the year 1992. From 202,141 deaths, diabetes was mentioned in 13,786 (6.8%), being the underlying cause in 5,303 (2.6%). The proportion was higher for women than men (10.1 vs 4.6% as mentioned and 6.1 vs. 2.9% as underlying cause). Among deaths with mention of diabetes, the main underlying causes were diabetes (38.5%), cardiovascular (37.2%) and respiratory diseases (8.5%), and neoplasias (3.8%). When diabetes was the underlying cause, the most common associated causes were cardiovascular (42.2%) genitourinary (10.8%) and respiratory diseases (10.7%). As associated cause, the most common underlying causes were cardiovascular (62.9%) and respiratory diseases (12.6%), and neoplasias (6.9%). Among deaths by neoplasias, with diabetes associated, it calls attention the high proportion of pancreatic cancer (10%), corroborating the hypothesis of a possible role of diabetes as a risk factor for this neoplasia. In spite the limitations of death certificates they could provide useful information about the importance of diabetes as a health problem. Also, the analysis by multiple causes of death gives an idea of the morbidity profile associated with diabetes by the time of the death, emphasizing the high frequency of cardiovascular diseases among these individuals.

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EPIDEMIOLOGY OF DIABETES , IMPAIRED GLUCOSE TOLERANCE AND CARDIOVASCULAR RISK FACTORS IN NORTH-EAST MALAYSIA

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Two thousand five hundred and eight subjects from the state of Kelantan in North-East Peninsular Malaysia were included in this study to determine the prevalence of diabetes mellitus and impaired glucose tolerance and their association with cardiovascular risk factors. Oral glucose tolerance test was performed on all subjects. The overall prevalence of diabetes mellitus was 10.5% and impaired glucose tolerance was 16.5%. There was no difference in the prevalence of diabetes mellitus between the males (10.5%) and females (10.5%) but the prevalence of impaired glucose tolerance was higher in the females (19.0%) than in the males (11.5%). The prevalence of both diabetes mellitus and impaired glucose tolerance was higher in those above 60 years' age group (DM- 30.3%; IGT-36.9%). Subjects with diabetes mellitus were more obese (38.4%) than normal subjects (24.1%). They also had a higher prevalence of hypertension (14.8%) and hypercholesterolaemia (71.9%) than normal subjects (hypertension-7.0%; hypercholesterolaemia-57.0%). Subjects with impaired glucose tolerance also had a higher prevalence of obesity (35.5%), hypertension (10.4%) and hypercholesterolaemia (63.0%) than normal subjects. Females were more obese than males in the diabetes mellitus (42.6% vs 29.9%), impaired glucose tolerance (38.5% vs 26.3%) and normal subjects (27.4% vs 18.1%).

In conclusion, the prevalence of diabetes mellitus and impaired glucose tolerance was high and they were associated with a high prevalence of obesity, hypertension and hypercholesterolaemia.

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MORTALITY OF JAPANESES-BRAZILIAN WITH AND WITHOUT NON-INSULIN DEPENDENT DIABETES MELLITUS IN A COHORT STUDY.

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As a part of a prospective study on Japanese migrants, living in a developed city in the Southeast of Brazil, we describe and compare four-years experience of mortality among diabetic and non-diabetic subjects. In 1993, a cohort of 530 first and second-generation of Japanese-Brazilians of both sex, aged 40 to 79 years old, were identified; at the beginning, 91 subjects (17%) were classified (according to WHO criteria) as non insulin-dependent diabetics (NIDDM), 90 (17%) as impaired glucose tolerance (IGT) and 349 (66%) as normals. An up date of mailing list allowed to identify those who died during this period. Family information and death certificates were used to record the date and the causes of death. The mortality rates for all causes and for specific causes (ICD-9 code 390-459: circulatory disease and ICD-9 code 580-599: renal disease) were obtained for the three groups of subjects. Proportional hazards regression models were used to compare the mortality rates, adjusted to several covariates (sex, age, generation, hypertension, dyslipidemia, obesity and plasma creatinin). The crude mortality rates for all causes for NIDDM, IGT and normal subjects were 31.5, 6.9 and 10.7 per 1,000 person-year and the mortality rates for specific causes were 24.7, 3.4 and 5.4 per 1,000 person-year, respectively. After simultaneous adjustments to the covariates, no difference was observed in the mortality experience for all causes between NIDDM and IGT subjects as compared with normals. However, higher mortality rates due to specific causes were observed among NIDDM than in the normal subjects (Incidence Density Ratio: 3.86; 95%CI: 1.11 - 13.38). These results in Japanese-Brazilians are consistent with previous reports of increased mortality in other diabetic populations, independently of other classical cardiovascular risk factors.

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NON-INSULIN DEPENDENT DIABETES MELLITUS IN KUWAIT - A PREVALENCE STUDY

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Non-insulin dependent diabetes mellitus (NIDDM) is a major public health problem in Kuwait. The objective of the study is the estimation of the prevalence rate of glucose intolerance among a sample of the national adult population aged 20 years and above in two out of the five Governorates of Kuwait. A total of 3007 subjects (1106 men and 1901 women) were interviewed by a physician who completed a standard questionnaire and carried out a physical examination which included height, weight and blood pressure measurements. Blood tubes were centrifuged immediately and refrigerated. Interpretation of oral glucose tolerance tests were based on the WHO diagnostic criteria for DM, 1985. The study span was between September 1995 to June 1996. The denominator used for computing the prevalence was obtained from the 1995 Kuwait census. The overall prevalence of glucose intolerance (IGT and NIDDM) in this study was found to be 17.78 (17.3% in men , 18.1% in women). In the population survey glucose intolerance was presented at a relatively young age, prevalence rate in the age group 20-39 was 8.18% (95% CI, 6.6 - 9.7) and in the age group 40 - 59 was 22.10% (95% CI 19.6 - 24.6). Obesity was a significant risk factor, ($p < 0.001$; as a consequence of recent life style and dietary changes. The strong association in patients with a positive family history of NIDDM (adjusted odds ratio = 2.4, $p < 0.001$) strongly suggests a genetic component. Hypertension was markedly associated with the relative risk of developing NIDDM (adjusted odds ratio = 1.5, $p < 0.001$) with the demographic transition which already started among the Kuwaiti population and if the prevalence of glucose intolerance remains the same, ageing of the population will contribute to an even more accentuated upward trend in the prevalence of glucose intolerance and its serious impact on the morbidity and mortality among the Kuwaiti population. The strong association between hypertension and NIDDM may recommend a common approach to the prevention and control of these two conditions.

This study was supported by grant MM 024 from Kuwait Universiy.

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TRENDS OF METABOLIC EMERGENCIES ADMITTED IN A CENTRALISED INTENSIVE CARE UNIT FOR DIABETIC PATIENTS. M.Grigorescu, E. Farcasiu, A. Barnea, I.Limbasanu and C.Ionescu-Tirgoviste, Clinic of Diabetes, Institute "N.C.Paulescu", Bucharest, Romania

The aim of our study was to analyse the demographic characteristics of diabetic patients admitted consecutively for various types of metabolic emergencies in three different years at ten years interval (1974,1984,1994). The main data are presented in the following table:

Characteristics	1974	1984	1994
No. of cases	825	931	762
Sex distribution (M/F)	382/543	411/520	351/411
Mean age ± SD	42.3 ± 21.6	49.5 ± 19.6	56.5 ± 22.1
< 20 yrs.	292	274	82
21-42 Yrs.	316	317	199
> 40 yrs.	217	340	481
Type of diabetes:			
IDDM	572	478	346
NIDDM	253	453	416
Type of emergency:			
- Diabetic ketoacidosis	511	391	232
- Hypoglycemic coma	76	85	109
- Hyperosmolar coma	29	56	72
- Myocardial infarction	28	76	114
- Cerebrovascular accident	67	71	94
- Others	114	252	141

Conclusions: The principal changes noted along the years were: dimunition of the frequency of emergencies in youths and an increase in older age groups; reduction of the percentage of ketoacidosis with an increase in the percentage of hypoglycaemic and hyperosmolar comas (the latter is often associated with myocardial infarction and cerebrovascular accidents). Mortality in the first 24 hrs. decreased from 12%,7% and 5% respectively.

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INCIDENCE OF NIDDM AND THE EFFECTS OF GENDER, OBESITY AND HYPERINSULINEMIA IN TAIWAN CHINESE S.-L. Wang and W.-H. Pan. Institute of Biomedical Sciences, Academia Sinica, Taipei, TAIWAN

Objective: The aim of this study is to determine NIDDM incidence in Taiwan and to examine its relation to obesity and hyperinsulinemia in men and women. **Methods:** A total number of 995 men and 1195 women aged 35-74 years free from diabetes in two townships in Taiwan were followed up to 5 years from 1991 to 1996. Baseline data include a questionnaire on general and metabolic information, the assessment of detailed anthropometric parameters and plasma glucose and insulin. Multivariate regression analysis was performed to examine the effect of the risk variables on NIDDM incidence. **Results:** The age-standardized incidence rate was 9.45/1,000 in men and 9.13/1,000 in women. Plasma glucose at baseline had RR of 3.0 in men and 2.9 in women. In men, waist circumference (RR=2.2) and plasma insulin level (RR=2.1) had the highest standardized RR values for diabetes incidence next to glucose. In women, both sub-scapular skinfold and Body Mass Index (BMI) had a notable impact on diabetes incidence with relative risk of 3.1 (C.I. 2.1-4.6) and 2.8(C.I. 2.1-3.8) respectively; whereas insulin had a moderate effect with RR of 1.5 (C.I. 1.1-1.9). In women the incidence increased with a dual occurrence of obesity and hyperinsulinemia (12 folds, compared to 3 folds in men) compared to those with only obesity (2.8 folds, 3 in men) or hyperinsulinemia (4.4 folds, 3 in men). **Discussion:** The present study demonstrated a slightly larger incidence of NIDDM in Taiwan than in western countries with comparable age distributions. The importance of obesity is indicated in preventing NIDDM in the community. Hyperinsulinemia played a significant role in NIDDM incidence independent of obesity. Moreover, the effect of synergistic interaction of hyperinsulinemia and obesity was profound in women.

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Diabetic admissions in rural Zaire.

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Case records of diabetic admissions to the Centre Médical Evangelique were reviewed in the light of clinical observation that suggested diabetres presented in a different manner to that seen in 'Western countries'. The case records were retrieved for 60 out of 61 diabetic admissions between November 1994 and February 1996. 53 patients were admitted once, 2 were admitted twice and 1 patient was admitted 3 times. 56 individuals needed inpatient treatment for their diabetes during the study period. 59% were male and 41% were female. Median age at admission was 47 years (range 16 - 74 years). Diabetes was relatively uncommon below the age of 35 years (17% stating an age of onset below 35 years). 35 % of admissions were new cases of diabetes. The main reason for admission was symptomatic hyperglycaemia without ketoacidosis (71.6%). Other reasons for admission included ketoacidosis (8.3%) and various infections (11.7%). 5 (8.3%) patients died during their admission, 2 from renal failure and three from infection. There were no deaths from ketoacidosis. On admission the majority of patients were taking no treatment but 71.7% needed insulin to control their symptoms and return the blood glucose to normal. The two most striking observations were firstly the rarity of ketoacidosis even amongst younger diabetics and secondly the high proportion of patients who needed insulin to control their symptoms and normalise their blood glucose levels. Insulin was relatively expensive ($20-30 a month) during the study period and so it was only used after a full trial of available oral hypoglycaemic agents had failed to control the situation. Many of these patients do not fit readily into Type-1 or Type-2 diabetes and it may be that so-called 'Tropical Diabetes' is the predominant form of the disease seen in Northeast Zaire. Tropical diabetes has been linked to cassava consumption and this crop provides the main staple diet in the region. A further prospective trial may help to clarify the situation.

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DIABETES IN JAPANESE POPULATIONS - A COMPARISON BETWEEN JAPAN AND BRAZIL. LC Iochida, LF Marcopito, LJ Franco, A Sekikawa, M Tominaga, H Eguchi and H Sasaki. Universidade Federal de Sao Paulo, Sao Paulo, Brazil and University of Yamagata, Yamagata, Japan.

Migrant studies are powerful tools in epidemiological studies. However, comparisons between data obtained in different countries are difficult, due to differences in methods and populations. Japanese were considered of relative low risk for NIDDM, until the early 1980's, but ethnic Japanese migrants showed very high prevalences of the disease. This study compares two genetically identical Japanese populations (Yamagata, Japan and Bauru, Brazil), in the same age-group and with the same diagnostic criteria. Data were collected during 1991-93 in Japan and in 1993 in Brazil. Individuals of both sexes, aged 40-79 years, were submitted to OGTT with 75g glucose load after fasting for 10-16 hs. Plasma glucose was determine by the glucose-oxidase method on fasting and 2h after load samples. DM and IGT were diagnosed by WHO criteria. Poisson regression was used to analyze the data. Participation rate was of 74.5% in Japan and 83.3% Brasil. Number of participants (n) and cases, and prevalences of DM and IGT (%), are shown in the table.

	Japan			Brazil		
	n	DM	IGT	n	DM	IGT
Men	1146	104 / 9.1	137 / 12.0	263	60 / 22.8*	37 / 14.1
Women	1478	160 / 10.8	244 / 16.5	267	33 / 12.4	49 / 18.4
Total	2624	264 / 10.1	381 / 14.5	530	93 / 17.6*	86 / 16.2

*p<0.05

The Japanese-Brazilians showed significantly higher prevalence of DM, with a higher prevalence in males, controlling for age. The higher prevalence of DM in the Japanese-Brazilians points to the influence of the "western" environment in the development of the disease.

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Hospitalized diabetic patients. Clinical epidemiology

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The aim of the study was to determine the prevalence and clinical characteristics of the diabetic patients hospitalized in the Hospital de Clínicas and to compare them with the non diabetic patients in a one day survey. A census was taked at the whole hospitalized population. The patients were divided in: 1) Non diabetics (Nd) and 2) Diabetics (D).The group Nd was analyzed in age, sex and hospitalization length. The group D was studied about living place, hospitalization area and clinical and diabetic characteristics (type, metabolic control, chronic complications, treatment). The statistic analysis was the non paired t test (significative $p \leq 0.05$). The prevalence was 13.3% of hospitalized D patients (37:279). The duration of hospitalization was 25 days for D vs. 21 Nd. The age was 65 years vs. 61 in D and Nd respectively. The ratio male/female 2,7/1 in D y 0,98/1 in Nd. The admission was related with diabetes in 51% (59% in male and 30% in women). The chronic complications and predominant related illness were: hypertension (19%), peripheral artery disease (12%), infections (12%), coronary artery disease (11%), diabetic foot (11%), retinopathy (10%), amputation (10%).The ratio of diagnostic age <30/>30 years was 1/10. The diabetes duration was over 10 years in 46%, 27% between 1 and 10 years and 27% less than 1 year. There were 24% insulin users (71% pork), 66% take oral hypoglycemic agents and the 85% were in a dietetic plan. The diabetic population of the Hospital de Clínicas has its own characteristics related with the kind of patients that consult or are referred, and the specialized servicies. There is a high prevalence of diabetes in hospitalized patients, 20% in males, mainly with podal and vascular pathology. Frecuently coexist cardiovascular disease, insulinic treatment and frecuent hospitalizations, denoting a high risk population that need special atention.

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NORTHERN CYPRUS: ANOTHER HIGH PREVALENCE AREA OF DIABETES AND IMPAIRED GLUCOSE TOLERANCE IN THE MEDITERRANEAN

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A nationwide epidemiology study was carried-out to determine the prevalence of diabetes mellitus and impaired glucose tolerance (IGT) in randomly selected 2726 (urban; 52%, rural; 48%) Cypriot subjects, aged 20 years (overall response rate 73%). Female to male ratio was 1678/1048. Based on WHO's criteria (2h capillary blood glucose after 75g OGTT), subjects were defined as diabetic (DGT), IGT, or normal glucose tolerant (NGT). According to "SEGI's world population" (95% confidence limits), an overall prevalence of 11.3% (11.3% female, 11.3% male) for DGT and 13.5% (16.1% female, 9.2% male) for IGT was found. No urban/rural difference was observed. Diabetes prevalence increased with age, being highest in the 75 years age group (36%), no gender difference was noticed. In contrast, IGT was found to be highest in the 70-74 years age group and more prevalent among female than in male (20% vs. 15%). The prevalence of known diabetes was 7.3%, additionaly nearly one-third of the diabetics was unaware of their disease (4%). Of the screened population, a positive family history for diabetes was found in 49% of DGT, in 47% of IGT and in 41% of NGT subjects. Prevalence of hypertension was higher both in DGT (34%) and in IGT (20%) than in NGT (13%). The age-standardized prevalence of diabetes increased clearly across tertiles of both body mass index and waist to hip ratio and it was also found to be higher in subjects with lower physical activity, lower income and lower education (p=10-8). Compared to other Eastern Mediterranean countries, particularly to Turkey, this study have indicated that diabetes is a common health problem in Northern Cyprus. The finding of higher prevalence rate of both DGT and IGT will certainly have an implication in establishing a national diabetes program in order to improve the quality of diabetes care in this country.

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NONINSULIN DEPENDENT DIABETES OF YOUNG (NIDDY)-OVERLAP WITH MALNUTRITION RELATED DIABETES(MRDM) : S.S.GUPTA, DIABETES CARE CENTRE, NAGPUR, INDIA

Study aims to correlate factors which might be responsible for some of differences seen between young diabetics of tropics and of West. Of 1760 diabetics, 237 NIDDY and MRDM with onset of diabetes(DM) before 40Yrs age were selected and correlated clinically. NIDDY were 190(80.2%), PDDM 39(16.5%) FCPD 8(3.3%). BMI in NIDDY male (m)-24.7±4.3kg/m^2,female(f)-26.9±5.4;kg/m^2;PDDM m-17.9±3.4,f-16.9±2.5;FCPD m-20±2.3,f-18.2 Waist hip ratio in NIDDY m-0.97±0.07,f-0.95±0.13;PDDM m-0.89±0.05,f-0.87±0.06;FCPD m-0.92±0.02,f-0.86. Duration of DM in NIDDY-4.4±5.6Yr PDDM 2.8±3.7Yr FCPD-4.3±4.9Yr. Family history of DM present in 69.7% NIDDY and 25%FCPD while absent in PDDM.80% NIDDY and 50% FCPD responded to sulfonylurea(SU) while PDDM didn't. 25.8% NIDDY had hypertension (HT),3.7% had coronary artery disease which were absent in MRDM. Macroangiopathy was absent in NIDDY;12.5% FCPD & 5.1% PDDM had retinopathy + nephropathy.Thus, tropical NIDDY have more of central than generalised obesity seen in West. FCPD have genetic predisposition, they do respond to SU,thus overlap NIDDY.Microangiopathy appear early while macroangiopathy and HT is rare in MRDM. Meaning,tropical diabetes, though not a homogenous entity, does have certain characteristics of it's own.

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PREVALENCE OF DM IN DEAN FUNES,CORDOBA,ARGENTINA.De Loredo,L;Luquez,H;Madoery,R;Rolter,H;Sereday,M de;Libman,C. Universidad Nacional de Córdoba.

The objective was to assess the prevalence of DM and his association with others risk factors of cardiovascular disease,mainly obesity, hypertension, dislipidaemia,insulin resistance and sedentarism.A random sample of 388 persons aged 30-70 y, was selected by a multietapic,sistematic and sex-age representative method,from a population of 18511 inh.with 95 and 2.3% precision.Fasting capillary glucose was measured by Haemoglukotest 20-800 and Reflolux II.Values≥7.8 mmol/l were, confirmed by venous plasma glucose by autoanalizer.Values≥5.5<7.8 mmol/l and one every six <5.6 mmol/l were submitted to the OGTT.WHO recommendations and diagnostic criteria for DM were applied.Known diabetics were identified as having a confirmed diagnosis and were treated.Obesity was diagnosed by NHANES III criterion:BMI≥27.3 for women and≥27.8 for men.Insulin-resistance was measured by fasting plasma insulin by ELISA.WHO criteria was applied for hypertension and Argentine Forum for Dislipidaemia criteria for lipids alteration.The X^2 test was used to characterize significant differences.The total prevalence of DM was 8.2%(9.7%M,6.9%W)and increased with age from 3.4%(30-39y)to 19.2%(60-69y);62.5% were known diabetics.Obesity was present in 68.8% of diabetics(D)and 47.2% of non diabetics(ND)(RR 1.45 CI 1.12-1.88).Hypertension was associated with DM in 56.3% and in 38% with ND.(RR 1.48 CI 1.06-2.0).Fasting insulin was higher in D:$\bar{x}$ 14.06 μU/ml(SE 1.52) than in ND:$\bar{x}$ 10.84 μU/ml(SE 0.40)p<0.02.There was sedentarism in 43,8% of D and in 27,2% of ND(RR 1.94 CI 1.0-3.7).There was no significant differences for dislipidaemias.In conclusion the prevalence was similar to the most recent data from South America.As a expected there was a significant association of DM with obesity,hypertension,fasting insulin and sedentarism.

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PREVALENCE OF DIABETES BY THE CAPTURE-RECAPTURE TECHNIQUE-ADVANTAGES AND LIMITATIONS

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Capture-recapture (C-R) is a promising method for assessment of diabetes prevalence, but has not been widely used or validated. Aims: We have explored the accuracy and feasibility of C-R in determining community diabetes prevalence in an urban area of North Liverpool, UK. In a recent 12 months period, we obtained lists of diabetic patients from three sources - Diabetic Clinic, Hospital Discharges and General Practioner (GP) lists. Other clinical data was recorded where possible. Names were computer-matched by surname, date of birth and post-code. C-R calculations were determined by log-linear modelling. Total population sampled was 178,365. Diabetes "captures" were 1,692 (Diabetes Centre), 486 (Hospital Discharges) and 702 (GP lists). Estimated known diabetes prevalence was 2.63% (95%CI 2.56-2.70%). Mean age was 57.6 years (SD=16.1); female:male ratio was 1:1.2; 82% were NIDDM and 18% IDDM; treatment was diet 25%, tablets 42%, insulin 33%; and prevalence increased markedly with age. This study shows a relatively high diabetes prevalence, with close confidence intervals. C-R is a rapid and cheap method for diabetes prevalence determination, provided easily computer-accessible lists are available. Ideally, results should be validated by other epidemiological techniques.

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HIGH PREVALENCE OF DIABETES MELLITUS SECONDARY TO ACUTE NECROTIZING PANCREATITIS

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The prevalence of diabetes mellitus (DM) secondary to chronic pancreatitis is known to be high, whereas few and controversial information exists about the diabetogenic potency of acute necrotizing pancreatitis (ANP).
A follow-up study was performed to investigate prevalence and risk factors of impaired glucose tolerance (IGT) in patients after ANP. Of 124 patients with a single episode of ANP 28 had died, 19 were lost for follow-up and from the remaining 76 patients glucose tolerance and clinical parameters were assessed in 37 patients (follow up: 4.0±2.9 years). After exclusion of patients with overt diabetes an oGTT was performed according to WHO criteria, with a result defined as borderline if blood glucose was >200mg/dl in any of the measurements. A clinical risk score including family history of DM, signs of metabolic syndrome before ANP and initial therapy was defined for each patient (range: 0-5).
Only 11 (30%) out of 37 patients had normal glucose tolerance, 14 (38%) had overt diabetes, 6 impaired (16%) and 6 borderline (16%) glucose tolerance. All patients with risk scores 0 and 1 (n=8) had normal glucose tolerance, whereas risk scores 4 and 5 (n=9) were 100% predictive for IGT/DM. In surgically treated patients (n=30) IGT/DM was found significantly more often than in patients with a conservative (n=7) treatment (67% vs. 0%, $p<0.002$). Fasting blood glucose (117±35 mg/dl vs. 98±8, $p<0.02$) and fructosamine levels were higher (262±68 mol/l vs. 218±12, $p<0.03$), whereas fasting insulin was lower (11±6 μU/ml vs. 17±5, $p<0.03$) in surgically treated patients, with no differences in age (46±11 years vs. 51±20) and follow up duration (4.3±3 years vs. 2.9±2).
In summary, we found a high prevalence of abnormal glucose tolerance in patients after a single episode of ANP, comparable to the prevalence found in chronic pancreatitis. Patients at risk were identified with an easily applicable clinical score. Our data strongly support recent trends in the initial treatment of patients with ANP towards a conservative approach.

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PREVALENCE OF DIABETES MELLITUS AND IMPAIRED GLUCOSE TOLERANCE UTILIZING TWO DIFFERENT METHODS

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The aim of this work was to evaluate the size of the problem of both diabetes mellitus (DM) and impaired glucose tolerance (IGT), as diagnosed according to the WHO (1985) criteria, in an Egyptian rural area. Five hundreds villagers, chosen at random from a village in Dakahlia province, were screened by measuring blood glucose both fasting and 2-hour following a 75 gm glucose load (Method 1). Another group of 500 age- and sex-matched villagers were screened by measuring blood glucose both fasting and post prandial following the ordinary breakfast meal (method 2). The prevalence of DM was found to be 6.2% and that of IGT 7% by method 1, while by method 2 the respective prevalences were 5.2% and 6.2%. The differences between both methods of screening were found statistically insignificant ($p>0.05$) in both conditions. This suggests that the 2-hour post prandial blood glucose estimation can be a good substitute for that following a 75 gram-glucose load. The former is certainly more convenient, physiological and palatable, and less expensive method for screening in mass epidemiological studies.

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PREVALENCE OF DIABETES MELLITUS AND VASCULAR RISK FACTORS IN THE URBAN POPULATION OF THE NORTHWEST OF B.A. PROVINCE, ARGENTINE REPUBLIC.

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OBJETIVES: To establish prevalence of DM and IGT in the city of Pehuajo(B.A.) and its spreading according to sex and age. To know the percentage of IDDM, NIDDM and IGT. To set the proportions of new diabetics and the known ones. To establish the prevalence of other factors of vascular risk and its association with Diabetes Mellitus.

MATERIAL AND METHODS: Random sample from subjets aged 20-80 (from no Institutions), located in Pehuajo, was determined by a multistage technic, by ranking sex and age (n: 603), a glucemia postloading was done, according to WHO recommendations. Cholesterol, triglycerides, blood pressure and BMI were tested.

RESULT: Prevalence of DM: 7.8% (n:47); 5.3% known and 2.5% new diabetics. Prevalence of IGT: 1%. The 25% of the known diabetics are under insuline treatment. The risk of presenting arterial hypertension increases according to age, high cholesterol and high BMI, and so is the risk of developing a heart attack as becoming older and DM. (Multivariate Logistic Regression).

CONCLUSION: An increase in the prevalence of DM is observed in relation to previous research carried out in Latinàmerica The high percentage of known diabetics, puts in evidence the efficient community educational work. The Diabetes Mellitus, high BMI and high Cholesterol increase the profile of the vascular risk.

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APPROPRIATE FASTING PLASMA GLUCOSE LEVEL FOR DETECTION OF DIABETES MELLITUS

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Inconsistency in the prevalence of diabetes mellitus (DM) diagnosed by current WHO recommended guideline has been observed. This study was aimed to determine fasting plasma glucose (FPG) level that can be appropriately used for early detection of DM. Standard 75 gm OGTT was performed in 393 subjects. They were 84 males and 309 females, aged 14-76 years old (mean±SD = 43.8±12.7 years). All of them had one or more of the following indications to undergo OGTT: DM in first degree relatives, overweight, hypertension, dyslipidemia and previous abnormality of glucose tolerance. Plasma glucose levels were determined in NaF preserved plasma using glucose oxidase method. Plasma glucose levels of ≥11.1 mmol/l at two hours after 75 gm oral glucose load were used as a gold standard for diagnosis of DM. Diagnosis of DM was made in 24.7% of cases by gold standard criteria compared to that of 5.8% by FPG values of ≥7.8 mmol/l (WHO criteria). Using various FPG values as cut-off points for diagnosis of DM gave different prevalence rates with varying degree of sensitivity and specificity as shown in the Table below. Diagnosis of DM by FPG of ≥7.0 mmol/l had improved sensitivity of 37.1% while retaining specificity at 100%. Cut-off value of FPG at 6.0 mmol/l gave nearest prevalence rate of DM to the gold standard criteria with Youden index (sensitivity + specificity - 1) of 0.63. Receiver operating characteristic curve revealed the best cut-off value being 6.2 mmol/l. We concluded that FPG of 7.0 mmol/l or more can be accurately used for detection of DM. Individuals with FPG values between 6.0-6.9 mmol/l should have OGTT for definitive diagnosis of DM.

FPG(mmol/l)	5.9	6.0	6.2	6.6	7.0	7.4	7.8
Prevalence of DM	25.4	21.6	17.8	14.2	9.2	7.9	5.8
Sensitivity	74.2	69.0	63.9	54.6	37.1	32.0	23.7
Specificity	90.5	93.9	97.3	99.0	100	100	100

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CLINICAL CHARACTERISTICS OF CHINESE YOUNG NON-INSULIN-DEPENDENT DIABETICS IN TAIWAN

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To know the clinical characterastics of young non-insulin-dependent diabetics (NIDD), we studied 50 cases (24M, 26F) who had been admitted to our metabolic ward from 1985 to 1996. The criteria for patient selection included: onset of diabetes before the age 30 years; absence of history of diabetic ketoacidosis; fasting or postprandial serum C-peptide $\geq$ 1.0 ng/ml; and no insulin requirement for over 2 years. There were 60% patients had body mass index (BMI) over 27 kg/m^2, and 54% had hypertriglycemia. Family history of diabetes in first degree relatives was noted in 35 (70%) patients. As compared with patients with shorter duration, those cases with history over 10 years had lower BMI (25.1 vs 30.0 kg/m^2, p=0.04), lower triglyceride (143.8 vs 310.0 mg/dl, p=0.006), and higher occurrence of retinopathy (50.0% vs 18.4%, p=0.03). In conclusion, NIDDM may develope in young age among Chinese. Most of them are obese, hyperlipidemic and family history of diabetes. Retinopathy occurs significantly higher in patients with longer duration.

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FREQUENCY OF CARBOHYDRATE METABOLIC DISORDERS IN PERIMENOPAUSAL AND POSTMENOPAUSAL WOMEN IN ŁÓDŹ (POLAND)

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The prevelance of diabetes mellitus (DM) and impaired glucose tollerance (IGT) in perimenopausal and postmenopausal women in Poland is unknown.
Aim of study:Evaluate the prevelance of carbohydrate metabolic disorders in perimenopausal and postmenopausal women admitted to Polish Mother's Memorial Hospital in Łódź during 1995-96
Materials and methods: The study group consists of 500 women aged between 45 to 65, mean 52,3 ± 5,3 admitted to the Department of Menopausal Diseases and Outpatient Clinic of PMMH for menopausal signs and symptoms. All women underwent clinical examination. Fasting blood glucose levels were evaluated in all cases. When FBG level was more than 105mg%, 75 gms OGTT was administered according to WHO protocol guidelines. Selected diabetes mellitus risk factors were evaluated.
Results: In the study group DM was diagnosed in 4,8%. For nearly 2/3 of the women this was the first time DM had been diagnosed. IGT was diagnosed in another 8%. In 87,2% of the women there was no carbohydrate metabolic disorder. Elevated Body Mass Index (BMI) was noted in 52,4% of women. Hypertension was observed in 18,2% of women and coexisted with DM and IGT in 13 and 10 cases respectively. Hypercholesterolaemia and hypertriglyceridaemia was noted in 27% of DM and 7% of IGT women. In the study group lipid metabolic disorders occured in 25 (39,1%) of women with carbohydrate disorders.
Conclusion: Screening for DM and IGT leads to the detection of most unknown cases of these deseases. Approximately 5% of perimenopausal and postmenopausal women have DM and additional 8% have IGT. Common associated disorders were hypertension and dyslipidaemia.

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CLINICAL PRESENTATION OF YOUNG DIABETES IN BANGLADESH

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Four hundred and fifty three diabetic subjects with onset on age below 30 years of age has been studied on the first day of diagonsis. 67 (14.8%), 22 (4.9%), 159 (35.1%) and 205 (45.3%) were diagnosed as FCPD, IDDM, PDDM and NIDDY subjects respectively according to WHO criteria. Male predominancy was found in FCPD, IDDM and PDDM and female in NIDDY subjects. Subjects of first three classes reported mostly from rural areas and NIDDY mostly from urban areas. NIDDY subjects had higher age 26±2 (M±SD) than FCPD, IDDM and PDDM subjects with identical age 21±1 (M±SD) years (p<0.05). Sixty six percent of FCPD, 46% of IDDM and 53% of PDDM subjects had education below class V contrast to 83% of NIDDY subjects above class V. Fifty two percent FCPD, 46% IDDM and 43% PDDM subjects were unskilled labour. Mean annual income (in US Dollar) of FCPD (813±136), IDDM (873±213) and PDDM (904±94) subjects were much lower than NIDDY (2258±163) subjects (p<0.05). Typical symptoms of diabetes mellitus (DM) were obtained in 97%, 95% and 93% of FCPD, IDDM, PDDM subjects respectively in comparison to 63% of NIDDY subjects. Signs of malnutrition were revealed in 93% of FCPD, 91% of IDDM, 92% of PDDM subjects in contrast to 40% of NIDDY subjects. Ninety four percent of FCPD and 91% of IDDM subject had BMI<19 whereas only 27.3% of NIDDY and 4.5% of IDDM subjects had BMI >25. History of DM in family were obtianed in 56% of NIDDY, 19% of FCPD, 14% of IDDM and 24% of PDDM subjects. Glycemic status (FPG and HBA1c) was significantly higher in FCPD (21.9±0.8, 11.3±1.1), IDDM (22.2±1.5, 11.3±1.2) and PDDM (20.0±0.6, 10.6±0.4) subjects compared to NIDDY (11.4±0.3, 8.1±0.2) subjects (p<0.05). Retinopathy was observed in 6%, 3.8% and 2.9% in FCPD, PDDM and NIDDY subjects respectively. Neuropathy was found in 56%, 45%, 43% and 4.5% of FCPD, IDDM, PDDM and NIDDY subjects. Twenty three percenr of IDDM, 7.5% FCPD, 8.8%PDDM and 9.3% NIDDY subjets had clinical proteinuria. Detailed biochemical investigation is needed to correlate clinical behavior and etiopathogenic mechanism.

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THE EFFICACY OF A SINGLE SCREENING METHOD FOR THE METABOLIC SYNDROME X

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A simple method was adopted for studying the occurrence of the features of syndrome X and for early detection of the syndrome in jeopardized individuals.Out of 343 patients 255 with central obesity and hypertension /group 1/,24 with obesity /gr.2/,35 with hypertension /gr.3/,29 without obesity and hypertension /gr.4/ were examined. Blood sugar and immunoreactive insulin levels during oral glucose tolerance test,fasting plasma lipids,body mass index,waist/hip ratio,blood pressure were determined.Hypertension,central obesity and high blood sugar levels for corresponding insulin levels were regarded as basic criteria of the syndrome.89% of gr.1 patients met these criteria.Type 2 diabetes mellitus,impaired glucose tolerance or"diabetoid"glucose curves could be detected in in 53-45-28-20%,hyperinsulinism in85-70-61-41%,dyslipidemia in 80-62-71-45%, high blood sugar / insulin levels (presumably insulin resistance)in 90-83-66-52% of the groups 1-2-3 and 4.Syndrome X could be effectively screened byinspection and hypertension in 89% of gr.1,but fragments of the syndrome were present in lesser amount in the other groups as well.A delayed peak and slow decline of insulin curves could be seen in patients with glucose intolerance including "diabetoid"individuals compared to normals suggesting an early abnormality of the beta-cell function and/or insulin resistance

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METABOLIC CONTROL AND QUALITY OF LIFE FOR DIABETES PATIENTS IN VILJANDI, ESTONIA – A POPULATION BASED STUDY

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Objective – To investigate aspects of metabolic control, treatment and complications, as well as the quality of life, in patients with diabetes mellitus from a defined population in Estonia.

Design – We invited 220 randomly selected diabetes patients from a local diabetes register of 1,100 patients, to a clinical investigation.

Setting – The city Policlinic in Viljandi, Estonia.

Main outcome measures – Medical history, physical examination (height, weight, blood pressure), laboratory variables (blood glucose and glucated hemoglobin A_1, serum cholesterol and creatinine, urinary protein), questionnaire on quality of life variables.

Results – In all 181 diabetes patients were investigated, of whom 90% were non–insulin–dependent diabetes mellitus (NIDDM). Mean diabetes was 8.9 years, and mean HbA_1 level 7.3%. The overall proportion of patients treated with insulin was 20.4%, and with anti–hypertensive drugs 27.6%. Smoking was present in 14%. The proportion of patients with various diabetes complications was high (73.5%), mostly consisting of different manifestations of cardiovascular disease. Foot ulcers or gangraende were observed in 11.7%. A low level of quality of life was registered in many patients, mostly due to difficult living conditions. QoL and subjectively perceived health was significantly lower than for Swedish patients.

Conclusion – Diabetes patients in Viljandi showed an acceptable degree of glucose metabolic control, but reported a high degree of diabetes complications, as well as impaired quality of life. The diabetes complications may therefore be due to other detrimental factors than hyperglycaemia, e.g. the standard of care during previous years and current living conditions.

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IMPACT OF GENDER AND OVERWEIGHT ON PARAMETERS OF THE METABOLIC SYNDROME IN NIDDM RELATIVES.

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First degree relatives of NIDDM patients present metabolic disturbances that can be taken as predictors for developing diabetes, depending of ethnicity. Being hispanic more prone to develop NIDDM we studied 24 non-obese first degree relatives (14 F/10 M) of NIDDM patients (DR) matched for age, gender and body mass index (BMI) with 22 controls (12 F/ 10M) from Venezuelan families, to compare with other hispanic groups. In DR, the subscapular (SC) and tricipital (TC) skinfolds measurements were higher. Separating by gender and BMI, this increase was observed mainly in overweight women (SC = 30.5 ± 1.9 *vs* 19.3 ± 3.5 mm TC = 27.9 ± 3 *vs* 20.0 ± 2.8 mm) indicating upper body and peripheral adiposity. Diastolic blood pressure (DBP) was elevated in DR but mainly in overweight men, who represented 70% of the total. None of DR had glucose intolerance, however the insulin was elevated at fasting and in response to a glucose load, resulting a high I/G ratio. The ΔI30/ ΔG30, indirect measure of insulin secretion, was elevated in overweigth men (87.9 ± 3.4 *vs* 76.2 ± 2.9). In women, it was observed a lower increment in the first phase insulin release as the BMI increases. Most lipid parameters were elevated in DR, except Chol and LDL-C. TG was elevated in normal as well as in overweight women (< 25 BMI 1.5 ± 0.2 *vs* 0.7 ± 0.1 mmol/l; > 25 BMI 2.3 ± 0.5 *vs* 1.3 ± 0.2 mmol/l). Low HDL-C was seen in both, women and men (women 1.0 ± 0.1 *vs* 1.2 ± 0.1 mmol/; men 0.8 ± 0.1 *vs* 1 ± 0.1 mmol). Insulin was significantly correlated with TC and lipid parameters in women, mainly in overweight, while in men the association was observed with lipids and DBP. (CONDES-CONICIT).

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PREVALENCE OF DIABETES MELLITUS ON PYGMIES IN KRIBI SUBDIVISION, SOUTH CAMEROON (CENTRAL AFRICA)

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On June 29th and 30th 1996, we conducted a screening exercice for diabetes mellitus in the pygmy population in Kribi Sub-division South Cameroon (Central Africa). The objectives were to find out if this condition existed the pygmy population and determine it's prevalence. Five camping villages were visited. Two of them were located deep into the forest, while the three others because of their proximity to the roads and Kribi toen, have undergone relatively on modernization (Lende, Dibamba and Bibuba). Capillary blood glucose, analysis, with rapid reading on Gucometer (AMES) was used as a scrrening tool. The population was informed to come fasting, but because we reached Kilombo and Bibuba late, the villagers had eaten two to three hours prior to our arrival. A total of 108 Pygmies were examined, comprising 63 females and 45 males, 41 of whom were children under fifteen years, while 67 were adults. We found 5 cases of high blood glucose levels (>1.40 g/l). None however attained 1.80 g/l. We estimated the diabetes prevalence at 4.6% for the whole and 7.5% if only the adult population was considered. All the positive cases were observed in the relatively modernized camping villages: Lende (5 cases), Dibambe (1 case) and Divuba (3 cases).In these camping villages, we noticed the presence of non-Pygmies who came for native treatment (the Pygmies are reputed to be very good herbalists). From this intermingling may result changes in food habits of Pygmies or mixted born babies into the Pygmy population. No child was seen with signs or symptoms of diabetes; probably because insulin dependent diabetes is absent in this population or because affected childen die rapidly due to the lack of insulin. All the adults with abnormal blood sugars were asymptomatic. Was this because the renal threshold for glucose was lower than 1.8 g/l or did the Pygmies benefit from some protection against diabetes mellitus through the use of traditional drugs. This study shows that diabetes exist among the Pygmies. The prevalence found in this study was 4.6%. However these are preliminary results which must be confirmed by other studies using more standardized methodolgy.

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SYNDROME X IN JAPANESE-BRAZILIANS: IS INSULIN OR ITS PRECURSOR INDEPENDENTLY ASSOCIATED TO HYPERTENSION?

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Hyperinsulinemia and hypertension are associated in the spectrum of syndrome X, but large studies adjusting for confounders are still required. Japaneses living outside Japan are more susceptible to chronic diseases; our group has previously reported high prevalence of NIDDM among Japanese-Brazilians. The present study aimed to evaluate if insulin (I) or proinsulin (PI) is associated with hypertension after adjustment for other risk factors, in first (1G) (n=238) and second (2G) (n=292) generation Japanese-Brazilians, aged 40-79 yrs. Blood pressure (BP) was measured by random-zero sphygmomanometer. Persons with systolic/diastolic BP ≥ 140/90 mmHg or taking antihypertensive drugs, were considered hypertensives. Diagnosis of diabetes was based on oral GTT by WHO criteria. Fasting and 2-hr after glucose load I and PI were determined by immunofluorimetric assays. 1G was older than the 2G (65.6±9.2 vs 53.6±8.4 yrs, $p<0.01$) and male/female ratios were 1.14 and 0.87, respectively. Age-adjusted prevalence of hypertension was 29.2% with no difference between genders or generations. Higher BMI, waist/hip ratio, plasma glucose and cholesterol were found among the hypertensives. Logistic regression showed that 2-hr I remained associated with hypertension (OR=1.43, 95%CI 1.13-1.81) after adjustment for age, gender, generation, family history, smoking, obesity, waist/hip ratio, glucose intolerance, and dyslipidemia. Japanese-Brazilians have higher prevalence of hypertension than the general population in Brazil. This finding, associated to the increased rates of NIDDM, reinforced the unfavorable impact of westernization to syndrome X. 2-hr hyperinsulinemia, seen in hypertensives, may be interpreted as an independent risk factor for hypertension in this population.

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EPIDEMIOLOGY OF ALLOPATHIC ANTIDIABETIC TREATMENT IN INDIA

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With estimated 20 million people with diabetes, India is already world's most populated country as regards people with diabetes. Even though traditional systems of medicine are widely practiced and are the mainline systems in rural areas, they do not offer a single effective antidiabetic medication. We decided to estimate the number of Indians with diabetes, who are on allopathic antidiabetic medications[Oral Hypoglycemic Agents (OHA), belonging to sulphonylurea and biguanide families and insulins.]The Operation Research Group published all India Sales figures of all the OHAs and insulins marketed in India, were studied to work out total number of OHA tablets/capsules and insulin vials sold in India during October'95 to September '96. By allocating average daily dose for each agent, we estimated the number of patients on each antidiabetic medication. We then worked out formulas to account for polytherapy and calculated total number of patients with allopathic antidiabetic medications. In our estimation only 474000 patients (out of 20 million) were receiving allopathic antidiabetic medications. We conclude that only a small fraction (approx. 2.5%) of Indian patients with diabetes are taking allopathic medications. The dual task of identifying undiagnosed and untreated persons with diabetes and providing them with appropriate medications, needs to be tackled on war footing.

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BASAL INSULIN LEVELS ACCORDING TO AGE, SEX AND BODY MASS INDEX IN NORMAL SUBJECTS IN KOREA

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To determine the basal insulin levels in normal Korean subjects and the factors influencing with basal insulin levels, 1917 normal subjects whose fasting blood sugar levels were below 6.4 mmol/L, were assessed basal insulin levels, total cholesterol, triglyceride and their anthropometric charateristics (body weight, height and body mass index). Mean basal insulin levels was 34 pmol/L, the basal insulin levels in men and women were 35±1 and 32±1 pmol/L, respectively. Fasting blood sugar levels was 5.4±1 mmol/L and insulin(uU/ml) to glucose(mg/dl) ratio was 0.06. The basal insulin levels of obese (BMI≥ 25) subjects was significantly higher than that of non-obese subjects (43 ± 1 pmol/L vs 31 pmol/L p< 0.001). Basal insulin levels according to age and body mass index was as follows;

Insulin(pmol/L)* / Age (years)	Men (n=1235)		Women (n=682)	
	obese (BMI≥25)	non-obese	obese (BMI≥25)	non-obese
20-29	74±13 (n=12)	29± 3 (n=34)	45± 18(n=2)	30±2 (n=54)
30-39	48±2 (n=121)	32±1 (n=269)	39± 5 (n=22)	29±1 (n=153)
40-49	42±2 (n=142)	31±1 (n=322)	39± 4 (n=46)	29±1 (n=172)
50-59	41±2 (n=73)	30±1 (n=183)	37± 2 (n=60)	31±2 (n=113)
≥60	36±5 (n=21)	29±2 (n=51)	44± 5 (n=15)	35±2 (n=46)

* Values are mean±S.E.M.

There were no significant difference in the basal insulin levels between the age group, however the basal insulin levels of obese group in each age group was significantly higher than that of nonobese group. In a multiple stepwise regression analysis, body mass index, fasting blood sugar and triglyceride were significantly positive contributing factors on the basal insulin levels, and age was significantly negative contributing factors on the basal insulin levels.

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DIABETES MELLITUS IN THREE ALASKAN ESKIMO POPULATIONS

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Diabetes has historically been relatively uncommon among Alaskan Eskimos compared to other populations. However, no large scale screening involving glucose tolerance testing of all subjects had been carried out. The objective of this study, a population based survey, was to determine the prevalence of glucose tolerance abnormalities, body fat content and distribution, and insulin levels in three Alaskan Eskimo populations, >=25 years age, in the Bering Straits region of northwestern Alaska. Data included body mass index, waist-hip ratio, glucose tolerance status by World Health Organization criteria, fasting insulin and lipid levels, and family, dietary, and exercise histories. A total of 454 of 899 (51%) eligible participants were screened, including 239 Siberian Yupik, 106 Central Yupik, and 109 Inupiat Eskimo people. The prevalences of diabetes in these groups were 9.6%, 2.8%, and 3.7% respectively, while the rates of impaired glucose tolerance were 7.5%, 13.2%, and 6.4%. Mean insulin levels were 45 and 38 pmol/l for men and women respectively. Age-adjusted overall rates of diabetes in these populations were 9.2% for women and 6.6% for men, similar to 8.8% and 7.8% for U.S. white women and men. Compared with Siberian Natives across the Bering Straits ages 45-64, our diabetes rate was much higher (8.2% versus 0.8%). Our mean insulin levels were much lower than those in the Strong Heart Study of American Indians which ranged from 114 to 165 for women and 97 to 128 for men (measured in the same laboratory, same units). In summary, our data indicates that: diabetes among Alaskan Eskimo people is more common than previously thought; our rates are higher than those among closely related populations across the Bering Straits; and lower insulin levels may indicate a lower degree of insulin resistance than among American Indians. Further comparisons of our findings to those among other indigenous populations should help define the relative contributions of genetic and life style related risk factors, which hopefully can lead to preventive strategies.

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PREVALENCE OF INSULIN RESISTANCE IN METABOLIC DISEASES. RESULTS FROM A POPULATION-BASED STUDY (BRUNECK STUDY).

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Insulin resistance is thought to be a common finding of several clinical conditions, including IGT, NIDDM, hypertension, dyslipidemia and hyperuricemia. This concept emerged from case-control studies in which insulin sensitivity was assessed by insulin clamp or alternative methods. We examined the prevalence rates of insulin resistance in the above mentioned clinical conditions among subjects participating in the Bruneck Study, a population-based study including 888 subjects aged 40-79 years in whom insulin sensitivity was estimated by homeostasis model assessment (HOMA). Subjects in the top quintile of HOMA distribution values (n=177) were regarded as insulin resistant. The prevalence of IGT, NIDDM, hypercholesterolemia, hypertrigliceridemia, low HDL cholesterol, hyperuricemia and hypertension (as defined by conventional criteria) significantly increased (p=0.05-0.0001) across quintiles of HOMA. This was found in either nonobese and obese individuals, in both men and women and in younger (40-59 years) like in older (60-79 years) subjects. The prevalence of insulin resistance was 41.2% in IGT, 62.9% in NIDDM, 24.6% in hypercholesterolemia, 53.9% in hypertriglyceridemia, 57.1% in subjects with low HDL cholesterol, 37.2% in hyperuricemia, 29.3% in hypertension. The prevalence of insulin resistance in subjects with the combination of glucose intolerance, dyslipidemia, hyperuricemia and hypertension (n=21, 2.4% of the entire population) was 81%. Twenty-eigth subjects (3.1% of the whole population) were insulin resistant but free of IGT, NIDDM, dyslipidemia, hyperuricemia and hypertension. These results document in a population-based sample that i) insulin resistance in hypertriglyceridemia is as common as in glucose intolerance (50-60%) whereas it is less frequent in hypercholesterolemia, hyperuricemia and hypertension (25 to 35%); ii) the vast majority of subjects with multiple metabolic defects are insulin resistant; iii) in a significant proportion of the general population insulin resistance can be found even in the absence of any metabolic diseases.

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INCIDENCE OF DIABETES AND ITS RISK FACTORS IN AMERICAN INDIANS -- THE STRONG HEART STUDY

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Diabetes mellitus (DM) is very common in American Indians. Results reported are from the Strong Heart Study (SHS), a cohort study of cardiovascular disease and its risk factors in American Indians. Phase I (baseline) data of the SHS show that the prevalence rates of diabetes in American Indians, aged 45-74 years, in Arizona (AZ), Oklahoma (OK), and South and North Dakota (SD/ND) were 70%, 40%, and 40%, respectively. The prevalence rates of impaired glucose tolerance (IGT) were respectively 14%, 17%, and 17%. DM and IGT status were defined by the WHO criteria. At Phase II, after an average of 4±0.7 years of follow-up, 3638 of the original 4549 participants (413 died before examination, 499 refused re-examination) completed the study. The cumulative incidence of DM from participants with normal glucose tolerance (NGT) and IGT are given below:

	WOMEN			MEN		
	AZ	OK	SD/ND	AZ	OK	SD/ND
NGT to DM	17%	10%	17%	8%	8%	11%
IGT to DM	45%	33%	33%	53%	36%	27%

Stepwise logistic regression was used to determine the risk factors for the development of DM. In women with NGT at baseline, fasting plasma insulin level and degree of Indian blood were found to be significantly associated with the incidence of DM. Whereas in men, only body mass index was significant. In women with IGT at baseline, only fasting plasma insulin level was significantly associated with the development of DM. In men with IGT, age was the only significant risk factor. The data indicate that diabetes is continuing to plague the American Indians. There is an urgent need to implement effective preventive programs and intervention strategies to reduce the burden of this devastating disease in this population.

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BLACK-WHITE DIFFERENCES IN INSULIN METABOLISM IN YOUNG NON-DIABETIC SUBJECTS.

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As part of a study on the pathogenesis of coronary artery disease, we profiled glucose handling and β-cell secretory dynamics in 79 Blacks (36 males, 43 females) and 84 Whites (54 males, 30 females) ≤55 years old (mean 47) without diabetes. Subjects underwent a 3hr, 75gm oGTT with measurement of glucose, specific insulin (INS,μU/ml) and C-peptide (C-pep). Fasting glucose and glucose tolerance were similar across ethnic groups (mean fasting glucose 100 mg/dL in Blacks and 99 mg/dL in Whites). Fasting INS was higher in Black vs. White males (15.5±9.2 vs 11.3±7.0, p<.01) and Black vs. White females (15.2±7.7 vs 13.0 ± 10.4, p= .04); however, Black females had the highest BMI (32.2 kg/m²). After adjusting for BMI, fasting and post glucose load INS (±SE) were compared by race and gender: (*=p<.05 Blacks vs. Whites)

	Fasting INS		30 min INS		Area under curve	
	Males	Females	Males	Females	Males	Females
Blacks	14.3 ± 1.1*	13.8 ± 1.1	72.5 ± 9.7	72.6 ± 7.4	17760 ± 2006*	15625 ± 1446
Whites	11.9 ± 0.9	15.2 ± 1.3	51.7 ± 7.9	63.6 ± 9.2	11455 ± 1634	14514 ± 1753

Although differences disappeared between females after adjustment for BMI, differences in fasting, 30 minute, and AUC INS persisted at $p < 0.05$ when adjusted only for waist/hip ratio. β-cell secretion did not differ as manifested by similar glucose and C-pep responses. We conclude that INS elevation observed in these Black women is attributable to obesity, independent of body fat distribution. In contrast, fasting and AUC INS are high in Black men without increased C-pep, independent of either obesity or body fat distribution. These finding suggest greater intrinsic insulin resistance and reduced INS distribution and/or metabolism in non-diabetic Black males.

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CLINICAL CHARACTERISTICS AND STATISTICAL EVALUATION OF 93.000 DIABETIC PATIENTS FROM AN ITALIAN REGION

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Following a national legislation that hypotizes a personal card for each diabetic patient (D), in our region (Campania in southern Italy) we activated a regional data-bank with anagraphical, anthropometric and therapeutical informations. From 1989 to 1994 we collected data about 92.758 D (F 56.3%, M 43.7%); we believe that our sample can represent half of diabetic population of the region (inhabitants=5.2 milions): the aim of this study was to evaluate these data. Statistical univariate analysis performed on all data showed that the distribution for age is different between the two sex (males are more represented in age's range 0-49 yrs, over this age the ratio is inverted). The 11.7% of patients were insulin-dependent (ID), 88.1% were non-insulin-dependent (NID) and 0.2% had a secondary diabetes. The D were treated by General Practitioners (GPs) in 57.9% of cases and in 25.1% by specialists, diabetologists or diabetes centre (DC), 17% of D were treated by both. Referring to therapy 6.5% of D was only on diet, 66.5% was in therapy with oral agents (OH), 22.4% was on insulin (I) therapy (median dosage 32 U.I./die) and 4.6% was in therapy with both drugs. Only 20.7% of D performed blood glucose self-monitoring. Body mass index (BMI) calculated for each patient showed that 42% of D was overweight and 28% was obese with an high prevalence of females among obese(F 33% vs M 21.6%). We found a statistical significative difference ($p<0.001$) between the BMI values of patients from different towns (obeses in the 5 region's chief towns 27.8% vs obeses in the other towns and rural zone 31.4%). On 4093 randomly selected patients we performed a stepwise logistic regression using as dependent variable the BMI (non obese <30, obese ≥30) and as independent variables: sex, age, type of therapy, type of diabetes, and glycaemic self-control. The results showed an higher risk of obesity among the females (Odds ratio=2.00), among the diabetics in therapy with OH (OR=1.64) or with OH+I (OR=1.96). The risk of obesity is lower among ID (OR=0.4).

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GLUCOSE TOLERANCE IN THE ELDERLY (>40 YRS) IN RURAL INDIA.

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We studied glucose tolerance in 321 subjects >40y in the village of Pimpale Jagtap near Pune (158 men: 58 y, 1.63 m, 52 kg, BMI 19.5 kg/m^2 and WHR 0.88; 163 women: 53 y, 1.50m, 45 kg, 19.8 kg/m^2 and 0.78). In men 2h plasma glucose was related to age ($r=0.24$, $p<0.01$), BMI ($r=0.23$, $p<0.01$), WHR ($r=0.21$, $p<0.01$), and to height ($r=-0.16$, $p<0.05$); in women to BMI ($r=0.15$) and head circumference ($r=-0.15$, $p=0.07$ both). Four percent (7 men, 7 women) had diabetes, 4% (5 men, 8 women) had IGT (WHO 1985). Hyperglycaemic subjects (IGT+diabetic) were older and more 'obese' (men 22.3 & women 21.6 kg/m^2), men had higher WHR and women a smaller head circumference compared to normoglycaemic subjects; they also had higher 2h plasma immunoreactive insulin & triglycerides, lower HDL cholesterol and higher blood pressure. Thus, in the elderly of rural India diabetes and IGT are relatively rare but are associated with other cardiovascular risk factors suggesting a metabolic syndrome.

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Diabetes mellitus Survey in Zhejiang Province

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According to the operating rules of national dibetes epidemiologic survey formulated in 1993. We investigated over 25yrs 7949 subjects in Zhejiang province, among them, 1060 persons in Hangzhou city, 1158 people in Xhao Shen Village, 1846 subjects in Wenzhou City and fisherman village, 1846 subjects in Ningpo Urban and rural areas, Dong Yong Heng Dian industrial group and 1000 subjects in pu two fisherman Village.

In this survey we discovered newly diagnosed Diabetes mellitus 73 cases (54 males, 14 females) , Known diabetes 93 cases (male 65, female 28) all diabetic patients were NIDDM except one was IDDM. The overall diabetic prevalence is 2. 71%, standardized prevalence is 3.1% (male 3.40%, female 2.54%), newly diagnosed IGT 99 case (66 males, 33 females). The prevalence is 2. 09% (male 2. 6%, famele 1.64%). Standardized prevalence is 2. 04% (male 2.33%, female 1.47%).

The diabetic prevalence in those over 55 increases sharply, peaking at 64-74 yrs in both sex. The ratio between the prevalence in overweight and nonoverweight group after standardization is 3.54%.

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PREVALENCE OF GLUCOSE INTOLERANCE IN BERMUDA.

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Diabetes is a major health problem in Bermuda. To assess its magnitude we determined the prevalence of glucose intolerance (known diabetes [KD], undiagnosed diabetes [UD] and glucose intolerance [IGT]) in a random sample (n=999) of adults (19+ years) in a nation-wide survey. All subjects, except those with known diabetes, had a 75 gm oral glucose tolerance test. The prevalence of KD = 8.5%, UD = 3.3% and IGT = 6.2%, giving an overall prevalence of glucose intolerance of 18.0%. For each group, the prevalence was higher in the females (KD: 5.5 vs 3.0, UD: 2.2 vs 1.1 & IGT: 3.4 vs 2.8%). The prevalence of KD & IGT were low in the 19-30 yrs group (2.1 & 2.1% respectively), peaked in the 61-70 yrs group (15.1 & 13.2%) and decreased thereafter (11.5 & 10.2%). The prevalence of UD increased steadily from 0 % in the 19-30 yrs group to 0.5% in the 31-40 yrs group and to 14.1% in the 71+ yrs group. All 3 groups had higher serum total cholesterol [KD = 5.34 + 0.21 mM, M + SEM, UD = 5.14 + 0.16 & IGT = 5.24 + 0.11 vs Normal = 4.77 + 0.03], and triglycerides [KD = 1.69 + 0.15 mM, UD = 1.72 + 0.15 & IGT = 1.65 + 0.16 vs Normal = 1.09 + 0.02] but lower HDL-cholesterol [KD = 1.1 + 0.04, UD = 1.12 + 0.08 & IGT = 1.1 + 0.05 vs Normal = 1.19 + 0.01] than the normal group. Only the KD [3.5 + 0.19 mM] and IGT [3.43 + 0.1] had higher LDL-cholesterol than the Normal [3.08 + 0.03] group. The glucose intolerant groups did not have higher serum apo A-I levels than the Normal group. However, all 3 groups had higher serum apo-B levels [KD = 112 + 3, UD = 108 + 5 & IGT = 112 + 3 mg/DL] than the Normal group [97 + 1]. Glucose intolerance is common in Bermuda. The prevalence of UD is highest in the 71+ yrs group. Bermudians with KD, UD, and IGT have higher serum cholesterol, triglycerides, LDL-cholesterol and apo-B but lower HDL-cholesterol levels than those without glucose intolerance.

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Prevalence and incidence of diabetes mellitus in Japan

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The purpose of this study is to obtain accurate data on the prevalence and incidence of IDDM and NIDDM. In order to make international comparisons, the survey had to be performed to the same internationally recognized standard methods using WHO criteria. The data are taken from 1989 to 1995. The prevalence and incidence of childhood IDDM are chiefly derived from the records of patients who received free medical care for diabetes, which is available for children younger than 18 years of age.

The mean prevalence of childhood IDDM per 100000 is 14.7 which is rather uniform for the different areas of Japan. From 1974 to 1994, childhood diabetes increased from 577 to 6146 children. The incidence per year was about one tenth of the prevalence, that is to say about 1.5 per 100000.

The population-based survey of NIDDM among adults aged 40 years or older using WHO criteria showed mean 9.4% (Male 10.0% Female 6.0%) from northern Japan(YAMAGATA) to Southern Japan(OKINAWA).

The incidence rate of adult NIDDM calculated from the new occurrence of diabetes in subjects who had previously been examined was 0.8 to 4%. On the other hand, the person year incidence rate calculated from several year's trends showed 2-3 per 1000 person years. The IGT group comprises the group at high risk of developing diabetes and macrovascular diseases. The prevalence of IGT ranged from 17 to 26% in most surveys, about 2 to 2.5 times higher than diabetes. These studies showed the number of Japanese diabetic patients reached more the 6 million people. Another survey of our study group showed Japanese Americans who live in Hawaii and Los Angels suffered from diabetes 2 to 3 times more than Japanese in Japan. These data suggest that a westernized life style promotes the development of diabetes in Japan. The traditional Japanese life style may prevent the incidence of diabetes and its complications.

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PREVALENCE OF GLUCOSE INTOLERANCE IN NORTH EASTERN REGION OF INDIA

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There is no epidemiological data on the prevalence of diabetes from North Eastern India. Therefore, a survey was conducted in the urban population of the city of Guwahati, Assam to evaluate the prevalence of glucose intolerance and associated risk factors. In a total of 1016 randomly selected adults aged 20 years & above (595 men, 421 women) glucose tolerance was tested by 2 hr post glucose (75 gm) plasma glucose estimation (WHO criteria). The age-adjusted prevalence of NIDDM was 8.2% in total, 8.7% in men, 7.8% in women. The age-adjusted prevalence of IGT was 4%, 4.1% in men and 3.4% in women. In 83.3% of NIDDM, diabetes was detected earlier. In the multiple regression analysis age, family history, increasing socio-economic strata and decreasing physical activity were significantly associated with NIDDM. Sex and BMI were not contributory. In the IGT group, the results were similar to NIDDM but BMI also was a contributory factor. This study showed that the prevalence of NIDDM in urban areas of Assam was also high, as reported from urban population in Southern India.

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TROPICAL PANCREATITIS FROM WESTERN INDIA

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AIM: To study Tropical pancreatitis and prevalance of Malnutrition - Related Diabetes Mellitus (MRDM) from ethnic population of Western India.

METHODS & MATERIALS :-40 cases meeting the following inclusion criteria viz (1) Abdominal pain (2) Evidence of chronic pancreatitis on ERCP (3) Evidence of Pancreatic calcification on plain x-ray abdomen, ultrasound, CT scan (4) Excluding all known causes of chronic pancreatitis viz alcoholism, biliary etc. were included in the study.

OBSERVATIONS: There were 36 males and 4 females .30 were from Maharashtra (18 Maharashtrians, 8 Gujarathi,3 UP ites, 3 Apites); 8 from Gujarat (7 Gujarathi,1 Kerla) &1 each from MP and UP. Abdominal pain was the predominant presenting complaint. Diabetes was seen only in (3/40) 7.5% and Malabsorption (5/40) 12.5 %. ERCP revealed (22/40) 55% had dilated pancreatic ducts ,(12/40) 30% stricutre in pancreatic duct and (6/40) 15% normal pancreatic duct apart from calcification in all. Mean duration of symptoms in dilated pancreatic duct group was 42 months, 9 months in stricture pancreatic duct group and 23 months in normal pancreatic duct group. Mean duration of follow-up was 70 months.

CONCLUSION : This study highlights that western Indian population behaves differently in its manifestations of tropical pancreatitis & MRDM as compared to classical features as described from South India. Abdominal pain predominates, malnutrition, parotid enlargement and diabetes were rare and overall prognosis was good in the Western Indian population.

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A SEARCH FOR IMPAIRED GLUCOSE TOLERANCE IN INDIVIDUALS WITH HYPERTENSION AND OBESITY AND THEIR OFFSPRING.

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During the examination of 11 000 villagers from Eastern Poland we estimated the prevalence of hypertension at 22.5%, obesity at 40%, overweight at 30 %, diabetes at 2,3% and impaired glucose tolerance at 8,3%. In one of the local districts of ca. 5000 inhabitants, we undertook an active search for hypertension and obesity in families from rural areas. Family nurses visited all the families in the district in their households and measured body weight, waist to hip ratio and blood pressure. People with earlier and newly diagnosed hypertension and obesity (BMI>30) as well as their parents and children consequently underwent clinical and laboratory examinations. Oral glucose tolerance test, estimation of total cholesterol, HDL cholesterol, triglicerides, fasting serum insulin level and urine albumin excretion were performed. The analysis was made in case of 30 three-generation families where parents with hypertension and/or obesity were the probants and who had at least three children aged 7 - 16. None of the 105 examined children did not have hypertension or state of impaired glucose tolerance. The average concentration of HDL cholesterol in children was 0,94 ± 0,45 mM/L and was substantially lower as compared with the one in the control group (1,43 ± 0,24 mM/L). Percentage of HDL cholesterol in the investigated group was 26,2% and 34,1 in the control group. In 7,3% of the examined children proved to have microalbuminuria > 50 µg/ml. 39% of the children had elevated fasting serum insulin level over 10 µJ/ml. Our results point to the necessity of introduction of early preventive measures in offspring of parents with hypertension and obesity.

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EPIDEMIOLOGICAL ASPECTS OF NONINSULIN DEPENDENT DIABETES MELLITUS IN MOSCOW REGION.

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The first stage of this work was the creation of the NIDDM patient's register in Mytishinsky district of Moscow Region. Up to now all the NIDDM's patients are registered. In total the quantity of these patients is 1345 persons. The anticipated results of this work were the description of general situation of mobidity in Mytishinsky district and the treatment received data as a model. Computer's treatment of the register's data has permit to represent the invalidity structure of the NIDDM's patients. Desease, which are not connected with diabetes mellitus, is at the first place in this structure. Diabetes mellitus, as a reason of invalidity, is on the second place. Cardiac decease is on the third place. From this, NIDDM is developing on the background of the existing hard deseases. The carried out large-scale investigation have permitted prevalence of complication among NIDDM's patients The quantity of autonomous neuropathy was negligible. Which can be connected with imperfection of the diagnostic methods. The analysis of medicines, which subscribed of the patients, had show the following: injections of insulin were subscribed 1.43% of patients, first generation's sulfanilamides were used by 8,4%, 71.68% got second generation sulfa drugs, 1,18% got biguanids drugs. These will permit to formulate the nessasary recommendations as well.

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RETROSPECTIVE STUDY OF GLUCOSE INTOLERANCE AMONG WOMEN ATTENDING FOR ANTENATAL CHECKUP

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AIMS OF STUDY: To evaluate the prevalence of Glucose intolerance in high risk group of women attending for Antenatal Checkup (A.N.C.)

DESIGN METHODS: Women attending for A.N.C. were subjected to a common questionnaire. Data regarding family history of Diabetes, Past Obstetric history, height, weight, B.P. and B.M.I. were documented. Ultra sound screening was done on routine basis. Risk group women were identified and were subjected to O.G.T.T. according to N.D.D.G criteria.

RESULTS:	TOTAL NO.OF PATIENTS	835	--
	TOTAL O.G.T.Ts DONE	324	38.75%
	I.G.T.	34	4.06%
	G.D.M.	11	1.31%
	KNOWN CASES OF NIDDM	4	0.57%

Out of 49 patients with Glucose intolerance, 17 patients required insulin for better Glycemic control.

CONCLUSION: The prevalence of glucose intolerance among women attending for A.N.C. at an Obstetric clinic in Nellore is 5.86%

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EPIDEMIOLOGICAL CHARACTERISTICS OF DIABETES MELLITUS IN CHINA

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To investigate the prevalence characteristics of diabetes, a community based prevalence study on diabetes mellitus was carried out in 12 provinces of China. the study population comprised 45,000 persons, who lived in the community for no less than five years, and with age of 20-74 years. 2-hour venous blood was collected after an oral glucose load (75 g) for each subject. Diabetes and IGT was defined according to diagnostic criteria proposed by the WHO Study Group on diabetes mellitus. The preliminary data showed: 1.The prevalence rate of diabetes was 3.4% for males, 3.0% for females, and it was 3.1% for the whole population. The prevalence rates of IGT were 4.1%, 4.4% and 4.3% respectively. 2.The prevalence rates of diabetes or IGT were related to the economic status, diet habits, physical activities and education levels Detailed information on nutrition and diet was also investigated. 3.There was a positive correlation between age and the prevalence rate of both diabetes and IGT. The highest age-specific prevalence rate was 10.1% for diabetes, and 12.1% for IGT, both of which were found in the age-group of 60 years or more. 4.The prevalence rates also varied in different ethnic groups.

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Prevention and Risk Factors for NIDDM

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LOWERED CARBOHYDRATE INTAKE IMPAIRS GLUCOSE TOLERANCE IN HEALTHY ADULTS.

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Eight volunteers (21.6±1.2 years, 58.9±11 kg, 3 females and 5 males) were recruited for this study. They underwent medical examinations and found to be "healthy". Written informed consent was obtained from each participant. Eight subjects consumed either a high-carbohydrate (CH) diet (CH calorie; 80 %) or a low-CH diet (CH calorie; 10 %) at predetermined times (08:30, 13:00, 18:00) for 3 days with a 2-week interval. Total caloric intake of the each diet was 30 kcal/kg/day. They were told not to exercise and consume any other food or beverages except for non-caloric drinks during the experimental periods. Subjects underwent 75 g oral glucose tolerance test (OGTT) at 10:00 on the day following the end of the diet. The result of OGTT was that 6 subjects showed impaired glucose tolerance (5 borderline and 1 diabetic) after a low-CH diet, although all subjects showed normal glucose tolerance after a high-CH diet. Hemostatic model assessment and fasting insulin values (both; indices of insulin sensitivity) did not significantly differ between the two test diets. However, insulinogenic index (ability to secrete insulin) was significantly different between the two test diets (low-CH diet < high-CH diet). Fasting plasma free fatty acid level after a low-CH diet was significantly higher than that after a high-CH diet. Fasting plasma triglyceride and total-cholesterol levels were not significantly different between the two test diets. These results indicate that decreased insulin secretion, not deterioration of insulin sensitivity, is likely associated with impaired glucose tolerance after a low-CH diet. Randle effect, which is the activation of glucose-free fatty acid cycle, is thought to be one of the mechanism of impaired glucose tolerance after a low-CH diet.

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THE PROGRESSION OF IGT TO NIDDM IN CHINESE - IS THERE A ROLE IN DIET AND DRUG TREATMENT?

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The effect of drug treatment on the progression of IGT to NIDDM remains controversial. We examined the effects of glipizide on the progression of glucose intolerance over a seven-year period in 68 Chinese subjects diagnosed to have IGT according to the WHO criteria. The study design was single blind, randomized and placebo-controlled. 35 subjects received glipizide 2.5 mg daily and 33 subjects received placebo. Neither of the two groups received dietary advice. A 75g OGTT was performed yearly. At each 3-monthly follow up visit, fasting plasma glucose, blood pressure, heart rate and BMI were measured and diet and drug compliance was assessed by direct questioning and tablet counting. All data are expressed as mean±SEM. The treatment group had similar clinical and biochemical characteristics as the control group. No difference was found in the age(37±1 vs 34±1 years,p=0.11), BMI(25.98±0.73 vs 25.40±0.79kg/m^2, p=0.59), systolic BP(117±3 vs 108±4 mmHg,p=0.06), diastolic BP(76±2 vs 71±3mmHg, p=0.17), fasting plasma glucose (5.6±0.1 vs 5.6±0.9 mmol/l,p=0.72)and insulin(11.9±1.7 vs 17.9±5.8μU/ml, p= 0.33).The mean follow up period was 3.15 ± 0.21years. The last available OGTT was used for analysis. Of the 68 subjects, 57 were followed up for 2 years or more and 43 subjects were followed up for 3 years or more.During the study period, 14 subjects (40%) in the treatment group and 13 subjects (39.4%) in the control group developed NIDDM. Six subjects(17.1%) in the treatment group and eight subjects(24.2%) in the control group remained having IGT. 15 subjects(42.9%) and 12 subjects (36.4%) in the treatment and control group had normal OGTT(p=0.74).The mean time to develop NIDDM was 4.74 and 4.96 years for the treatment and control group (p=0.75) respectively.The rate of conversion of IGT to NIDDM is 12.7 % per year, which is comparable to other ethnic groups such as the Dutch(13.8%) and the South African Indians(12.6%).Our study suggests that there is no effect of glipizide on the progression of IGT to NIDDM. However, since the number of our patients is relatively small, further large scale studies are necessary.

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DISORDERS OF CARBOHYDRATE AND LIPID METABOLISM IN RELATION TO CHROMIUM STATUS. M.Vrtovec[1], J.Štupar[2], A.Briški[3], A.Kocijančič[1], A.Gantar[4]. Dept.of Endocrinol. & Metab.[1], University Medical Centre, Ljubljana, Institute Jožef Stefan[2], Ljubljana, Laboratory of the University Medical Centre Ljubljana[3], Laboratory of the IUV Tannery[4], Vrhnika, Slovenia.

The presence of chromium in man has been shown to be necessary for maintaining normal carbohydrate and lipid metabolism. In our study, the incidence of disorders of carbohydrate and lipid metabolism was compared between the population of tannery workers (n=149, M58/F91) and workers in wood industry (n=150, M55/F95) from the same region. There were no statistically significant differences between the two groups regarding age (40.6 ± 8.11 Vs 41.5 ± 7.04 years), sex and body mass index (27.28 ± 4.34 Vs 27.48 ± 4.52 kgm^{-2}). Assessment of the chromium status of both populations was made on the basis of chromium content of their scalp hair. The mean chromium content in tannery workers was significantly higher (2.68 ± 1.85 Vs 0.24 ± 0.32 μgCr/g). We found significantly lower fasting blood glucose (5.36 ± 0.86 Vs 5.71 ± 1.3 mmol/l) and lower incidence of diabetes mellitus (3 Vs 5) in the group of tannery workers but higher incidence of impaired glucose tolerance (6 Vs 4). Serious hypertriglyceridemia (TG > 2.2 mMol/l) was also less frequent among tannery workers (12.1 % Vs 18 %; average 1.57 ± 1.17 Vs 1.85 ± 1.81). Lower incidence of serious hypercholesterolemia (chol > 6.5mMol/l) was also found among tannery workers (10.7% Vs 12.7%; average 5.32 ± 1.19 Vs 5.42 ± 0.97) but it can be at least partly attributed to the lower HDL cholesterol levels (1.15 ± 0.33 Vs 1.32 ± 0.29 mmol/l) in the group of tannery workers ($p < 0.05$). Conclusion: The lower fasting blood glucose values and lower incidence of serious hypertrigliceridemia in tannery workers could be attributed with high probability to the protective role of chromium in human metabolism.

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DIET IN ASIAN INDIANS WITH GLUCOSE INTOLERANCE.

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Diet information in Asian Indians by recall, estimates of cooked food in a day and monthly purchases was obtained from 85 diabetics at four teaching hospitals in a 1988 Pilot Study, 142 subjects with abnormal glucose tolerance by OGTT in the National Diabetes Survey of 1989, 10 IGT and 4 diabetics diagnosed in a High Altitude Center Study of 1989, and 37 newly ascertained diabetics of a Tropical Diabetes Study of 1991. The minimum and maximum mean daily intakes among these populations were - total calories (1156-2586 C), carbohydrates (720-1981 C, 53-69 %), cereals or complex carbohydrates (486-1478 C, 55-70 % of total carbohydrates), refined sugars (0-137 C, 0-8 % of total carbohydrates), proteins (177-302 C, 11-16 %), animal proteins (52-121 C, 18-46 % of total proteins), fats (259-693 C, 17-32 %), animal fats (54-503 C, 30-54 % of total fats). These population nutrient intakes among rural Asian Indians were within the limits set by WHO recommendations for 'population nutrient goals' in relation to effects of chronic, non-deficiency diseases in 1990. There is as yet insufficient evidence to allow specific dietary goals in primary prevention of diabetes to be given in Asian Indians.

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PROINSULIN, IMMUNOREACTIVE INSULIN AND SPECIFIC INSULIN IN RELATION TO CONVERSION TO NON-INSULIN DEPENDENT DIABETES MELLITUS: THE MEXICO CITY DIABETES STUDY

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Although insulin resistance and decreased insulin secretion are characteristic of established non-insulin dependent diabetes mellitus (NIDDM), which of these metabolic abnormalities is the primary determinant of NIDDM is still controversial. A disproportionate increase in the proinsulin to insulin ratio has been proposed as a marker for compromised insulin secretion. We examined the association of fasting immunoreactive insulin (which cross-reacts with proinsulin), specific insulin (which does not cross-react with proinsulin), proinsulin, and the fasting proinsulin/specific insulin ratio to the risk of developing NIDDM in the 3.25 year follow-up of the Mexico City diabetes Study. These measurements were made in 85 subjects who subsequently converted to NIDDM("prediabetics") and in 95 age and gender matched subjects who remained non-diabetic at follow-up ("controls"). Immunoreactive insulin, proinsulin and the proinsulin/specific insulin ratio were significantly higher in prediabetic than in control subjects. However, the relation between specific insulin and the development of NIDDM was weaker than for proinsulin or immunoreactive insulin. After further adjustment for obesity, body fat distribution and glucose tolerance status, proinsulin and the proinsulin/specific insulin ratio (but not specific or immunoreactive insulin) predicted conversion to NIDDM. A high proinsulin/specific insulin ratio predicted conversion to NIDDM both in subjects with normal and those with impaired glucose tolerance at baseline. We conclude that in prediabetic subjects increased proinsulin, a marker of islet cell distress or compromised insulin secretion, is associated with rapid conversion (within 3.25 years) to NIDDM even in obese populations.

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DYNAMIC OBSERVATION OF PATIENTS WITH IMPAIRED GLUCOSE TOLERANCE

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The paper is concerned with preventing diabetes mellitus (DM) at early stages. Our aim was to assess the effect of diet and biguanides on patients with impared glucose tolerance (IGT). The method of patient selection was employed, where 275 patients with IGT were diagnosted among 3,312 people underwent an epidemiologic study with the standard glucose tolerance test (GTT). To estimate the reliability of statistical results, the χ^2- and Fisher criteria were employed. The patients have been under our observation during 3 years. A diet instruction was provided for all the patients, but only 91 (33%) followed these recomendations. 154 patients (56%) run there usual meal habits, and 30 patients (11%) were treated with diet and biguanide during 6 months. All these groups had no statistically significant differences in family histories of diabetes mellitus, ages, and obesity. During the observation period none of the biguanide treated patients had developed DM. The GTT normalization was observed in 21 cases (70%), and 9 cases (30%) remained with IGT. Among those followed diet, 1 (1%) developed NIDDM, 64 (70%) had normal GTT, and 26 (29%) remained with IGT. Among 154 patients which run their usual diet, NIDDM was diagnosted in 15 cases (10%), while normal GTT in 70 cases (45%); 69 patients (45%) remained with IGT. In the group treated with biguanide, weight loss and positive changes in lipidogramms were more significant than in the group treated with diet only ($P \leq 0.05\%$). In the group with free diet, nonsignificant weight gain was observed. As a conclusion, we would like to emphasize that biguanide treatment may be useful in the DM prevention for patients with IGT, obesity, and abnormal lipidogramms.

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GUT HYPERPLASIA AS A POSSIBLE FACTOR TO INDUCE POSTPRANDIAL HYPERGLYCEMIA IN NIDDM MODEL RATS.

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Otsuka-Long-Evance-Tokushima-Fatty (OLETF) rats are useful model animals for the study of the development of non-insulin-dependent diabetes mellitus (NIDDM) with hyperphagia and obesity. We found that gut in OLETF rats was enlarged compared with that in its non-diabetic control (LETO; Long-Evance-Tokushima-Otsuka) rats. To clarify the relation of intestinal enlargement and glucose absorption efficacy, we examined oral glucose tolerance test (OGTT) and oral xylose test (OXT) in these animals. Both rats were pair-fed for 2 weeks (4-6 week age). Significant increases of intestinal area (1.4-fold), wet weight (1.3-fold) and cell numbers on a villus column (1.3-fold) were observed in OLETF compared with LETO, indicating that epithelial hyperplasia was independent of hyperphagia in OLETF ($p<0.01$). Though OLETF in this stage did not have increased fasting plasma glucose level and was free from insulin resistance by glucose-clamp technique, both plasma glucose level at 1 hr by OGTT and plasma xylose level at 1 hr by OXT were significantly ($p<0.01$) increased in OLETF in comparison with LETO by 1.5-fold and 1.4-fold, respectively. Since plasma insulin levels during OGTT had no significant difference among those animals, and increased plasma xylose levels during OXT did not cause the increase in plasma insulin levels, glucose and xylose increments after oral loading in OLETF may reflect increased glucose absorption from the small intestine. These results indicate that gut epithelial hyperplasia may have important roles for the pathogenesis in both the postprandial hyperglycemia and the resulting NIDDM in this model animal.

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INSULIN RESISTANCE AND METFORMIN

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Objective: To investigate in a group of patients without Diabetes Mellitus but insulin resistance, anthropometric (weight, body mass index [BMI]), ratio of waist to hip circunference [RWH]), metabolic (glucose, insulin, ratio glucose/insulin, cholesterol, HDL, LDL, VLDL cholesterol, triglicerides, urea, uric acid and aspartate aminotransferase [AST]) and clinical changes (tolerance, side effects, vital signs, etc.) with the administration of different doses and withdrew period of Metformin. **Participants:** 12 obesity and/or hypertensive patients without diabetes mellitus, age average 49 years old (24 to 72 years), with BMI 31.24 +/- 5.4 and RWH 0.95 +/- 0.13. They were in good arterial hypertensive control. Measurments and laboratory described above were taken basal and at the end of each period (1 month each). We considered insulin resistance those with ratio of glucose/insulin < 6. We prescribed metformin 850 mg/day the first month. Increase the dose to 850 mg twice a day the second month and 850 mg tid the third month, and at the end of the 3 months we withdrew the metformin and take the measurments at the end of the fourth month. **Results:** Metformin lowered the levels of insulin (178.2+/-84 vs. 55.2+/-32.4 pmol/l, P=0.0007),cholesterol, triglicerides and LDL cholesterol; raised the ratio glucose/insulin from 3.4+/-1.1 to 10.56+/-6.2. P=0.001, was well tolerated and minimun of side effects. 850 mg/day of metformin got an important decline of the variables above. No hypoglucemia was reported. **Conclusions:** Metformin is an effective drug to lower insulin resistance and the metabolic changes seen in obese or/and hypertensive patients. Maybe it's possible with the correction of this abnormality to prevent or modify the natural clinical history of non-insulin dependent diabetes mellitus, and other entities related to insulin resistance.

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POSSIBLE ASSOCIATION OF DIABETES MELLITUS AND HELLP SYNDROME

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Following the observation of development of type 1 diabetes mellitus in a patient with HELLP syndrome, a rare but severe form of preeclampsia with haemolysis, elevated liver enzymes and low platelet count, we assumed a possible disease association based on autoimmune pathogenesis. We therefore investigated all 55 women with HELLP syndrome who were admitted to our hospital during the last 4 years for the presence of diabetes and autoimmune diseases. An oral glucose tolerance test (OGTT) with 75g glucose load, HbA1c, islet cell antibodies (ICA), insulin auto-antibodies (IAA), anti-glutamate decarboxylase antibodies (GAD), free thyroxine index, T3, thyroid stimulating hormone, thyroglobuline antibodies, thyroperoxidase antibodies, antinuclear antibodies (ANA), anti DNA antibodies and routine laboratory parameters were determined during their post partum stay in hospital. In 5 patients diabetes mellitus was diagnosed, 13 patients showed impaired glucose tolerance, only one being borderline-positive for ICA and antiGAD. In 6 of these cases a positive history of type 2 diabetes in parents or grandparents was reported. 7 patients were repeatedly found positive for ANA titers of 1:160 to 1:2560, four of which were patients within the group who's glucose tolerance was impaired. 3 patients had a history of Hashimoto's thyreoiditis. In 4 patients hypothyroidism was detected, 2 patients of which had impaired glucose tolerance. Thus we found abnormal glucose tolerance in about 30% and features of autoimmunity other than preeclampsia in about 20% of patients. In conclusion we may say that our data shows evidence for a correlation of autoimmune disease other than diabetes with HELLP syndrome. In addition a high prevalence of impaired glucose tolerance was found, but a true association with autoimmune diabetes seems unlikely. If impaired glucose tolerance is due to insulin resistance or other causes remains to be determined by further investigation.

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THE COMMUNITY DIABETES PREVENTION PROJECT (CDPP)

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The CDPP characterized a population at risk for NIDDM before the development of the complete insulin resistance syndrome (IRS). CDPP is using lifestyle interventions to alter this progression. Using a diabetes risk assessment tool, we identified 418 people (321 F, 91 M, mean age of 46 ± 9.8 yrs.) After a baseline evaluation, they were randomized into a standard (S) and intervention (I) group. The I group received an annual assessment with feedback, quarterly newsletters, behavior change program and monthly phone calls for motivation to change diet, exercise and stress behaviors. After one year 7 people developed NIDDM, 5 had IGT, 27 dropped and 13 pregnant couldn't be assessed. Below, are the baseline and year 1 data.

	Baseline		Year One	
Variables	Intervention	Standard	Intervention	Standard
BMI (kg/m^2)	29.6 ± 6.5	29.1 ± 5.6	29.6 ± 6.8	29.0 ± 6.0
Cholesterol (mg/dl)	207 ± 37	209 ± 37	205 ± 37	214 ± 45
LDL-C (mg/dl)	130 ± 31	128 ± 31	134 ± 28	129 ± 32
HDL-C (mg/dl)	48 ± 17	46 ± 15	46 ± 14	46 ± 14
Triglyceride (mg/dl)	166 ± 108	155 ± 110	166 ± 98	149 ± 91
Glucose (mg/dl)	99 ± 10	100 ± 12	99 ± 10	101 ± 13
BP Systolic (mmHg)	126 ± 15	127 ± 14	125 ± 14	123 ± 16
BP Diastolic (mmHg)	80 ± 8	81 ± 8	80 ± 8	78 ± 11
Waist/Hip Ratio	84 ± 8	84 ± 9	83 ± 12	82 ± 10
VO_2max (mg/kg/min)	21 ± 6	22 ± 7	21 ± 7	21 ± 8
Insulin (μU/ml)	7.9 ± 5.5	7.3 ± 5.4	7.4 ± 4.8	7.4 ± 4.8
C-Peptide (ng/ml)	2.7 ± 2.4	2.3 ± 1.3	2.2 ± 1.0	2.2 ± 0.9
HbA1c (%)	5.4 ± 0.4	5.3 ± 0.5	5.6 ± 0.4	5.5 ± 0.7
Alb./Creatinine Ratio	9.2 ± 11.4	9.3 ± 11.5	13.9 ± 33.5	8.5 ± 12

The CDPP study is defining the natural history of the development of IRS and whether lifestyle interventions over 5 years can prevent the progression to NIDDM.

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MATERNAL DIABETES STATUS DOES NOT INFLUENCE ENERGY EXPENDITURE NOR PHYSICAL ACTIVITY IN 5-Y OLD CHILDREN.
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Offspring of women who have diabetes during pregnancy have a greater prevalence of obesity in early adulthood than offspring of women who are normoglycemic. Obesity can result from excess food intake, low levels of energy expenditure, or both. We tested whether maternal diabetes status influences resting metabolic rate (RMR, by ventilated hood), total energy expenditure (TEE, by doubly-labeled water) or the level of physical activity (TEE/RMR) in 95 5-y old Pima Indian children. Maternal diabetes status was assessed by an oral glucose tolerance test using a 75 g glucose load during pregnancy. Twenty-four women had diabetes (2 h plasma glucose ≥11.1 mmol/l) and 71 had normal glucose tolerance (2 h plasma glucose <7.8 mmol/l with no past history of abnormal glucose tolerance).

	Diabetic Mother		Normoglycemic Mother
Number/Sex of Offspring	(13 M/11 F)		(31 M/40 F)
Weight (kg)	26.4 ± 6.9		24.1 ± 6.0
Relative Weight (%)	127 ± 25	p =0.07	118 ± 22
Percent Fat (^{18}O)	33 ± 8		31 ± 7
Birth Weight (kg)	3.9 ± 0.8	p <0.02	3.5 ± 0.5
TEE (kJ/d)	6508 ± 1109		6097 ± 957
RMR (kJ/d)	4674 ± 786		4491 ± 678
TEE/RMR	1.40 ± 0.12		1.36 ± 0.13

TEE and RMR were similar in both groups after adjustment for body size and sex by linear regression analysis, indicating that maternal diabetes does not affect energy expenditure nor physical activity in young children. Thus, increased energy intake is the likely cause of obesity in early adulthood among offspring of women with diabetes.

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LIFE STYLE INTERVENTION IN NIDDM: FIRST REPORT OF JAPAN DIABETES COMPLICATIONS STUDY (JDCS)
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Rapid changes in life style such as diet and physical activity in last 50 years are a major cause of an increasing number of diabetic subjects with diabetic complications in Japan. In the present study, we have performed life style modification to prevent the progression of diabetic complications including retinopathy, nephropathy, and macroangiopathy in 2066 non-insulin dependent diabetes mellitus without complications or with background retinopathy whose HbA1c is greater than 6.5%. 2066 diabetic subjects were randomly divided to two groups; a group of intensive life style modification and a group of conventional treatment. In a group of intensive life style modification, diet and physical activity are intensively managed to achieve a following goal of treatment; HbA1c, BMI, blood pressure, plasma cholesterol, triglyceride levels, W/H ratio should be less than 6.0%, 22 kg/m^2, 140/85 mmHg, 220 mg/dl, 150 mg/dl, 0.9 for men, 0.8 for women, respectively, and plasma HDL cholesterol level should be greater than 40 mg/dl, and patients should quit drinking alcohol and smoking. We have started the JDCS since April in 1996, and initial HbA1c was 7.8 ± 1.2% in intensive group and 7.9 ± 1.3% in conventional group. This is the first report of JDCS which is a prospective study performing 6 year-follow up.

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AUTOIMMUNE PROFILE OF NON-INSULIN-DEPENDENT DIABETES IN NORTH INDIANS
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Non-insulin-dependent diabetes in the young (NIDDY) is frequent among North Indian Asians and differs clinically from MODY. NIDDY may have a heterogenous etiology. We studied pancreatic islet cell antibodies (ICA) and glutamic acid decarboxylase antibodies (GADA) in NIDDY subjects and in patients with Type 1 diabetes and healthy controls. ICA were measured by indirect immunofluorescence and GADA by immunoprecipitation of 35 S recombinant human GAD. Results: The frequency of autoantibodies is shown in the table.

	NIDDY	Controls	Type 1 DM
ICA	1/49 (2%)	4/130 (3%)	22/62 (36%)*
GADA	16/28 (57%)**	1/67 (1%)	-

* p<0.001 vs NIDDY ** p < 0.001 vs controls

When NIDDY patients having duration of diabetes of > 2 years (42/49) were compared to Type 1 diabetic patients of similar duration the ICA frequency was similar (1/42 vs 4/20, p=0.06). Mean GADA titre in NIDDY subjects was 25±33 U vs 3±2 U in controls (p < 0.001). Conclusion: A significant proportion of NIDDY patients have GADA positivity, suggesting that in North Indian Asians this entity may be heterogenous.

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LONG-TERM TOLERABILITY PROFILE OF ORLISTAT, AN INTESTINAL LIPASE INHIBITOR.
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The risk of Type 2 diabetes is increased 2-10 times in obese individuals, as a result of various factors, including insulin resistance, impaired glucose tolerance and dyslipidaemia. In a 1-year Phase III study of 322 diabetic obese patients, orlistat 120 mg tid in conjunction with a hypocaloric diet (estimated energy expenditure minus 600 kcal/day, minimum 1200 kcal/day, with 30% of calories as fat) resulted in maintained weight loss and significantly improved glycaemic control. The long-term tolerability of orlistat was assessed in a meta-analysis including this study and 6 Phase III studies of obese non-diabetic patients. All studies had a 4 or 5 week single-blind lead-in period with placebo and a hypocaloric diet. Patients were then randomised to a double-blind, placebo-controlled treatment period lasting up to 2 years. A total of 1740 patients received placebo; 2038 patients received 120 mg orlistat tid with meals. Significantly increased incidences of adverse events (AEs) were only reported in the gastrointestinal (GI) tract, as expected from orlistat's mode of action and negligible absorption. Most of the GI AEs were of mild intensity and experienced within the first week of treatment. The incidence of GI AEs reduced greatly after 12 weeks of treatment, indicating that long-term treatment does not increase the risk for these events. Indeed, there was a substantial decrease in reported GI AEs in year 2 compared with year 1; GI AEs with the highest frequencies (8-27%) in year 1 decreased by 6-22 percentage points in year 2. During year 1, 35% of patients in the placebo group withdrew prematurely from the trial versus 29% of orlistat-treated patients. The rates of premature withdrawal were lower for all treatment groups in year 2 compared with year 1. Moreover, over 1 year of treatment, only 7% of patients in the orlistat 120 mg group withdrew because of GI AEs. Statistically significant decreases in plasma levels of vitamins D, E and beta-carotene were reported following orlistat treatment, however, these values remained within the normal clinical range. In conclusion, a large patient population with extensive exposure to orlistat has been assessed for tolerability. Although there was an increase in GI AEs, these were well tolerated and did not lead to an increased drop-out rate overall compared with placebo. There were no major CNS or cardiovascular events. Orlistat 120 mg tid is well tolerated for long-term use in the treatment of obese patients at risk of Type 2 diabetes.

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TITLE: FACTORS INFLUENCING THE CONVERSION OF IMPAIRED GLUCOSE TOLERANCE TO DIABETES MELLITUS

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Abstract: The aim of this study was to find out the infulence of certain factors like age, sex, family history of diabetes, blood pressure, obesity and glycaemic status on the conversion of impaired glucose tolerance (IGT) to diabetes mellitus. All newly diagnosed IGT (according to WHO criteria) subjects attending the outpatient depertment of BIRDEM, Dhaka, Bangladesh were considered for the study. One hundred thirteen subjects with IGT were followed for 4 to 13 months (mean 10.3 months). Diabetes mellitus developed in 23 (20.35%), glucose tolerance remained impaired in 69 (61.07%) and glucose tolerance returned to normal in 21 (18.58%). Of the baseline variables examined , plasma glucose and HbA_{1c} levels at the time of the initial examination were consistent in predicting conversion of IGT to diabetes, when the data were examined by univariate method. Both fasting (P< 0.001) and 2-h (P< 0.001) plasma glucose levels were useful predictors. Although HbA_{1c} level was within the normal range, but it was highly predictive in conversion to diabetes (P< 0.001). The IGT subjects with family history of diabetes in their first degree relatives showed a higher tendericy to become converted to diabetes. None of the other baseline variables was found predictive for conversion of IGT to diabetes. Chi-square and student's 't' test were used for statistical analysis. We conclude from our study that glycaemic status at the initial examination in subjects with IGT has an influence on the conversion of IGT to diabetes mellitus. We also conclude that heredity may be a predictive factor in conversion of IGT to diabetes mellitus.

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THE LONG TERM EFFECTS OF ORLISTAT, A NEW LIPASE INHIBITOR, IN TREATMENT OF OBESE NIDDM PATIENTS

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A strong association between obesity and the development of NIDDM has been well established. Weight loss can improve glycaemic control and lower cardiovascular risk in such patients. Orlistat, a new agent for the long-term management of obesity, decreases the absorption of fat through inhibition of lipase in the gut. A 57-week multicentre, randomised double-blind, placebo-controlled group study was undertaken to determine its effect in obese NIDDM patients treated with oral hypoglycaemic agents. After one year of treatment, patients receiving 120 mg orlistat tid (n = 162) lost an average of 6.2±5.7% of initial body weight compared with 4.3±6.2% for placebo (n = 159), a difference of 2.4 kg ($p<0.05$). Moreover, 49% of orlistat-treated patients lost more than 5% of their initial body weight versus 23% of the placebo group. Fasting blood glucose fell by 0.02 mmol/l in the orlistat group and increased in the placebo group by 0.54 mmol/l ($p<0.05$). A significant improvement in HbA1c was seen in the orlistat group, a decrease of 0.2% compared to an increase of 0.3% in the placebo group ($p < 0.01$). Patients with HbA1c >8% at baseline on orlistat had a mean decrease of 0.5% compared to a mean decrease of 0.05% in the placebo group. A significant reduction in the dose of oral agent was seen in the orlistat group ($p=0.0019$). Improvement of mean levels of total cholesterol, LDL-cholesterol, triglycerides, LDL/HDL ratio and apoliprotein B were seen in the orlistat group as compared to the placebo group ($p\leq0.04$). The incidence of adverse events was similar in both treatment groups in all body systems other than the gastrointestinal (GI) system. As was expected, due to the mechanism of the drug, a higher incidence of mild GI events, 20.5% greater than the placebo group, were reported in the orlistat group. There were no clinically significant decreases in vitamin levels in the orlistat-treated patients. The study showed that orlistat is well tolerated and effective in causing weight loss in NIDDM patients with attendant improvement of glycaemic control and lipid parameters. It also demonstrates that anti-obesity drugs provide an additional approach for the treatment of NIDDM.

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SMOKING, GLUCOSE INTOLERANCE AND INSULIN LEVELS IN SWEDISH MIDDLE-AGED MEN.

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Smoking is suggested to cause non-insulin-dependent diabetes (NIDDM) by way of insulin resistance. It is not known whether smoking also affects insulin secretion. The aim of this study was to study the association between glucose tolerance, insulin levels and smoking accounting for body mass index (BMI) and family history of diabetes. We performed a cross-sectional study of 3129 men aged 35-56 years selected from four communities in Stockholm. The sample consists of 52% with at least one first or two second degree relatives with diabetes and 48% without relatives with diabetes. In a glucose tolerance test (WHO) we detected 55 men with NIDDM (previously not diagnosed) and 172 with impaired glucose tolerance (IGT). We also identified 502 men with glucose concentrations in the upper part of the normal range defined as 2h plasma glucose levels 5.8-7.7mM. Information on body weight, length, cigarette smoking and other life habits were obtained by a questionnaire. The analyses were done with adjustment for age, BMI and family history of diabetes. The relative risk of NIDDM was 1.7 (95% confidence interval 0.8-3.4) for cigarette smokers and 2.7 (1.3-5.7) for men smoking 16 or more cigarettes per day. Corresponding estimates for IGT were 1.1 (0.7-1.6) and 1.2 (0.7-1.9). These estimates were also close to unity for high normal glucose concentrations. The relative risks associated with smoking were more pronounced for lean men and for men without a family history of diabetes in NIDDM. The results indicate that smoking was not significantly associated with indicators of insulin resistance (fasting insulin ≥21mU/l) or low insulin response (delta 2h insulin ≤60mU/l) in IGT. In conclusion, high consumers of cigarettes have increased risk of NIDDM after controlling for age, BMI and diabetes in the family. It is unclear, however, whether this increase in risk is due to insulin resistance, a decreased insulin response or both.

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THE EFFECT OF ORLISTAT ON GLUCOSE TOLERANCE IN OBESE NON-DIABETIC INDIVIDUALS. T. Taylor, Georgetown University Medical Center, Division of Endocrinology, Washington, D.C.

Obesity is often associated with a series of metabolic disturbances, including insulin resistance and impaired glucose tolerance. Indeed, insulin resistance and impaired glucose tolerance are the fundamental links between obesity and the development of diabetes. A new compound, orlistat, an inhibitor of dietary fat absorption, has been shown to promote maintained weight loss in obese diabetic patients. This weight loss was also associated with a significant improvement in patients' glycemic control. In order to investigate the effects of orlistat on glucose tolerance in obese (average BMI 35 kg/m^2) non-diabetic patients, a meta-analysis of 1 and 2-year Phase III USA and European clinical trials has been undertaken. In total, 1096 patients received placebo and 1521 patients received orlistat 120 mg tid, both in conjunction with a hypocaloric diet in year 1 and a eucaloric diet in year 2. After 1 year, the average loss from initial body weight was 6.2% for orlistat-treated patients and 4.3% for placebo ($p < 0.05$). After 2 years, orlistat-treated patients had lost 6.7% of initial body weight versus 3.7% for placebo ($p < 0.05$). Of patients who had normal OGTT values at baseline, 1.3% of the placebo sub-group and none of the orlistat sub-group were classed as diabetic at the end of 104 weeks. Similarly, of patients with impaired glucose tolerance at baseline, 25% of the placebo sub-group and 4.3% of the orlistat sub-group were diabetic at the end of 104 weeks. The overall changes in fasting insulin after 104 weeks from initial values for placebo and orlistat were -8.9 and -20.3 pmol/L, respectively. There was a statistically significant difference in fasting glucose between the placebo and orlistat groups after 1 year ($p=0.001$; difference -0.07 mmol/L). Also after 1 year, oral glucose tolerance tests (OGTT) revealed there was a mean increase in incremental glucose area under the curve (AUC) level of 0.23 for placebo-treated patients compared with a mean decrease from baseline of -1.51 for orlistat treated patients ($p<0.001$). Furthermore, in year 1, insulin AUC only decreased by 18.26 in placebo recipients compared with 263.8 in the orlistat group ($p<0.001$).These results suggest that orlistat may assist in reverting impaired glucose tolerance to normal, which may delay or prevent progression to Type 2 diabetes in non-diabetic obese individuals.

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PREDICTING CHANGE IN VISCERAL ADIPOSITY: A PROSPECTIVE STUDY IN JAPANESE AMERICANS

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We hypothesized that baseline insulin resistance and lower insulin secretion would predict change in visceral adiposity over 6 years of follow-up in third generation Japanese Americans. At baseline, 115 nondiabetic men and 115 nondiabetic women had a 75 g oral glucose tolerance test (WHO criteria for glucose tolerance); body mass index (BMI) (weight in kg/height in m2); visceral adiposity as measured by intra-abdominal fat area (IAF) at the level of the umbilicus using computed tomography; and fasting and 30 minute plasma glucose, insulin, and C-peptide levels. Insulin secretion (IS) was defined as the (30 - 0 minute insulin)/30 minute glucose. Measurement of BMI and IAF was repeated after 6 years of follow-up. The outcome was defined as change in IAF (ΔIAF) over 6 years of follow-up (6 year IAF - baseline IAF). Baseline characteristics were as follows: mean age women (W) 40.1 yr, men (M) 40.1 yr; mean BMI W 22.8, M 24.9; mean IAF W 42.6 cm2, M 74.8 cm2; IGT prevalence W 31.1%, M 19.1%. Overall and visceral fatness increased on average over 6 years (ΔBMI W 1.3, M 1.0; ΔIAF W 19.0 cm2, M 13.5 cm2). Among women, ΔIAF was positively correlated with the following baseline variables: fasting insulin (FI) (r=0.27, p=0.008), fasting C-peptide (FC) (r=0.29, p=0.004), IAF (r=0.31, p<0.001), and BMI (r=0.33, p=0.001). In a multiple linear regression model among women that adjusted for baseline age, IAF, and IGT status, both baseline FI (coefficient (B) =0.130, 95% confidence limits (CL) 0.003 to 1.257, p=0.046) and IS (B= -0.14, 95% CL -0.25 to -0.04, p=0.009) were significantly related to ΔIAF. Among men, similar weaker, nonsignificant associations were noted between ΔIAF and baseline FI, FC, and IS in univariate and multivariate analyses. We conclude that gain in visceral adiposity was related to higher fasting insulin (reflecting lower insulin sensitivity) and lower insulin secretion in women, but this effect was not observed to the same degree in men. In this population, patterns of insulin sensitivity and secretion associated with higher subsequent NIDDM risk predict gain in visceral adiposity.

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GLUTAMATE DECARBOXYLASE ANTIBODIES IN CHINESE PATIENTS WITH NIDDM DO NOT IDENTIFY LATENT IDDM

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NIDDM is a heterogeneous condition, with patients having varying age-at-onset, body weight and need for insulin treatment. It has been suggested that in Asians patients with NIDDM, a significant subset of patients may have latent or slow-onset IDDM, characterised by presence of autoantibodies to GAD, eventual loss of ß-cell function and need for insulin treatment. The objective of this study was to determine the prevalence of GADab in Chinese NIDDM patients and evaluate them with patients with classical IDDM. Autoantibodies to GAD were tested in 168 Chinese NIDDM patients (mean age 40±15.3yr, mean duration 2.3±4.3yr), and 134 IDDM patients (mean age 22.1±12.9yr, mean duration 4.6±6.6yr). GADab was measured with an ELISA (rated 83% valid, 100% specific and 75% sensitive;1st GAD Proficiency Program, USA). The prevalence of GADab was 16.1% in the NIDDM patients and 39.6% in IDDM patients (p<0.0001). GADab titre in NIDDM was similar to IDDM (31.3±29.4 vs 42.6±41.1 relative units). GADab[+] NIDDM patients compared to those GADab[-] had similar age-onset (39.3±16.5 vs 38.3±15.1yr), body weight (BMI 23.2±4.0 vs 24.6±4.0kg/m2), duration of diabetes (2.8±5.2 vs 2.2±4.3yr) and fasting C-peptide concentrations (0.62±0.30 vs 0.69±0.32 nmol/l). In contrast, GADab[+] IDDM had significantly younger age-onset (18.1±11.5yr), lower body mass index (18.1±3.3 kg/m2) and fasting C-peptide concentrations (0.20±0.1nmol/l) compared to both the GADab[+] and GADab[-] NIDDM patients.Six of 27 (22.2%) GADab[+] NIDDM patients required insulin treatment for glycaemic control compared to 26 of 141 (18.4%) GAD[-] NIDDM patients (p=NS). In conclusion, 16%of our Chinese NIDDM patients had presence of GADab compared to 1.7% reported in Koreans and 8.3% in Thais.The presence of GADab in Chinese NIDDM patients did not distinguish a sub-group who may have latent or slow-onset IDDM.

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SICK GENES, SICK INDIVIDUALS OR SICK POPULATIONS WITH CHRONIC DISEASE? AN INTERNATIONAL EXAMPLE FROM STUDYING DIABETES.

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Intensive searches for genes predisposing to or "causing" chronic disease are based on a premise that familial patterns indicate gene-based inheritance. Rose's alternative, less popular with clinical scientists thinking in individuals only, is that populations give rise to their deviations and extreme values who become patients. NIDDM is an example where population-based twin registers (e.g. Denmark) show little mono-dizygotic difference, suggesting major hospital ascertainment bias in ascribing a genetic basis. Here, we examined geographically dispersed populations of West African origin of similar genetic background to test how much environmental exposures can contribute to diabetes prevalence. Careful representative samples (n=400 per site, 200 men) aged 25-74 years were drawn from local population registers at rural and urban sites in Cameroon, Jamaica and among African-Caribbean (AfC) migrants to Manchester, UK who are mainly of Jamaican birth. Standardised methods, including cross-site training, were used to ascertain blood pressure (BP) and diabetes status by 2 hour GTT, on WHO criteria. Nutritional intakes were also characterized in each site. World age-adjusted NIDDM prevalence rose considerably within Cameroon, rural (0.8%, 95% CI 0.1-3%) to urban (2, 0.4-5 %) despite younger urban mean age, was higher again in Jamaica (7.4, 4-12 %) where male body mass indices (BMI) were lower than in Yaounde perhaps related to recent economic difficulties. Results were highest in Manchester (11.2, 8-19,%) where physical activity rates were minimal. Across-site population attributable risk for known diabetes from obesity (>25kg/m^2) ranged from 20-40%. Hypertension rates also rose sharply across site (6% rural Cam.-28%,UK), closely related to BMI. Occurring on closely similar genetic backgrounds within Cameroon and between Jamaica and Manchester suggests factors affecting energy balance (intake versus expenditure) rather than gene differences determine diabetes and hypertension rates in these and likely in most populations.

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IS INSULINOPENIA IN AFRICAN NIDDM IMPORTANT?

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To test the hypothesis that severe hyperglycaemia at presentation in Africans with NIDDM (**A**) is a consequence of insulinopenia, associations with plasma glucose at diagnosis were sought in 127 **A** and 74 caucasian patients (**C**) with similar BMI (±30 kg/m^2). **A** had higher glucose (**A** 18.2, IQR 13-32 mmol/l; **C** 15.0, 12-19 mmol/l, p<0.001); were more often ketonuric (**A** 35.2%; **C** 14.3%, p<0.001), acidotic (**A** 16.5%; **C** 8.5%, p=0.015), and significantly infected (**A** 29.1%; **C** 13.5%, p=0.01). Ischaemic heart disease was present in 15% of **C**, but was absent in **A**. Regression analysis showed independent associations with glucose in **A** only - with infections, p=0.003, and with BMI (neg) p=0.02). After stabilization C-peptide was 1.9 ng/ml in **A**,. vs 4.0 ng/ml in **C** (p<0.001), and was associated with BMI in both **A** (glucose 10.2 mmol/l) and **C** (glucose 10.2 mmol/l). In a parallel study, C-peptide was significantly (p<0.05) lower in **A** vs **C** in each quartile of BMI. *Conclusions*: at diagnosis of NIDDM in **A**, severe hyper-glycaemia 1) is more frequent in **C**; 2) is associated with infections and weight loss; 3) is not associated with C-peptide levels. Insulinopenia does not cause severe hyperglycaemia in **A**, who are more insulin sensitive than **C**.

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Sequential changes in serum insulin concentration during development of non-insulin dependent diabetes in Japanese

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Changes in serum insulin concentrations during deterioration of glucose tolerance were studied in 38 male Japanese workers serving in a Japanese company who changed from IGT to NIDDM according to WHO criteria. When they had IGT, they were followed at 0.5 to 1 year intervals and had an OGTT, in which venous plasma glucose and serum insulin concentration were measured after an overnight fast and 1 and 2h after ingestion of a 75 g glucose. In 23 subjects(48.0±6.5years and body mass index,BMI 23.8±2.4,means±SD) fasting and glucose-stimulated insulin concentrations decreased progressly with increased glycaemia until glucose concentrations reached the range that defines IGT. Progression from IGT to NIDDMM was associated a further decrease in fasting insulin concentrations and insulin response. On the other hand,in 8 subjects(46.9±6.4 years and BMI 28.1±3.6) the onset of IGT or NIDDM was associated with an increase in fasting and glucose-stimulated insulin concentrations. In 7 subjects(50.4±3.2 years and BMI 24.1±1.7) the onset of IGT was associated with high insulin concentrations, but progression from IGT to NIDDM was associated with a decline of insulin concentrations. In Japanese NIDDM, there is heterogeneity in the patogenesis of NIDDM. Some people destined to develop diabetes have primary defect in β-cell function, some people have primary defect in insulin sensitivity and others have diabetes because of both diminished insulin sensitivity and impaired β-cell function.

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GLUCOSE TOLERANCE AND OTHER METABOLIC PARAMETERS IN SIBLINGS OF NIDDM PATIENTS (PRELIMINARY RESULTS)

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Aim of the study was to investigate in which extent relatives of NIDDM patients present an impaired metabolic profile. 17 siblings of NIDDM patients and 16 controls, matched for sex, age and BMI, underwent oGTT (WHO criteria). Lipids and uric acid (UA) were also measured. Male siblings, as compared to controls, had higher concentrations of UA (303.35±27.58 vs. 249.82±21.44μmol/l, p=0.029). No significant differences were noted in triglycerides, total-, HDL-, LDL-cholesterol, apoA1 and apoB. Female relatives had higher waist-to-hip ratio (W/HR) and fasting c-peptide levels than respective controls (0.88±0.075 vs. 0.79±0.085, p=0.018 and 2.51±0.96 vs. 1.45±0.45ng/ml, p=0.008 respectively). Areas under glucose curves (AUGC) were significantly higher in siblings (1208.48±213.59vs. 988.82±174.86 mmol/l/120min, p=0.003). In addition, 13 out of 17 siblings but only 2 out of 16 controls had impaired glucose tolerance (IGT), (Chi-square=13.60, p=0.0002, RR=6.12). Relation between BMI and AUGC was significant in siblings (r=0.72, p=0.003) but not in controls (r=0.18, p=0.512). In addition c-peptide was positively related to age in controls (r=0.59, p=0.031) and negatively in siblings (r= -0.68, p=0.007).This was confirmed by subsequent multiple regression analysis (independent variables: age, BMI, WHR). **Conclusions:** Siblings, compared to controls, present a) higher frequency of IGT, b) greater AUGC values, c) higher UA(males), d) higher fasting c-peptide levels (females), e) similar lipid levels. Furthermore, there was a positive relationship between c-peptide and age in controls and a negative one in siblings.

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RISK FACTORS OF NON-INSULIN DEPENDENT DIABETES MELLITUS, IMPAIRED GLUCOSE INTOLERANCE AND HYPERTRIGLYCERIDEMIA IN CHINESE

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In order to ascertain whether visceral obesity is a risk factor for non-insulin dependent diabetes mellitus (NIDDM), impaired glucose intolerance (IGT) and hypertriglyceridemia in Chinese, regional adipose tissue distribution was measured by magnetic resonance imaging (MRI) in 44 patients with NIDDM, 28 with IGT, 18 with hypertriglyceridemia and 79 healthy controls.All were male with age from 40 to 70 years old.The influences of insulin-glucose homeostasis indices, body mass index (BMI), blood pressure, serum lipids and regional adipose tissue distribution indices on NIDDM, IGT and hyperglyceridemia were analyzed by logistic regression method.Results: 1.Fasting plasma insulin (P=0.0004), ratio of insulin to glucose at 30' after 75g oral glucose load(P=0.0183), fasting insulin resistance index (FIRI=fasting glucose×fasting insulin/25) (P=0.0000), molar ratio of C-peptide to insulin area under curve after glucose challenge (P=0.042), serum triglyceride (P=0.0139) and systolic blood pressure (P=0.0176) were independent variables for NIDDM. 2.C-peptide at 2h after glucose challenge (P=0.0536), FIRI (P=0.0001), molar ratio of C-peptide to insulin in fasting state (P=0.0815), systolic blood pressure (P=0.0776), BMI (P=0.0011) and visceral adipose tissue area (P=0.09) were risk factors for IGT. 3.Visceral adipose tissue area (P=0.0145) and molar ratio of C-peptide to insulin in fasting state (P=0.0575) were independent variables for hypertriglyceridemia. Our study suggested that in Chinese insulin resistance,abnormalities of insulin secretion and insulin clearance were also the common characteristics of NIDDM and IGT, while visceral adipose tissue accumulation was an important risk factor for IGT and hypertriglyceridemia.

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ARE NUTRITIONAL FACTORS INVOLVED IN THE INCREASED PREVALENCE OF NIDDM IN JAPANESE MIGRANTS IN BRAZIL?

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Genetic and environmental factors may be involved on the genesis of NIDDM. We confirmed the increased prevalence of glucose intolerance in a Japanese community aged 40-79 yrs living in Brazil, using OGTT and WHO criteria. The present study aimed to evaluate the association of nutritional aspects with glucose intolerance in this population. Nutritional questionnaires (food frequency data from the 2 months) were obtained from 506 subjects and computed by specific software. 335 subjects were normals, 88 impaired glucose tolerants (IGT) and 83 were diabetics, being 51 previously and 32 newly diagnosed (ND). Nutrients intake and clinical parameters of ND and IGT subjects (intolerant group) were compared with the normals. Intolerant group showed higher BMI, waist/hip ratio, plasma cholesterol and triglyceride and higher frequency of obesity, hypertension and dyslipidemia (p<0 05). Normal and intolerant groups, respectively, did not differ concerning total energy intake (EI) (medians: 2802 and 2779 Kcal), percentages of energy derived from fat (FI) (31.9 and 31.6%), protein (14.2% for both) and carbohydrate (53.5 and 53.2%), polyunsaturated / saturated ratio (P/S) (0.53 and 0.51) and had similar acculturation scores. Intolerant group tended to have lower level of physical activity. Considering adjustments for sex, age, generation, EI, FI, P/S, hypertension, waist/hip, physical activity and acculturation, logistic regression showed that only hypertension and waist/hip remained associated with glucose intolerance and reinforce a lack of association with FI. This methodology did not differentiate the groups by food intake patterns, which could be in part due to the sample size. On the other hand, our data could be reflecting the importance of genetic factors for NIDDM, expressed by a western type diet.

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THE PRELIMINARY EPIDEMIOLOGIC STUDY OF RISK FACTORS IN 50 CHINESE NIDDM FAMILIES

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Diabetes has become a major public health problem for people of any age and in any country in the world. In order to estimate the environmental factors for NIDDM patients, investigation was carried out in 50 Chinese NIDDM families. There are at least two sips with NIDDM in every family. The other members of the family were divided into normal or IGT (impaired glucose tolerance) group according to the test of OGTT. Comparison of the means was done by X test using SPSS package. The results showed that ages, BMI, w/t rates and blood pressure in NIDDM patients (119 cases) have significant difference compared with those of the normal group (131 cases). We also found 41 IGT patient in those families. It is suggested that the development of NIDDM was closed associated with ages, obesity and hypertension. Further studies are proceeding, especially in screening of the candidate genes in these families.

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EVIDENCE OF ACCULTURATION IN FIRST AND SECOND-GENERATION JAPANESE AND JAPANESE-BRAZILIANS: ASSOCIATION WITH NIDDM?

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Japanese migrants are more susceptible to NIDDM, that may occur in response to environmental factors, many of which reflect acculturation. We have reported increased prevalence of NIDDM in Japanese-Brazilians especially in the second-generation (IIG). The present study evaluate the acculturation process from the first (IG) to the IIG and a possible association with NIDDM. The sample comprised 238 IG and 292 IIG subjects; male/female ratios were 1.14 and 0.87, and mean ages were 66±9 years and 53±8 years, respectively. Diagnosis of NIDDM was based on oral GTT using WHO criteria. An acculturation score (0 or 1 or 2) was attributed to each subject based on a sociodemographic questionnaire, using a sociometric scale. Score 2 corresponded to those who considerably changed to western lifestyle. Higher number of subjects with score 0 was seen in the IG when compared to IIG (47% vs 26%) and higher frequency of score 2 in the IIG (20% vs 42%) ($p<0.0001$). Dividing in three groups (normals: n=349; impaired glucose intolerants (IGT): n=90 and diabetics: n=91), the same pattern of acculturation score was observed for each single group and no association with their glucose tolerance state. We conclude that our methodology was able to demonstrate the higher degree of acculturation in the IIG Japanese-Brazilians, but not to differentiate the 3 groups according to the acculturation scores. However, our results could be pointing to the importance of genetic factors, whose expression was manifested when a high risk population is exposed to an unfavorable environment.

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BODY MASS INDEX, WAIST TO HIP RATIO AND OTHER RISK FACTORS FOR DIABETES IN CHONGQING, CHINA

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Objectives: To investigate the prevalence of diabetes mellitus (DM) and its risk factors in Chinese people. **Methods**: A cross-sectional survey was conducted in 1996 in Chongqing people, aged ≥35 years old. DM was diagnosed based on 2-hour plasma glucose ≥11.1 mmol/L or DM patients on treatment. Age-adjusted mean was used in statistic test. **Results**: (1) A total of 489 subjects was randomly selected (241 in men and 248 in women). Mean (SD) age was 49 (10.3) in men and 48 (9.6) in women. (2) Prevalence of DM was 12.5% in men and 9.7% in women. (3) Age-adjusted mean (SE) for significant factors between subjects with and without DM is shows in Table 1.

Table 1 Age-adjusted mean (SE) in subjects with and without diabetes by sex

	Non-Diabetes	Diabetes	P
Males			
Body mass index (BMI)	23.98 (0.23)	26.29 (0.63)	<0.001
Waist to hip ratio (WHR)	0.88 (0.01)	0.93 (0.01)	<0.001
HDL-cholesterol (HDL)	1.20 (0.02)	0.97 (0.06)	<0.001
Triglyceride (TG)	1.40 (0.07)	2.08 (0.19)	<0.001
Females			
Waist to hip ratio (WHR)	0.81 (0.004)	0.85 (0.01)	<0.001
Systolic BP (SBP)	118.32 (1.19)	132.17 (3.73)	<0.001
Diastolic BP (DBP)	74.63 (0.71)	79.51 (2.23)	<0.05
HDL-cholesterol (HDL)	1.39 (0.02)	1.19 (0.07)	<0.01
Triglyceride (TG)	1.05 (0.04)	1.79 (0.13)	<0.01

Conclusion: The findings suggest that increased WHR, TG and decreased HDL are associated with risk of DM in both sexes. BMI in men and blood pressure in women are associated with risk of DM, respectively.

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WESTERNIZED LIFESTYLE INCREASING INSULIN RESISTANCE DURING DEVELOPMENT OF NIDDM IN JAPANESE SUBJECTS.

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Both insulin resistance (IR) and insufficient insulin secretion play an important role in the development of NIDDM. The present study investigates how more westernized lifestyle has influenced the association between IR and NIDDM in Japanese subjects. Japanese - American originally from Hiroshima who are now living in Los Angeles (JA, N = 1,020, age 61± 8 (SD) yrs) and Japanese currently residing in Hiroshima (JH, N = 962, age 59 ± 4 yrs) were surveyed between 1980 and 1993. Serum glucose (SG), insulin (IRI) and C - peptide (CPR) levels were measured before and after a 75-g glucose load. In the 1980 study, IRI and the SG ratio were measured over the first 30 min after the glucose load (Δ I / G30) as a marker of insulin secretion. Age•sex -adjusted prevalence of diabetes (WHO criteria) was significantly higher in JA than JH (13.4% vs. 6.2%, $p < 0.01$). Waist -hip ratio, as well as fasting (F)-IRI, F-CPR, ∑-IRI and ∑-CPR were significantly higher and F-CPR / F-IRI molar ratios were significantly lower in JA than JH when age, BMI and glucose tolerance state were adjusted ($p < 0.05$ - $p < 0.001$). IR (evaluated by homeostasis model assessment) associated with increasing 2hr-SG post glucose load was significantly higher in JA ($p < 0.01$ - $p < 0.001$). The highest incidence of diabetes during the 6 ± 3 yr follow-up period was observed in the low Δ I / G30 (≤ 0.5) and high F-IRI (≥ 9 μU/ml, a marker of IR) group (36.4 per 1,000 person / year) followed by the low Δ I / G30 and low F-IRI group (16.1 per 1,000 person / year) . Thus, the hyperinsulinemia observed in JA appears to be associated with higher CPR levels, suggesting the presence of IR and compensatory excretion of insulin. Central obesity may also play an important role in IR in JA. The present results also suggest that increased IR associated with a westernized lifestyle in subjects with decreased insulin secretion play an important role in the development of diabetes in Japanese subjects. Thus, an increased westernized lifestyle appears to greatly impact the development of NIDDM through an increase in IR in Japanese subjects.

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HYPERINSULINAEMIA AND DIABETES:
A FAMILY STUDY OF NIDDM IN NEW ZEALAND MAORI

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Aim: We investigated a diabetes prone Maori family with a history of early onset diabetes to determine the prevalence of hyperinsulinaemia, niddm, and igt.
Methods: A multigenerational family was enrolled for this cross-sectional study All but one of the subjects studied had a physical examination and an oral GTT with total insulin) levels at time 0 (fasting), 30,60 and 120 minutes.
Results: 64 studied. Raised fi (fi>13μu/ml=2 SD above normal mean) was present in 34/64. 7/33 with raised fi had abnormal glucose tolerance(agt) and 5/31 of normal insulin grp. Generation 1(n=9):4 nidds, 2 igt, 3 normal oggt (ngt). Three nidds, 2 igt were newly diagnosed. Generation 2(n=31):3 nidds, 2 igt, 26 ngt. Two nidds and both igt were new. Prevalence of abnormal glucose tolerance varied from 16% (gen 2) to 67% (gen 1).Generation 3(n=26):26-ngt. Of 52 ngt: 26 (grp1) had raised fi and 26 normal fi (grp 2 controls). Relative risk for diabetes in grp 1 vs grp 2: 1.17(ci: 0.28 to 0.48). Factors associated with raised fi were: Grp 1: systolic bp (p<0 02,ci:0.1 to 0 9), Grp 2:bmi (p<0.03),age (p<0.03) and male gender. Oggt insulin levels at 30, 60 and 120 minutes but not fasting were reduced (p<0.01) in nidds and glucose levels were increased (p<0.0001) vs ngt. The area under the glucose and insulin curves were not different for grp 1 vs grp 2.
Conclusions: In this Maori pedigree,raised fi confers increased risk of diabetes and is inherited as autosomal dominant. Prevalence of agt increases by generation. Raised fi and systolic bp may be an early feature of a familial insulin resistance syndrome. Factors other than bmi, age, sex appear to determine the insulin resistance in a subgroup of this family with ngt. The high prevalence of undiagnosed diabetes suggests regular screening is required in these families.

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MATERNAL AND PATERNAL DIABETES CONFER SIMILAR RISKS OF TYPE II DIABETES TO CHILDREN.

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The occurrence of Type II diabetes in siblings of 743 Type II patients (aged 42-86 years and diagnosed between ages 35-74) was ascertained by questionnaire and verified by interview. For 681 families (91.7%), the index case had knowledge of the diabetes status of both parents as well as siblings. Siblings with diabetes were reported by 222 (32.5%) index cases, giving a study group of 362 cases among 1816 siblings (20%). Life table estimates of cumulative incidence of diabetes by age 70 years in siblings (excluding index cases) was used as the measure of lifetime risk of Type II diabetes according to parental diabetes status. For 44.5 % of the families, neither parent was affected, and for 8.7% both were. For the remaining 46.8%, only one was affected; mothers accounting for 30.2% and fathers for only 16.6%. The risk of Type II diabetes among siblings was significantly higher if both parents rather than one was affected (43±5 vs. 27±2, p=0.003), and significantly lower if none rather than one was affected (16±2 vs. 27±2, p=0.0002). If one parent was affected, the parent-specific risks were:
Diabetic Father only, 23±4, vs. Diabetic Mother only, 28±2; p=0.29.
The risk was similar regardless of which parent was affected . It is an error, therefore, to interpret the prevalences of maternal vs. paternal diabetes in cross sectional studies as a measure of probabilities of transmitting diabetes. Most likely, the difference is due to more complete ascertainment of maternal than paternal status.

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MASS SCREENING FOR NON INSULIN DEPENDENT DIABETES MELLITUS [NIDDM] AND RISK FACTORS IN THE COMMUNITY

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A mass screening programme was carried out at community level to explore the relationship between NIDDM and various risk factors.A previous national survey in the Island of Mauritius had revealed a high prevalence of NIDDM (12.8%) in the adult population aged 25 and over.Adults residing in the catchment area of sixteen area health centres throughout the island were selected for the present study.Methodology included measurement of blood pressure,body mass index,and determination of fasting plasma glucose and lipids.Of the 3929 adults screened ,57% were female,43% were males and 51% were aged between 30-50.NIDDM occurred in 8% of the screenees.30% of the subjects were overweight and 13 % obese.High blood pressure was common (16%). Significant hypertriglyceridemia(25%) and hypercholesterolemia(11%) were also found. Logistic regression analysis gave an estimated risk of each factor with an NIDDM as follows:Dependence of NIDDM on age was highly significant (chi-square 128.84, p< 0.0001);no significant difference in probability of NIDDM between sexes (chi-square=0.5);weight status was significant (chi-square =19.83,p<0.001);high blood pressure was found to be significant but less so than other factors (chi-sq= 6.12,p=0.01);a strong association was found with the triglyceride level (chi-sq= 39.44, p< 0.001).The study showed that "mass screening" programme in a community with a high prevalence rate of NIDDM can provide a simple means to investigate relationship of the disease with various risk factors.

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RISK FACTORS FOR DIABETES IN CHINESE PEOPLE

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Objectives: To investigate the risk factors for non-insulin-dependent diabetes mellitus (NIDDM) in Chinese people. **Methods**: A total of 48 NIDDM patients were newly diagnosed (27 in male and 21 in female). Controls (N=102) were randomly selected from healthy people and matched to cases based on the date, age at diagnosis and sex. **Results**: Mean body mass index (BMI), systolic and diastolic blood pressure and triglyceride were higher and high density lipoprotein (HDL) was lower in male cases than that in controls (P<0.05). In females, mean triglyceride was higher and HDL was lower in cases than that in controls (P<0.05). After adjustment for a wide range of covariates, multiple logistic regression analysis indicated that: (1) In males, BMI and triglyceride were independently associated with a high risk of NIDDM. (2) In females, only triglyceride showed to be an independent risk factor for NIDDM. (3) Significant interaction effects between triglyceride and total cholesterol (TC) on the risk of NIDDM were observed in both sexes. Table 1.

Table 1 Multiple logistic regression model for risk factors of NIDDM

	B (SE)	OR	OR 95%CI
Males			
Body mass index	0.31 (0.09)	1.36	1.14 - 1.63
Triglyceride	0.79 (0.28)	2.64	1.27 - 3.81
Triglyceride*TC	1.46 (0.66)	4.31	1.18 - 15.69
Females			
Triglyceride	1.38 (0.41)	3.96	1.78 - 8.89
Triglyceride*TC	1.69 (0.26)	5.43	3.26 - 9.02

Conclusion: The study suggests that BMI and triglyceride in men; and triglyceride in women are independent risk factors for NIDDM. The interaction effects between triglyceride and total cholesterol are associated with high risk of NIDDM in both sexes.

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SCREENING FOR NIDDM IN SUBJECTS WITH FASTING PLASMA GLUCOSE OF 110-139 MG/DL

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Subjects with fasting plasma glucose (FPG) of 110-139 mg/dl (SFPG) have a high prevalence of IGT and NIDDM. Screening requires oral glucose tolerance tests (OGTTs) which are time-consuming. To reduce OGTT number, tests may be performed mainly in high risk SFPG identified by other criteria. **Aim:** To find factors associated with NIDDM in SFPG. **Methods:** In 1995-96 SFPG were identified in a primary care unit of an urban area (15,000 people). NIDDM family history, physical activity, age, sex, BMI, blood pressure, ischaemic heart disease, ß blocker or thiazide treatment, FPG, lipid profile and HbA1c were recorded. All subjects underwent an OGTT not later than 3 months after FPG determination (WHO criteria). **Results:** 334 SFPG (63.4±0.7 yr, 53.0 % females) were evaluated and formed three groups: 139 (41.46%) had normal OGTT, 102 (30.5 %) IGT and 93 (27.8 %) NIDDM. NIDDM subjects were ($p<0.05$) older (59.6±1.2, 63.7±1.2, 68.5±1.2 yr, respectively), had hypertension (40.6, 43.1, 62.4 %) and were on ß blocker treatment (5.0, 4.9, 15.1 %) more frequently. They also had ($p<0.05$) higher FPG (117.9±0.5, 121.1±0.8, 125.5±0.9 mg/dl), HbA1c (5.2±0.1, 5.3±0.1, 5.8±0.1 %) and tryglicerides (128.0±6.3, 144.7±6.4, 162.1±8.9 mg/dl) and lower HDL (54.9±1.4, 51.9±1.6, 47.9±1.4 mg/dl). In the logistic regression model the presence of NIDDM was associated positively with FPG, HbA1c and ß blocker treatment; when HbA1c was excluded from the model, age (positively) and HDL (negatively) were included. **Conclusions:** NIDDM was positively associated with FPG, HBA1c, ß blocker treatment and age, and negatively with HDL.

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FASTING PLASMA TOTAL AMYLIN CONCENTRATION PREDICTS DETERIORATION IN GLUCOSE TOLERANCE OVER 4.5 YEARS.

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A strong cross-sectional association between fasting total plasma amylin concentration and glucose tolerance has been demonstrated. This prospective study was undertaken to examine whether baseline fasting total plasma amylin concentration predicts deterioration in glucose tolerance over 4.5 years. 723 volunteers who had a non-diabetic oral glucose tolerance test (OGTT) in 1990-92, were followed up at 4.5 years and their OGTT repeated. Fasting plasma amylin was measured at baseline by an assay which assessed both glycosylated and non-glycosylated amylin species.

Geometric mean fasting total amylin (and 95% confidence interval) (pmol/l) by baseline and follow-WHO category.

	Follow-WHO category		
Baseline WHO Category	Normal	IGT	NIDDM
IGT (n=129)	4.21 (3.36-5.28)	6.28 (4.68-8.41)	7.68 (5.33-11.03)
Normal (n = 594)	4.12 (3.81-4.45)	4.52 (3.27-6.24)	6.91 (4.78-9.97)

ANOVA of fasting total plasma amylin by follow-up WHO category (adjusting for baseline WHO category) was $F=4.04$, $p=0.02$. Subjects were grouped into quintiles by change in 120 min. plasma glucose, adjusted for regression to the mean. A significant association was found between baseline amylin and deterioration in glucose tolerance (odds ratio per quintile of fasting total plasma amylin concentration 1.16, 95% CI = 1.02-1.34). We conclude that baseline amylin concentration predicts change in glucose tolerance over 4.5 years. Whether this association reflects a causal relationship remains to be determined.

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IS IMPAIRED GLUCOSE TOLERANCE A RISK FACTOR FOR DEVELOPING TYPE II DIABETES MELLITUS?

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53 subjects of impaired glucose tolerance (IGT) and 43 of type II diabetes mellitus (NIDDM), over 40 years of age, were detected by stratified sampling of 1800 of adult population, diagnosed by WHO's criterious with standard 75g. –OGTT, in an epidemiological surveying in Jiu–Quan County, China. Statistical evaluation of blood pressure (B.P.), waist–hip ratio (WHR) and body mass index (BMI) of the IGT in compare with the NIDDM and 63 normals by multiple linear regression analysis studied in this paper to identify whether IGT is a risk factor for developing NIDDM. The results showed: 1)There were linear relationships in the regression analysis in the inspected subjects with the 3 factors (B.P., WHR, BMI). The variance analysis $F=12.03$, $P<0.01$. 2) The standardized regression coefficiency of the WHR was the largest (0.2709) which suggested that WHR was the most important factor of the three. 3) By t test: the WHR in IGT ($\bar{X}=0.91\pm 0.07$SD) and NIDDM (0.91 ± 0.07) were significantly larger than that of the normal (0.85 ± 0.07), $P<0.01$, but no differance between IGT and NIDDM, $P>0.05$. 4) There were no significant differences of BMI in between IGT, NIDDM and normal group (24.67 ± 4.04, 24.49 ± 3.16, 23.19 ± 3.12, respectively). In conclusion: IGT is a risk factor for developing NIDDM, and WHR is a critical risk factor for NIDDM rather than BMI.

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INSULIN RESISTANCE AND SECRETION INDEPENDENTLY PREDICT 4.5 YEAR DETERIORATION IN GLUCOSE TOLERANCE.

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To study the relative roles of insulin resistance and insulin secretion in the pathogenesis of glucose intolerance, a population-based cohort of 1071 caucasian subjects who had a non-diabetic oral glucose tolerance test in 1990-92 were traced and invited to attend for a repeat test 4.5 years later. 937 volunteers attended for rescreening. The crude incidence of diabetes in this population was 6.3 per 1000 person years of follow up. The probability of changing between WHO categories is a function of baseline glucose tolerance, regression to the mean and true change. To separate these effects, we analysed the 2 hour plasma glucose as a continuous variable after adjusting for regression to the mean. Subjects were grouped into quintiles for regression to the mean adjusted change in the 2 hour plasma glucose. Subjects in the top quintile were compared with those in the middle 3 quintiles by logistic regression. The probability of deterioration was independently associated with increasing baseline age, body mass index and 2 hour plasma glucose and change in body mass index from baseline to follow up. The probability of deterioration in glucose tolerance, adjusting for these factors, was independently associated with increasing baseline fasting insulin (Odds Ratio per log unit 2.08, $p<0.0001$), a measure of insulin resistance, and with a lower 30 minute insulin incremental response (Odds Ratio 0.54, $p<0.0001$), a measure of insulin secretion. We conclude that measures of insulin resistance and insulin secretion independently predict deterioration in glucose tolerance over 4.5 years.

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THE IMPACT OF MENOPAUSE AND AGING ON THE DEVELOPMENT OF GLUCOSE INTOLERANCE IN PERIMENOPAUSAL WOMEN

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There is a sharp rise of prevalence of diabetes mellitus and impaired glucose tolerance in women aged 45-55. To investigate the possible impact of menopause and aging on the development of glucose intolerance in perimenopausal women, a community-based epidemiologic study was conducted in Kinmen. 1100 adults (56.7% postmenopausal) were screened by single fasting plasma glucose (FPG) followed by an OGTT undertaken in subjects with FPG ≥5.5 and <7.8mM. According to the WHO criteria, the overall prevalences of DM and IGT are 7.2% and 5.8% in the age group of 45-49 (n=584) and 9.9% and 4.8% in the age group of 50-54 (n=516), respectively. With multivariate analysis, in women aged 45-49, hypertension (SBP≥160mmHg and/or DBP≥95mmHg) is significantly associated with the diagnosis of IGT (vs normal glucose tolerance, odds ratio=2.83) while hypertriglyceridemia (≥2.3mM, OR=11.59), family history of diabetes (OR=11.02) and menopause (OR=2.61) are associated with the development of diabetes mellitus. On the contrary, in women aged 50-54, family history (OR=8.67), hypertriglyceridemia (OR=5.53) and regional adiposity (waist-hip ratio ≥0.89, OR=2.36) are identified as risk factors for diabetes. It is concluded that relatively early menopause is an independent risk factor for diabetes in women less than 50 years of age.

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THE CHARACTERISTICS OF DIABETES IN THE INITIAL STAGE: CAN WE PREDICT THE EARLY GLYCEMIC STATUS FOLLOWING DETECTION?

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To determine the association of glycemia with the increasing age and obesity of newly detected non-insulin-diabetes mellitus (NIDDM) and to quantify the predictors of the risk variables for glycemic status in the initial stage we investigated 107,743 (men 71,512, women 36,231) subjects of age ≥30yr. According to WHO diagnostic criteria (1980), oral glucose tolerance test (OGTT) was done in these subjects during registration. The mean (SD) values for age (yr), blood glucose (mmol/l)), fasting (FBG) and 2h after glucose (2-hBG) of men were comparable to that of women 48.7 (10.8) vs 47.7 (10.7), 11.3 (4.7) vs 11.4 (4.5) and 18.6 (6.4) vs 18.8 (4.0), respectively. Although the men had significantly higher height (Ht, $p<0.0001$) and weight (Wt, $p<0.001$) than women the mean BMI (body mass index = kg/m sq) was significantly lower in men than women (22.4 (3.6) vs 24.4 (4.4), $p<0.001$). Glycemia was significantly associated with increasing age but only in women (FBG, <50 vs ≥50y: X^2 ,123 $p<0.0001$). Both FBG and 2-hBG showed significant negative correlation with Wt, Ht, BMI, systolic (SBP) and diastolic (DBP) blood pressure (for each variable, $p <0.001$) in either sex. These observations were further analyzed by stepwise multiple regression model which estimated these variables for the predictive values of 2-hBG and FBG as dependent variables. In men, the predictive value for log-2-hBG (mmol/l) was 57.5 + 0.090(wt, kg) + 0.026(DBP, mmHg) - 0.027(age, yr) - 0.680(BMI) - 0.038(SBP, mmHg) - 0.153(ht, cm) [Adjusted R sq = 0.09, F 1156, $p<0.0001$]. Similar observations were also noted in women. When FBG was taken as a dependent variable the results were almost the same. Regardless of sex the significant inverse relationship of glycemia with Ht, BMI and SBP may be attributed to the detection of diabetes in a very advanced stage with moderate to a very high blood glucose level in the study population. These findings suggest that the glycemic status in early stage of diabetes does not relate to obesity, particularly in developing communities, where the detection of diabetes are too late when hyperglycemia is not infrequently associated with low BMI.

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EFFECT OF YEARLY FOLLOW-UP OF POTENTIAL DIABETICS

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In 1967, we began to follow-up the patients with "border diabetes", since 1981 only with IGT, founded with "case finding" of clinically silent diabetics in an area of Prague-West since 1961. They were as potential diabetics educated and yearly (until to onset of NIDDM) examined. In 1993, all these living 331 patients (group AB) were matched against 654 Type 2 diabetics (group C) living in the same area, in whom this diagnosis (DG) was established at their first examination. Group AB was devided into 2: subgroups: A = 142 (43%) of them, in whom this DG was already established and B = other 189 (57%) still as IGT followed-up. The main results: at inclusion, group AB was younger than C (56.6±14.4 and 60.9±10.9 yrs $p < 0.001$), had a lower BMI (29.4±5.7 and 30.4±5.0 kg/m^2 $p < 0.01$) and the same duration of follow-up: 7.9±7.4 yrs and 8.0±6.4yrs. Subgroup A was younger than subgroup B (53.8±13.9 and 58.7±14.4 yrs $p <0.01$), but had a higher BMI already in younger age (30.5±5.5 and 28.5±5.8 kg/m^2 $p < 0.01$) and longer duration of follow up: A 12.4±7.9 yrs = 6.0±5.7 before + 6.4±5.3 yrs after the DG of NIDDM, B only 4.6±4.8 yrs, $p < 0.001$. In 1993, subgroup B was already 2,4 yrs older (63.3±13.7 yrs $p < 0.05$) than group C at the DG of NIDDM, but still only with IGT. At DG. of NIDDM, age of subgroup A (59.8±12.1 yrs) and that of C did not differ. BMI of A was also the same as of C, but A reached this value of BMI already in younger age. In 1993, duration of treatment after the DG of NIDDM was in A shorter than in C ($p <0.01$). At this time, more patients of group A needed still only diet to good control (55.5% and 33.0% in C, $p < 0.001$) and less OAD (31.1% and 49.7% $p < 0.001$). No significance was found in need of insulin: 13.4% and 17.3%! A shorter time since the DG of NIDDM to indication of insulin (or insulin + OAD) in A: 5.6±3.3 yrs than in C group: 8.2±6.1 yrs ($p < 0.05$), could correspond with more of patients with the LADA Type in subgroup A.

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DIABETIC PATIENTS ASSOCIATED WITH POSITIVE GAD-ANTIBODIES AND MITOCHONDRIAL DNA ABNORMALITY IN JAPAN

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Mitochondrial $tRNA^{Leu(UUR)}$ gene (mtDNA) mutation is one of important causes of diabetes in Japanese patients, and manifests a wide range of diabetic phenotype, from NIDDM to IDDM. Glutamic acid decarboxylase antibodies (GADab) is a useful screening test for IDDM. To study the frequency and the clinical characteristics in the diabetic patients associated with positive GADab and mtDNA mutation (A to G transition at nucleotide pair 3243), diabetic patients whose onset of the disease were under 50 years were examined. MtDNA was isolated from the peripheral leukocytes, and the mtDNA 3243 mutation was detected by Apa I digestion. GADab was measured by radioimmunoassay using Hoechst GAD kit. Four patients (3.3%) with mtDNA mutation and 10 (8.3%) GADab positive patients were found in 120 diabetic patients. There were no patients associated with both mtDNA mutation and positive GADab. The onset age and body mass index of 4 patients with mtDNA mutation were 29±6 years and 16.4±1.5 kg/m^2. The diabetic phenotype of 2 patients were clinically IDDM. The other 2 patients were NIDDM, but a patients started insulin injection at 11 years since onset of DM. Six of 10 patients with positive GADab showed IDDM phenotype and 3 patients had been considered to be NIDDM. The onset age in patients with positive GADab were 33±14 years, and 3 NIDDM patients were associated with obesity at the onset of DM. Eight of 10 patients were treated by insulin injection. In conclusion, mtDNA mutation and positive GADab account for 12% of early onset diabetes in Japan. The patients with mtDNA mutation are characterized by early onset diabetes with loss of body weight, and most of the patients with positive GADab show IDDM-like phenotype.

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GLUCOSE TOLERANCE STATUS OF BLACK SUBJECTS WITH IMPAIRED GLUCOSE TOLERANCE AFTER 4 YEARS. WF Mollentze, JM Koning, G Joubert, GM Oosthuizen, M Steyn, TI Muller, DJV Weich. Departments of Internal Medicine, Biostatistics and Chemical Pathology, University of the Free State, Bloemfontein, South Africa.

At least in Europids IGT confers an increased risk of progression to diabetes and also to the development of coronary heart disease. The meaning and longterm implications of IGT in black populations are still unknown. The aims of the study were to determine the glucose tolerance status of a group of black subjects with IGT after 4 years. The addresses of one hundred and three blacks (72 females, 31 males) with IGT identified 4 years earlier in a survey to determine the prevalence of diabetes mellitus in the black community of Mangaung were revisited by a health care worker. Available subjects were requested to undergo another standard 75g oral glucose tolerance test. They were also once more subjected to the same research protocol as was previously followed. Death certificates of deceased subjects were obtained. Seventy eight subjects (54 females, 24 males) participated in the follow-up study. Of the remaining 24 subjects, 12 had died, 4 had left the area, 6 refused and 2 were to ill to participate. Of the 78 subjects with IGT 12 (15%, Group 1) became diabetic, 26 (33%, Group 2) remained in the IGT category and 40 (52%, Group 3) reverted back to normal glucose tolerance. Median two-hour plasma glucose levels of the respective groups were 13.76 mmol/l, 8.51 mmol/l and 6.66 mmol/l. Median fasting serum insulin levels were significantly lower after 4 years in all three groups. Median differences between present and previous fasting serum insulin levels were -7.7 uIU/ml for Group 1 (95% CI -23.9; -5), -4.7 uIU/ml for Group 2 (95% Ci - 10.6; -1.8) and -5.2 uIU/ml for Group 3 (95% CI -9; -4.5). IGT also confers an increased risk of diabetes in black subjects. No direct cause-and-effect relationship between IGT and death was evident. Irrespective of glucose tolerance status subjects became significantly more insulinopaenic as judged by fasting serum insulin levels.

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Glucose tolerance in first degree relatives of NIDDM patients in a mediterranean area. Main determinants.

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First degree relatives of NIDDM patients are at increased risk to developing the disease. Metabolic abnormalities have already been described in such a subjects. The aim of our study was to characterize the main determinants of glucose tolerance in subjects with family history of NIDDM. One hundred and ninety one first degree relatives (38.8±14,8 year-old, 61 % women / 39 % men) were included in the study. In all of them body mass index and blood pressure were evaluated. After 12-h fasting, an oral glucose tolerance test was performed , obtaining basal glucose and insulin (IRI) in order to calculate fasting insulin resistance index (FIRI), % B (HOMA insulin secretion) and % S (HOMA insulin sensitivity). In 32 % of subjects , an abnormal glucose tolerance was found: 22 % impaired glucose tolerance and 10 % NIDDM (WHO criteria). A stepwise regression analysis demostrated that 120' glucemia was independently related to basal glycemia, FIRI, % B and % S. When those variables calculated from IRI determination were excluded, basal glucose was the only main determinat of glucose tolerance. Conclusions: Abnormal glucose tolerance is a highly common finding in subjects with family history of NIDDM; fasting glycemia, as well as, insulin sensitivity and secretion capacity being the major determinants of it.

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PROGRESSION OF IMPAIRED GLUCOSE TOLERANCE TO DIABETES MELLITUS IN NORTH-EASTERN PENINSULAR MALAYSIA

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Impaired glucose tolerance (IGT) is associated with an increased risk of atherosclerosis and of developing Non-Insulin Dependent Diabetes Mellitus (NIDDM). NIDDM has been found to occur in 10%-70% of patients with IGT followed for 14 years. In order to investigate the contributing factors and the conversion rate of IGT to NIDDM, we reexamined 79 IGT subjects diagnosed between 1991-1994 after interval of 20 months.
A total of 2284 subjects, aged more than 20 years old were chosen from 9 districts of the region by cluster sampling. 176(7.7%) patients were diagnosed to have NIDDM and 379 (16,6%) patients were IGT (using WHO criteria). Measurement of Body Mass Index (BMI), blood pressure (BP), oral glucose tolerance test (OGTT), lipid and lipoprotein levels were repeated.
After 20 months, 31(39.2%) of 79 subjects with IGT has normal glucose tolerance, 32(40.5%) maintained the IGT stage and 16(20.3%) persons developed to NIDDM. Fasting blood sugar (FBS) and BMI were positively correlated to the development of NIDDM ($p<0.007$ and $p<0.006$ respectively). However, other parameters such as age, 2h post-glucose challenge blood sugar, lipid, lipprotein levels and BP were not significantly correlated.
The result of the survey revealed a high prevalence of IGT in this region and 11.8% of these subjects developed NIDDM each year. BMI and FBS positively related to prognosis of IGT.

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HERITABILITY ESTIMATES AND ETIOLOGICAL MODELS FOR NON-INSULIN-DEPENDENT DIABETES MELLITUS (NIDDM) AND ASSOCIATED METABOLIC PARAMETERS.

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Non-insulin-dependent diabetes mellitus (NIDDM) is a heterogen disease with a multifactorial etiologi comprising genetical and environmental factors. Epidemiological and metabolic studies of twins and first degree relatives have indicated genetic components in the development of NIDDM. Among a population-based twin material (n=604 twins) we have estimated the heritability and best fitting models for NIDDM and indirect derivatives of glucose tolerance (0 min and 2 h OGTT plasma glucose), insulin secretion (30 min OGTT plasma insulin) and insulin resistance (0 min plasma insulin). The methodology permits models with additive genetic (A), dominance (D), shared environmental (C) or unique environmental (E) sources of variance to be fitted and the magnitude of each contributor to variance can be estimated.

Phenotype	Best model	Parameter estimates		
		a2	c2	e2
NIDDM	ACE	0.27	0.41	0.33
IGT+NIDDM	AE	0.61	-	0.39
F-glucose	AE	0.27	-	0.73
2h-glucose	AE	0.42	-	0.58
F-insulin	AE	0.53	-	0.47
30 min-insulin	AE	0.27	-	0.73

Concordance rates for NIDDM based on this population suggested a major importance of environmental factors in the etiology of NIDDM, which is supported by the estimated ACE model, in which additive genes only contribute 27% to the total variance, with 41% and 33% from common and unique environment, respectively. However, the model for IGT+NIDDM indicates a relatively greater genetical component in the etiology to abnormal glucose tolerance compared to NIDDM per se. The heritability estimates for the metabolic parameters suggest an importance of environmental factors for the phenotypes glucose (in)tolerance and insulin secretion, however for insulin resistance, the proportion of variance determined by genetic factors is greater, confirming the importance of a genetic component. In conclusion the results confirm the multifactorial etiology of NIDDM, but emphasizes the importance of environmental factors. "Susceptibility genes" for NIDDM appears to be found among genes coding for insulin resistance.

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PREDICTORS OF IMPAIRED GLUCOSE TOLERANCE IN OFFSPRING OF PARENTS WITH CONJUGAL NIDDM.

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In the group of 56 adult normoglycaemic non-diabetic subjects, whose both parents had NIDDM, impaired glucose tolerance (World Health Organization criteria) occurred during the 5-year follow-up (1990-95) in 18 (group A), and in 38 the tolerance to glucose remained normal. At the initial examination (1990) the group A didn't differ from the group B in mean age, BMI, serum C-peptide area under curve (AUC) after oral glucose (75g), nor in fasting serum levels of triglycerides, total cholesterol, LDL-cholesterol, apolipoprotein B and A1. The former group was, however, characterized by higher glycaemia AUC [18.3±0.67 (SEM) vs 16.7±0.40 mmol · l^{-1} · h^{-1}, p=0.019] and higher serum insulin (IRI) AUC [1.18±0.10 vs 0.98±0.40 nmol · l^{-1} · h^{-1}, p=0.027], and by lower serum level of HDL-cholesterol [1.05±0.07 vs 1.28±0.06 mmol · l^{-1}, p=0.019]. The onset of impaired glucose tolerance (1995) was associated in this group with the increase in serum insulin (IRI) AUC [1.8±0.22 vs 1.18±0.10 nmol l^{-1} · h^{-1}, p=0.008) and the decrease in serum C-peptide AUC [9.43±0.57 vs 10.75±0.58, nmol · l^{-1} · h^{-1}, p=0.057], and with increase in serum apolipoprotein B [164±8.6 vs 105±6.1 mg/dl, p=0.000] and the decrease in serum apolipoprotein A1 [135±6.2 vs 150±6.5 mg/dl, p=0.04]. Conclusion: The slight elevation of blood glucose (within the normal range) and of serum insulin (IRI), and the decrease in serum HDL-cholesterol seem to be significant predictors of impaired glucose tolerance in healthy offspring of parents with conjugal NIDDM.

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AN EPIDEMIOLOGICAL STUDY OF OBESITY AND DIABETES IN JAMAICA

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The aim of this survey was to determine the prevalence and pattern of obesity in the Jamaican adult population and how these relate to the incidence of diabetes mellitus .This Caribbean Isle has an area of 11,460 km², with a population of over 2 million, which is predominantly of Afro origin. A two stage stratified random sampling design, in which each dwelling had an equal probability of being selected, was employed. Selected households were visited in which subjects 15 years and over, from whom informed consent was obtained, were interviewed. The control group included one person in every twenty seen. A questionnaire which included medical and family histories was administered. In addition, anthropometric and fasting capillary blood sugar (FBS) measurements were done using standard techniques. An abbreviated oral glucose tolerance test, after an overnight fast, was performed on all subjects with FBS >6.1 mmol/L. The data was analysed using Epi 5, an advanced statistical program specific for epidemiological data. Non-response was documented and factored in the final analysis of the data. The prevalence of obesity in the adult population was 36.6 %; which had the following pattern: 30.0 % with Body Mass Index(BMI) > 25 kg/m² and normal Waist Hip Ratio (WHR), 3.0% had normal BMI but WHR ≥ 1, and 3.6% with BMI > 25 kg/m² and WHR ≥ 1; of which 22.0 %, 24.9 % and 46.0 % respectively were diabetic. The data of this study show that 36.6 % of the Jamaican adult population is obese 48.5 % of which is diabetic. It is also apparent that truncal obesity is a greater(P<0.05) risk factor, and WHR perhaps a better predictor, for diabetes in Afro Caribbean people.

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Epidemiology of Cardiovascular Risk Factors in NIDDM

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PHYSICAL ACTIVITY IN ELDERLY SUBJECTS WITH IMPAIRED GLUCOSE TOLERANCE OR NEWLY DIAGNOSED DIABETES MELLITUS

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Several studies report that physical inactivity is a risk factor for developing diabetes mellitus type II. However, these studies are based on adult populations, excluding elderly subjects. Therefore we studied the association between physical activity and diabetes mellitus in 1107 subjects, aged 55-80 years. The study was performed as part of the Rotterdam Study, a population-based cohort study in elderly subjects. The participants were classified as diabetes (known or newly diagnosed), impaired glucose tolerance and normal glucose tolerance, according to the WHO criteria. Physical activity was assessed with a self-administered questionnaire. Physical activity was expressed as hours/week as well as categories of intensity: light, moderate and heavy. For these analysis the known diabetic patients were excluded. The mean age of the study population was 66.8 years (SD 5.5), 745 subjects (68%) had normal glucose tolerance (NGT), 153 IGT (14%) and 118 newly diagnosed diabetes (11%). The mean total activity time was 22.9 hr/wk (SD 21.9) for newly diagnosed diabetes, 21.6 hr/wk (SD 18.3) for IGT and 25.5 hr/wk (SD 18.3) for NGT. Housekeeping, work (paid or volunteer), walking and bicycling together contributed 80% of the mean total activity time in all groups. Sporting activities contributed 4% to the total time spent on physical activity for NGT, 3% for IGT and 1% for newly diagnosed diabetes. The diabetic subjects (17%) and subjects with IGT (15%) spent less time on heavy activities as compared with the NGT (20%). After adjustment for age and sex, a significant trend for the three groups was shown for sporting activities (p=0.001) bicycling (p=0.03) and heavy activities (p=0.006). Preliminary results showed that physical activity in elderly subjects, newly diagnosed with diabetes, is lower as compared to subjects without diabetes, but this difference did not reach statistical significancy. However, intense physical activity was significantly less in newly diagnosed diabetes patients, which suggest that also at higher ages physical inactivity is associated with the development of diabetes mellitus.

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AGING AND VISCERAL ADIPOSITY ACROSS TWO GENERATIONS OF JAPANESE-AMERICAN WOMEN

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Increasing age and adiposity (subcutaneous and visceral) contribute to the risk of developing NIDDM and CHD. We explored the effects of these parameters on the risk factors of fasting insulin (FI), C-peptide (FC), triglycerides (Tg), LDL-Cholesterol (LDL-C) and HDL-Cholesterol (HDL-C) among 158 second-generation (Nisei) and 95 third-generation (Sansei) Japanese-American women. Glucose tolerance status was determined by a 75g oral glucose tolerance test and WHO criteria. Subcutaneous adiposity was represented by triceps skinfold thickness (Skfld) and visceral fat by intra-abdominal fat area (IAF) at the umbilical level as measured by computed tomography. Mean age of Nisei was 62 yrs vs Sansei, 40 yrs; frequencies of IGT and NIDDM were 37% and 29% (Nisei) vs 31% and 0 (Sansei), respectively. Nisei were significantly fatter than Sansei: Skfld 24 vs 22 mm, t-test p= .03; IAF 94 vs 44 cm^2, p <.0001. The two generations did not differ in FI or HDL, but Nisei had significantly higher FC (.93 vs .79 nM, p<.0001), Tg (131 vs 109 mg/dl, p= .0008) and LDL (145 vs 121 mg/dl), p<.0001. Within generation (Gn), subjects were assigned by terciles of IAF into high (H), medium (M) and low (L) groups (Gp) for which mean values of risk variables were calculated. Mean FI (pM) for Nisei by Gp: (L) 64 (M) 100 (H) 122, Sansei: (L) 64 (M) 62 (H) 120 (ANCOVA of Gp by Gn adjusted for Skfld: Gp effect p< .0001). A similar trend was seen for FC and Tg, and a reverse trend for HDL. The two H-Gps were indistinguishable by generation. In this population of Japanese-American women, the interactive effects of older age with high levels of visceral adiposity are associated with adverse risk profiles for NIDDM and CHD. A subset of premenopausal Sansei women, identified by high visceral adiposity, present a risk profile more typical of postmenopausal Nisei, who average over 20 years older.

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ASSOCIATION OF PHYSICAL ACTIVITY WITH GLUCOSE INTOLERANCE AMONG NATIVE HAWAIIANS IN NORTH KOHALA, HAWAII.

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Specific Aims: The Native Hawaiian Health Research (NHHR) Project characterized glucose intolerance and heart disease risk factors among 350 adult Hawaiians, ages ≥30 years old. Our clinic screening included an assessment of glucose tolerance, body mass index (BMI), waist-hip ratio (WHR) and physical activity levels. Physical activity has been associated with glucose intolerance in previous population-based studies. We report a comparison of physical activity among native Hawaiians with and without glucose intolerance. Methods: Glucose tolerance status was assessed by 2-hr OGTT using WHO criteria, or by medical history. Physical activity was estimated with the Pima Indian Questionnaire (PIQ) (Kriska, 1990), which was modified to include traditional Hawaiian activities. Modified versions of this instrument have been used in a variety of populations. Age, WHR, and BMI were regressed on metabolic equivalents (METS) for past week and past year activity calculated from the frequency and duration. Prevalence odds ratios for glucose intolerance were estimated using logistic regression. Results: WHR and BMI were not associated with either past year or past week activity. Age (mean=47.9±12.7 y.o.) was inversely and statistically associated with past week activity only (ß=-0.412,p≤0.001). Participants with glucose intolerance had significantly lower past week activity levels than participants with normal glucose tolerance (17.1±3.06 METS vs. 27.5±2.28 METS, respectively; p≤0.01). Prevalence of glucose intolerance was higher in less active participants (POR = 2.07, 95 Percent CI 1.33-3.23). The age-adjusted POR was unattenuated, and remained statistically significant (adjusted POR=1.95, 95 Percent CI 1.23-3.11). Conclusion: Prevalence of glucose intolerance was higher among adult native Hawaiians reporting lower levels of past week activity, even after adjustment for age. Lack of association between physical activity and BMI or WHR suggests that physical activity may be beneficial to glucose tolerance regardless of body mass.

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BODY FAT CHANGES WITH DIET AND EXERCISE DIFFER BETWEEN MEN AND WOMEN

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Change (Δ) in body fat deposits and general adiposity over 2.5 y were examined in relation to diet and exercise in non-diabetic Japanese American men (n=146) and women (n=130), ages 45-74 y at baseline. Computed tomography (CT) measured subcutaneous fat for the thorax (TX), abdomen (AB) and mid-thigh (TI), and intra-abdominal fat (IAF). Weight and height provided body mass index (BMI, kg/m^2). Food frequency questionnaires estimated baseline animal fat (BAF) and plant fat (BPF) intakes. Questions at follow-up on dietary changes targeted sources of fat (dairy, red meat, oils, etc.). Questionnaires covered daily activity levels which were converted to estimates of energy expenditure at baseline (BEE) and follow-up (ΔEE). Adjusting for baseline body fat values, partial correlation coefficients were calculated. BPF was significant only in women: ΔTX (-0.27, p<0.01), ΔAB (-0.19, p<0.05), ΔBMI (-0.23, p<0.05), and Δweight (-0.23, p<0.05). ΔEE was significant only in men: ΔTX (-0.22, p<0.01), ΔAB (-0.22, p<0.05), and ΔIAF (-0.18, p<0.05). Reduction in red meat intake was also only significant in men: ΔTX (-0.31, p<0.001), ΔAB (-0.18, p<0.05), ΔIAF (-0.21, p<0.05), ΔBMI (-0.27, p<0.001), and Δweight (-0.31, p<0.001). BEE was significantly correlated with ΔTX in women (-0.19, p<0.05) and men (-0.19, p<0.05). BAF was correlated with ΔAB in women (0.30, p<0.001) and men (0.20, p<0.05) and with ΔTX in women (0.32, p<0.001). No factors were significantly related to ΔTI. In summary, baseline energy expenditure and animal fat intake were associated with gains at subcutaneous truncal fat sites in both sexes. In addition, for men exercise and reduction in red meat intake appear to be the most important avenues toward adipose tissue reduction, while for women, baseline plant fat intake appeared to be most associated with loss of adipose tissue. Thus we conclude that in both men and women avoidance of gain of truncal fat may be best attained by exercise and low animal fat intake, whereas approaches to adipose tissue reduction seem to differ between men and women.

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RELATIONSHIP BETWEEN SPORTS ACTIVITY AND INSULIN RELEASE IN RELATIVES OF NIDDM PATIENTS

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The relationship between regular physical activity and insulin release after an oral glucose tolerance test (OGTT) was studied in first-degree relatives of NIDDM patients and in control subjects. Relatives (7M, 20F) and control subjects (5M, 17F) did not differ for age (45.4 ± 6.3 vs 46.2 ± 7.0 years), body mass index (BMI) (26.3 ± 3.8 vs 26.2 ± 3.3 kg/m^2), and waist-hip ratio (0.82 ± 0.07 vs 0.82 ± 0.07). Subjects underwent an OGTT (75 g) and completed a questionnaire for sports activity yielding a sports index (SI). Insulin sensitivity was assessed as glucose infusion rate (GIR) divided by mean plasma insulin (I) during the 2nd and 3rd hour of a hyperglycemic clamp (10 mmol/l, 180 minutes). All subjects had a normal glucose tolerance. There was no significant difference between relatives and controls for SI (2.5 ± 0.7 vs 2.6 ± 0.6). After the OGTT, the time-to-peak-insulin and the plasma insulin at 120 minutes were inversely correlated with the SI in relatives, but not in controls (r=-0.52, p=0.011 and r=-0.43, p=0.039; partial correlation, gender, age, and BMI as covariates). In relatives, the SI was positively related to the GIR/I during the 2nd (r=0.50, p=0.013) and 3rd (r=0.54, p=0.006) hour of the clamp. SI and GIR/I were not correlated in controls. In conclusion, in NIDDM relatives, but not in controls, regular sports participation is related to an earlier decline in insulin release after an OGTT, presumably due to an enhanced insulin sensitivity.

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OBESITY IN JAPANESE DIABETICS AND THE IMPLICATIONS.

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With rapidly developed life style into modernization-westernization in Japan, NIDDM is enormously increasing. In this study, we undertook to know what resultant obesity has influenced on diabetes. 100 diabetics(male 44 and female 56, mean age 62±13 yr, mean duration 10±6 yr) without other peculiar diseases were unintentionally chosen. They included NIDDM 58, insulin requiring diabetes(N/I) 25 and IDDM 17 and were subdivided into 4 groups by the present BMI(kg/m^2), Ⅰ(BMI≦18,n=2), Ⅱ(18<BMI≦26,n=74), Ⅲ(26<BMI≦30, =20) and Ⅳ(30<BMI,n=4),respectively. Their maximum BMI in the past were Ⅰ(n=0),Ⅱ(n=53),Ⅲ(n=26) and Ⅳ(n=21). Inherent diabetic pictures in 7 subgroups classified by the combination of both BMI were investigated. Personal history of obesity was recognized in only 3/17(17.6%) of IDDM, but in 40/83(42.8%) of both NIDDM and N/I. The more marked obesity in the past was, the higher prevalence of N/I was especialliy shown even if the present BMI is within normal limits. Microangiopathic complications were frequently shown in IDDM mostly classified in Ⅱ at present, but their frequencies were the highest in both NIDDM and N/I of Ⅱ at present whose BMI reduced from Ⅲ in the past because of poor diabetic control. Complications of ischemic heart disease, hypertesion and hyperlipidemia were more frequently involved in the groups not only being obese right now, but also being overweighted solely in the past. Detailed clinical courses in some obese NIDDM followed for a long term revealed a close parallel to these findings. It was suggested that we should have a strategy to prevent positively obesity and to provide against the explosive increase of diabetes, by asking ourselves to reconsider what the changed mode of life has brought on.

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EPIDEMIOLOGY OF NIDDM IN RELATION TO OTHER CARDIOVASCULAR RISK FACTORS IN LEBANON.

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The aim of the study is to define the prevalence and current epidemiological features of NIDDM, impaired glucose tolerance and other cardiovascular risk factors in the adult Lebanese population. Cluster sampling technique was used in two urban settings representing low and high socioeconomic groups and one semi-rural setting.Chi square and T-test was used in order to correlate Diabetes with other risk factors.A total of 2518 Lebanese subjects aged 30 years and above were studied and showed an over-all prevalence of diabetes and impaired glucose tolerance of 13.15 % and 6.0 % respectively with no differences between males and females. The results are similar to those reported from studies reported recently from some Middle Eastern and other countries. The prevalence was very low (< 1 %) below the age of 40 and increased steadily with age. The main risk factor was obesity (especially abdominal) which probably accounts, at least in part, to the observed increased prevalence with age and amongst lower socio-economic groups.A positive family history of diabetes but not consanguinity was another risk factor. Subjects with diabetes and IGT were more likely to have heart disease, had a slightly higher mean systolic and diastolic pressure and serum triglycerides. An increase in total and LDL-cholesterol and lower HDL-cholesterol was seen only in female but not in male subjects with diabetes and IGT .

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CARDIOVASCULAR RISK OF BODY MASS INDEX (BMI) IN ELDERLY RURAL INDIANS: A NEED TO REASSESS OBESITY CRITERIA?

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We studied glucose tolerance, plasma lipids, blood pressure and resting ECGs in people >40 y in the village of Pimpale Jagtap near Pune (158 men: 58 y, 1.63 m, 52 kg, BMI 19.5 kg/m^2 and WHR 0.88; 163 women: 53 y, 1.50m, 45 kg, 19.8 kg/m^2 and 0.78). Four percent had diabetes and 4% IGT, only 1 had cholesterol >6.2 mmol/l, 6% had triglycerides >2.3 mmol/l and 14% were hypertensive (>140/90 mm Hg and/or treatment). 15% (16 men & 31 women) showed an 'ischaemic' ECG (coronary probable 4, possible 43; Minnesota 1982). Plasma glucose, triglycerides, cholesterol and blood pressure were all related to BMI ($p<0.01$). There was a U shaped distribution for triglycerides, IGT and 'ischaemic' ECGs, but not for diabetes, hypertension and cholesterol which showed a continuous rise with BMI. In the highest quartile of BMI (median 22.9 kg/m^2 men, 24.5 kg/m^2women) 8% had IGT, 10% diabetes, 33% hypertension and 20% 'ischaemic' ECG. Thus, the cardiovascular risk in rural Indians is related to relatively low BMI. This could be related to their past nutritional experiences.(intrauterine as well as subsequent).

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CIGARETTE SMOKING IS THE RISK FACTOR FOR METABOLIC DISTURBANCES IN THE OBESE

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Cigarette smoking is well known risk factor for CVD. The aim of this paper was to find out its potential role in the metabolic disturbances especially those concerning glucose metabolism in the obese. The investigation was conducted in the group (N=146) of the obese persons (BMI>30 kg/m2) aged 20 to 64 years, devided in two subgroups: smokers(S) and nonsmokers (NS). BMI was used as an index of obesity, waist circumference (W), waist to hip ratio (WHR) and Sagittal diameter (Sd) as indices of fat distribution. The following parameters of glucose metabolism were measured: fasting glucose and inslin levels as well as glucose and insulin levels during OGTT. The results obtained show no difference in BMI between smokers and nonsmokers (35.5 v.s 35.2) but smokers tend to dispose more fat in deep abdominal region and all the parameters of fat distribution were significantly higher like W,WHR and Sagittal diameter ($p<0.02$, $p<0.009$, $p<0.0001$). Fasting glucose was 5.40 mmol/L v.s 4.69 what is significantly higher ($p<0.04$) as well as glucose area under the curve (930.5 v.s 790.7 mmol/L/min) and fasting insulin: 25.4 mU/L in smokers v.s 17.2 in nonsmokers ($p<0.001$). The insulin area under the curve was higher but not significantly. Systolic blood pressure was 141.7 mmHg v.s 132.5 ($p<0.004$) and diastolic was also higher but not significantly. It can be concluded that smoking cigarette is a risk factor for metabolic distrurbances especially in obese with abdominal type of fat distribution..

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COMPARISON OF BMI AND WHR BETWEEN JAPANESE POPULATIONS IN JAPAN AND BRAZIL

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Migrant studies measure the impact of environmental factors on the development of diseases. We reported that prevalence of NIDDM in Japanese Brazilian in Bauru, Brazil is much higher than that in Funagata, Japan (17.6 % vs 10.1 %). These two Japanese populations are considered genetically identical. Both are population-based study with high participation rates (74.5% in Japan and 83.2% in Brazil). Both employed a 75 g oral glucose tolerance test as a primary test with 1985 WHO diagnostic criteria. This study compares body mass index (BMI) and waist-to-hip ratio (WHR) between these two populations in the same age group (40-79). Body height (m) and weight (kg) were measured while subjects were wearing light clothing without shoes. Waist and hip circumferences were measured at the level of the umbilicus and at the level of the greater girth, respectively. BMI was calculated as weight divided by the square of height. WHR was calculated as waist divided by hip. Student's t-test was used to compare the data. Means and standard deviations are shown in the below table.

		Japan	Brazil
BMI	Men	23.5 (3.0)	24.2 (3.5)
	Women	23.9 (3.3)	24.3 (3.6)
WHR	Men	0.89 (0.06)	0.99 (0.08)
	Women	0.88 (0.06)	0.90 (0.08)

Both BMI and WHR showed significantly higher value in Japanese Brazilian than in Japanese in Japan. Higher prevalence of NIDDM in Japanese Brazilian can probably be attributed to obesity.

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DIABETIC CHRONIC COMPLICATIONS IN TYPE 2 DIABETES: A MULTICENTRIC STUDY. X. Mundet, JF. Cano,M. Mata, J. Franch, M. Bundó, M. Berenguer and the GedapS Group Catalan Famiy and Community Medicine Society. Barcelona (Spain)

Aims: To assess prevalence of diabetic chronic complications in type 2 diabetic patients attended in Primary Health Centres (PHC) and to know the compliance of screening measures.***Patients and methods***: Cross-sectional study carried out in 1993-94 in 76 PHC (34.1% rural and 65,9% urban) attending 1.092.532 people. Patients were selected by systematic sampling from 21,556 adults registered as type 2 diabetics. Final sample included 2,595 patients with a mean age of 66,8+/-10 years and a mean evolution time of 8,1+/-5,9 years from the diagnosis.***Results:*** Nephropathy: Screening for microalbuminuria was performed in 34% of diabetics. Microalbuminuria was confirmed in 17% (IC95%: 14-20), proteinuria in 5,3% (IC: 4-6) and renal insufficiency in 5%. Retinopathy: Fundoscopy was performed in 55% of patients, finding some kind of diabetic rethinopaty in 37% of them (IC: 34-40), blindness was present in 2%. Ischemic Heart and Cerebrovascular Disease were present in 12% (IC: 12-14) and 6% (IC: 5,3-8). Diabetic Foot was reported in 10% of cases (IC: 8,8-11,1), the acute lesions raised 7,9% (6,8-8,9) and amputations were 2,1% (IC: 1,5-2,6). Varience analysis showed a close relationship between microvascular complications and age (p: .006), diabetes average evolution time (p:.00001), current level of HbA1c (p:.0004) and systolic Blood Pressure (p:.0001). Macrovascular disease was correlated with age (p:.00001), evolution time of diabetes (p:.00001) and current level of total cholesterol (p:.0004). ***Conclusions:*** 1. The frequency of complications is similar to the found in other country studies. 2. The detections of the complications must be improved. 3. As it is already known, the age and the average evolution time of diabetes are strongly correlated to the complications. Furthermore, we found that the metabolic control, Total Cholesterol level and Systolic Pressure have an important influence in its development.

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Accuracy and Reliability of Dual Energy X-ray Absorptiometry (DEXA) for rat body composition measurement.

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Rat is a current model for nutrition studies. One limitation in its use is the accurate determination of the total body fat mass which can only be determined with the tiresome chemical extraction technique (CE) which requires sacrifice of the animal. Usefulness of DEXA in human nutrition investigations suggest that it could be suitable also for rat studies. Therefore we have tested intra- and inter- assay reliabilities of the DEXA technique (coefficient of variation: CV) and its accuracy as compared to the gold standard CE procedure (linear regression analysis with correlation coefficient: r). 26 rats (18 Wistar; 8 GK) with age ranging from 2 to 24 months and body weight (bw) ranging from 130 to 500 g, were screened in triplicate after anaesthesia on a QDR 4500 absorptiometer (Hologic). After sacrifice their lipid content was determined by CE (mean of 3 determinations/sample). 1/ Comparison of the two techniques (DEXA vs CE) showed a strong linear correlation ($r=0.975$; $p<0.0001$). 2/ During intracalibration studies, CV value for boneless lean mass was found lower than 1%, except for the case of animals with bw lower than 150g (CV: $1.4 \pm 0.9\%$, n=5). CV value for % of total body fat mass CV was $6.8 \pm 3.3\%$ for bw <150g (n=5), $4.2 \pm 3\%$ for bw in the range 150-250g (n=9), and $2.6 \pm 1.1\%$ for bw beyond 200g (n=14). There was no significant difference concerning reliability between the two techniques. 3/ Intercalibration reliability was lower and induced an increase of CV values (up to 8% for the % fat mass value, in the animals with bw beyond 200g). However, this limitation can be circumvented by using a quality control, and correcting the results using a standard. Therefore, DEXA can be considered as an accurate, reliable and non aggressive technique for body composition determination in rat, as far as rigorous methodology and inter and intra-assay quality controls are taken into account.

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DOES A CLINICAL PHENOTYPE AT RISK FOR DIABETIC COMPLICATIONS EXIST? A 20 YEAR FOLLOW UP STUDY IN A NORTH EASTERN ITALIAN REGION

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To verify if clinical phenotypes prognostic for diabetic complications exist or only the good metabolic control can prevent these alterations we 100 type 2 diabetic patients 59 males (M) and 41 females (F), randomly chosen on 540 patients who were diagnosed type 2 diabetes between 1974-1984,were analyzed with a retrospective study. The multiple logistic regression verifies the Odds ratio (OR) (I.C 5-95°) of some clinical parameters (CP);: A) age at the diagnosis (AD) BMI ; Systolic and diastolic blood pressure ; fasting glucose and glycated hemoglobin (Hba1c) (evaluated three time every year) respect to ischemic heart disease (angina or myocardial infarction), retinopathy (evaluated by fundus), Lower Extremity artery disease (LEAD) (evaluated by ankle brachial index (ABI), and neuropathy (evaluated by Diabetes neuropathic index and Biotesiometer).

Clinical Parameter	IHD	Retinopathy	LEAD	Neuropathy
AD (<55/>55)	0.69(0.18-2.5)	0.61 (0.19-1.9)	0.35 (0.24-1.0)	0.39 (0.11-1.0)
SEX (F/M)	0.11(0.01-0.9)	0.84 (0.25-2.8)	0.49 (0.15-1.5)	0.34 (0.13-0.9)
BMI(>26/<26)	1.12(0.26-4.7)	1.21 (0.33-4.3)	0.30 (0.10-0.9)	0.50 (0.18-1.3)
SDP(<160/>160)	1.61 (0.36-7.1)	2.61 (0.72-9.4)	3.20 (1.02-10)	1.40(0.40-4.1)
DBP(<90/>90)	4.59 (0.86-24)	1.41 (0.25-7.9)	1.83 (0.39-8.5)	1.42 (0.31-6.4)
FG (<140/>140)	0.77 (0.20-2.8)	1.77 (0.51-6.0)	1.92 (0.65-5.6)	1.61 (0.63-4.1)
HbA_1c(<7.1/>7.1)	0.86(0.23-3.2)	0.86 (0.23-3.2)	0.79(0.26-2.37)	0.67(0.22-1.6)

We conclude that in these patients with the diabetes showed a phenotype at risk for neuropathy and Lead (male, lower weight at the diagnosis); instead the metabolic control and blood pressure increased the risk to develop ischemich heart disease and retinopathy.

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INCREASED INCIDENCE OF HYPERTENSION AND DYSLIPIDEMIA IN MIDDLE-AGED MEN WITH HYPERINSULINEMIA OR DIABETES

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We investigated the association of diabetes and hyperinsulinemia with the incidence of future hypertension and dyslipidemia in the Kuopio Ischemic Heart Disease Study (KIHD), a population-based study of middle-aged men from eastern Finland. Subjects were 558 unmedicated men with resting blood pressure < 165/95 mmHg, or 786 men with serum triglycerides < 2.3 mmol/l and HDL cholesterol > 1.0 mmol/l at baseline and whose hypertensive status and blood lipids were assessed at 4-year follow-up. In logistic regression models adjusting for age, resting blood pressure, obesity, weight change and other predictors, diabetic men (blood glucose of ≥ 6.7 mmol/l or clinical diagnosis of diabetes with either dietary, oral or insulin treatment) had a 6.0-fold (95% confidence interval 1.8 to 20.2, p = 0.004) risk of subsequent hypertension (≥ 165/95 mmHg) compared to non-diabetic men. Men with hyperinsulinemia (fasting insulin of ≥ 12mU/l) had 2.0-fold (p = 0.015) risk of hypertension, 2.1-fold (p = 0.002) risk of dyslipidemia and 2.8-fold (p = 0.026) risk of the combination of these two insulin-resistance-related conditions in four years. These preliminary findings demonstrate the role of hyperinsulinemia and diabetes in hypertension and dyslipidemia and suggest that both hypertension and dyslipidemia can be associated with insulin metabolism disturbance, independent of body weight and obesity.

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MONITORING THE INCIDENCE OF LOWER EXTREMITY AMPUTATIONS: APPLICATIONS OF CAPTURE-RECAPTURE TECHNOLOGY.

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Lower Extremity Amputations (LEAs) are a major health problem in the diabetic population.The Capture-Recapture (C-R) method was used in this report to estimate the LEAs incidence rates in the city of Rio de Janeiro, for the 1992-4 period. This amputee register (AR) is carried out in the city of Rio de Janeiro (population of ± 9,500,000 inhabitants).Diagnosis were recorded according to the WHO ICD-9.The AR data were classified into:.peripheral vascular disease (PVD), diabetes mellitus (DM) trauma, neoplasms, gangraena emphysematosa (G.Emphy) and ostheomielitys (Osteo).The LEAs reporting cases came from: Source 1 (S1)-1191 cases from records of 23 hospitals, and Source 2 (S2)-157 cases from a limb fitting center. An additional source (S3) was a larg rehabilitation center (34 cases). Log-linear models were applyed to the C-R technique when more than two sources were used. Amputee death certificates from S1 were reviewed with the identification of 257 deaths.To estimate LEAs incidence models were evaluated with 2 and 3 sources,and including or excluding deaths. Applying C-R with 2 sources (S1 and S2), excluding deaths, the results are: S1=934 cases; S2=193 cases, and common to S1 and S2=50 cases, with an estimation of 3,555 cases (95% CI: 2,748 - 4,362). Applying log-linear models for S1 (1,191 cases), S2 (157 cases) and S3 (34 cases), the estimated total number is 5,040 cases. Excluding deaths, the total number of estimated cases are 3,954, that is an annual incidence rate of 13.8/100,000. Altogether, 1,383 patients with LEAs were identified as having: DM =379 (27.4%), PVD = 804 (58,1%) G.Emphy = 36 (2.6%), Osteo =44 (3.1%), trauma=103 (7.4%), neo=16(1.1%). These findings showed a high incidence of LEAs and that DM is one of the leading causes; must of the LEAs may be preventable with the provision of adequate health care.

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RELATIONSHIPS BETWEEN SOCIOECONOMIC STATUS AND CARDIOVASCULAR RISK FACTORS IN AFRICAN AMERICANS

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The rate of diabetes mellitus (DM) in African Americans (AA) is reaching epidemic proportions. AA with type 2 DM suffer more from cardiovascular diseases (CVD) associated with diabetes than the general population. Lower socioeconomic status (SES), family history, obesity, and hyperinsulinemia are often cited as contributory factors to the premature development of DM and CVD in this population. However, we are not aware of any study that has examined the relationships between SES and CVD risk factors (i.e. Syndrome X) in a genetically-enriched AA population at high risk for type 2 DM. **METHODS:** We studied 200 healthy first degree relatives of AA patients with type 2 DM, age range 25-65 years, 42 males and 158 females. Standard OGTT, metabolic and anthropometric parameters and questionnaires on SES and demographics were obtained in each subject. SES was divided into quartiles based, on annual income, ranging from <$10,000 to >$100,000. To examine the impact of insulin on CVD risk, we examined the clinical characteristics and metabolic parameters according to quartiles of fasting insulin concentrations. **RESULTS:** There were no significant differences in any of the clinical characteristics, metabolic, lipid and lipoprotein concentrations and anthropometric parameters among our SES quartiles. When examined as insulin quartiles, body mass index (BMI), waist hip ratio (WHR), % body fat tended to be greatest in the 4th quartile when compared to the lower quartiles. Fasting serum and postprandial glucose and c-peptide levels were significantly higher in the 4th quartile when compared to the lower quartiles. We observed greater very low density lipoprotein cholesterol (VLDL-C) and triglycerides (TRIG) and lower high density lipoprotein cholesterol (HDL-C) in the 4th when compared to the lower quartiles. Serum cholesterol (CHOL) and low density lipoprotein cholesterol (LDL-C) levels were not associated with increasing insulin concentrations. **CONCLUSION:** Our present study demonstrates no SES/income effect on CV risk factors in AA at high risk for type 2 DM. Clustering of the components of Syndrome X was only seen in those individuals in the highest quartile of insulin. We conclude that, AA at high risk for type 2 DM especially those with clinical and metabolic characteristics similar to those found in subjects in the 4th quartile should be evaluated for CV risk, as well as, considered for DM and CVD prevention programs, regardless of their SES.

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PREVALENCE OF DIABETES AND RISK FACTORS FOR CORONARY HEART DISEASE AND STROKE IN PERUVIAN MESTIZO POPULATION.

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Since 1994 has been designed a national epidemiologic study which aim is to examine the prevalence of the main risk factors which provides coronary heart disease (CHD) and stroke in our population.

The present pilot study was done at ingenieria urbanization, Lima which population is 26,497. A sample population of 158 people up to 18 years old was chosen by multiphase cluster through a descriptive, prospective and cross-sectional research during October-December 1995.

The chosen people was visited in their homes by a previously capacitated health team making them an epidemiologic questionnaire, anthropometric measures and determination fasting blood sample for blood glucose and lipidic profile. The statistic program STATA was used for calculated the distibution of frecuencies, Chi2, the correlation analysis and the association measures.

The prevalence of diabetes mellitus was 7.5%, hypertension 37.8%, hypercholesterolemia 21% and obesity 28.7%. 18% of subjects were smokers, 39% alcohol drinkers, 70% sedentarian life and 72% coffe drinkers.

The results explain the contribution of diabetes and associated risk factors in the elevated morbility and mortality by CHD and stroke in the peruvian mestizo population.

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SOCIO-ECONOMIC STATUS, PREVALENCE OF DIABETES AND OTHER CARDIOVASCULAR RISK FACTORS IN CHINESE

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Low socio-economic status is associated with increased prevalence of diabetes mellitus (DM), coronary heart disease and early mortality in Caucasians. We studied 2847 Hong Kong Chinese subjects undergoing oral glucose tolerance tests (OGTT) and examined the relationships between socio-economic status, glucose intolerance and other cardiovascular risk factors. Of the 2847 subjects, 473 (16.6%) were men and 2374 (83.4%) were women. They were classified according to their education level and occupation. When analysed according to education level and after adjustment for age, women in the lowest socio-economic group had the highest body mass index (BMI), systolic and diastolic blood pressure (BP), fasting and 2-hour plasma glucose (PG), prevalence of DM and obesity. After further adjustment for BMI, alcohol and smoking, the differences in systolic BP, 2-hour PG and prevalence of DM lost their significance. Men with the lowest education level had the highest prevalence of diabetes and smoking after age adjustment. The difference in prevalence of DM in men also lost significance when BMI, alcohol and smoking were adjusted as covariates. When categorised according to occupation and after adjusting for age, women in the lowest socio-economic group had the highest fasting and 2-hour PG, glycated haemoglobin, prevalence of DM, alcohol intake and obesity. After further adjustment for BMI, alcohol and smoking, women in the lowest socio-economic group still had the highest fasting PG and prevalence of DM. Men in the lowest socio-economic group had the highest prevalence of smoking and alcohol intake after adjusting for age and BMI. In conclusion, Hong Kong Chinese in the lower socio-economic group have increased risk of glucose intolerance and cardiovascular risks. Obesity, tobacco and alcohol are important factors within these associations.

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GLUCOSE INTOLERANCE AND PATIENTS WITH CORONARY ARTERY DISEASE: DO THE WHO CRITERIA NEED REEVALUATION?

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The aim of the present work was to study the role of glucose intolerance and the metabolic condition in patients with coronary artery disease (CAD). Forty four non diabetic patients with CAD, verified by coronary angiography, were screened with oral glucose tolerance testing. According to the WHO (1985) criteria, 11 (25%) patients were found to have impaired glucose tolerance (IGT group) while 33 (75%) were euglycemic (non-diabetic non-IGT group). A control group of 10 age- and sex-matched euglycemic subjects, with angiographic evidence of absent CAD, was also studied. All individuals were subjected to biochemical tests including complete lipid profile, oral glucose tolerance test and serum insulin and C-peptide levels, and anthropometric measurements including body mass index, waist/hip ratio and skin fold thickness, as well as estimation of body composition by near-infrared interactance technique to assess the percentage of total body as well as abdominal fat. The non-diabetic non-IGT CAD patients showed a significantly lower HDL ($p<0.001$) and a significantly higher glucose area ($p<0.05$) than the respective parameters of the matched control group, although both were not significantly different regarding the anthropometric measurements. This could suggest that values of glucose tolerance test lower than that of the WHO (1985) might still impose risk for developing CAD and raises the question whether the IGT figures of the WHO (1985) needs modification or a term like "pre-IGT" be considered, especially in CAD patients: A concept that still needs more work on a larger scale.

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LIPOPROTEIN(A) LEVELS IN OBESE SUBJECTS WITH AND WITHOUT NON INSULINDEPENDENT DIABETES MELLITUS

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The aim of this study is to compare lipoprotein(a) levels [Lp(a)] in obese individuals with non insulindependent diabetes mellitus with obese subjects without diabetes mellitus. We also describe if the increase in the body mass index [BMI] goes along with higher concentrations of Lp(a) in both groups. A cross-sectional survey was set out in our centre in which 224 obese subjects (BMI > 25 kg/m^2) were analyzed (56 subjects with non insulindependent diabetes mellitus and 168 without diabetes). t-tests and simple regression were performed; only p-values of less than 0.05 were reported as significant. Mean Lp(a) values were lower in obese people with non insulindependent diabetes (11.3 mg/dl versus 12.7 mg/dl). No statistical differences were observed comparing age and gender. Among people with BMI ranging from 25 to 29.9 kg/m^2, mean Lp(a) levels were 13.7 mg/dl in patients with non insulindependent diabetes and 14.9 mg/dl in those without diabetes mellitus. The results observed in subjects with BMI between 30 and 34.9 kg/m^2 were 12.4 mg/dl and 13 mg/dl and in people with BMI greater that thirty-five were 10.6 mg/dl and 11.8 mg/dl. The correlation observed comparing Lp(a) levels with BMI was the same in both groups (r:0.09). No statistical differences were achieved. Lp(a) is considered as an independent risk factor for developing ischaemic heart disease. Depending on the results observed in this survey mean Lp(a) values are not significantly modified when an increase in BMI is produced either in people with non insulindependent diabetes or without diabetes mellitus.

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STUDY ON DIAGNOSTIC VALUES FOR ORAL GLUCOSE TOLERANCE TEST BASED ON COMPLICATIONS

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To prevent DM complications, diagnostic values of GTT were investigated using long term follow-up observation. Subjects were 19,734 GTT examinees between 1965–1995. Diabetics under treatment and gastrectomy and liver dysfunction cases were excluded. Results :1) DM development rate increased with increasing 2hr-PG and reached a plateau at 2hr-PG of 180mg/dl or more. 2) Incidence of retinopathy rose with increasing 2hr-PG. In the 180–199 mg/dl 2hr-PG group the rate was 6.1/1000 PY, significantly higher than that for the 140–159mg/dl group but little different from that for the 200–239mg/dl group. 3) There was no difference with respect to incidence of hypercholesterolemia between the 180–199mg/dl 2hr-PG and the 200–239mg/dl groups. 4) Incidence of ischemic changes in ECG was significantly higher in the 180–199 mg/dl than in the normal group, but no difference was observed between the 180–199mg/dl and the 2hr-PG 200–239mg/dl groups. 5) Incidence of hypertension was higher in the 180 –199mg /dl than in the 140–159mg/dl group, but no difference was observed between the 180–199mg/dl and the 200–239mg/dl groups. 6) CHD mortality rate was higher in the 180–199 mg/dl than in the normal and 140–159mg/dl groups. Conclusion:DM diagnostic 2hr-PG value was 180mg/dl or more.

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SERUM LIPOPROTEIN(A) CONCENTRATION IN CHINESE NIDDM PATIENTS WITH ALBUMINURIA

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Our study was to determine the relationship and distribution of Lp(a) levels in NIDDM patients with different urinary albumin excretion rates (AER) compared with healthy nondiabetic subjects. Diabetic patients were grouped in normoalbuminuric, AER< 20 μ g/min (n=126), microalbuminuric, AER 20-200 μ g/min (n=56) and macroalbuminuric, AER> 200 μ g/min (n=52) categories. Within the diabetic patients' population, we examined the relationship between Lp(a) concentration and the rates of urinary albumin excretion. We measured lipids, lipoproteins, Lp(a) and blood pressure, and urinary albumin excretion in the 234 subjects with NIDDM and 100 healthy nondiabetic subjects. Non-insulin-dependent diabetic patients with normo-, micro- and macroalbuminuria were compared with healthy nondiabetic subjects. The macroalbuminuric diabetic group had significantly higher Lp(a) levels than the normoalbuminuric diabetic group and healthy nondiabetic subjects (28.0$\pm$19.5 vs 21.1$\pm$21.9 mg/dL; 28.0$\pm$19.5 vs 20.7$\pm$18.7mg/dL, respectively).($P<0.05$, $P<0.05$). The prevalence of is chemic heart disease was higher in macroalbuminuric as compared to normoalbuminuric diabetic patients ($P<0.05$). Importantly, there were positive correlations between Lp(a) and the urinary albumin excretion rate in the macroalbuminuric diabetic group ($P<0.00001$) and in all diabetic patients ($P<0.0001$). In conclusion, Lp(a) level is significantly elevated in NIDDM patients with macroalbuminuria.

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DETERMINANTS OF CHANGE IN PLASMA TRIGLYCERIDE LEVEL OVER 4.5 YEARS IN THE ELY PROSPECTIVE STUDY

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Increased plasma triglyceride (TG) levels are a cardinal feature of the insulin resistance syndrome (IRS) and therefore the increased risk of IHD associated with the IRS may be mediated by abnormalities of TG metabolism. To study determinants of change in plasma TG concentration with time we have undertaken analyis, stratified by gender of follow-up data from a population-based cohort, established in Ely, Cambs, UK. 1071 Caucasian subjects known to have either normal glucose tolerance or IGT in 1990-1992 were traced and invited to attend for a repeat test 4.5 years later. 937 volunteers attended for rescreening. In men the mean change in fasting plasma TG concentration was 0.026 mmol/l (range -4.6 to +10.5) and in women the mean change was 0.11 mmol/l (range -2.3 to +6.0). As the probability of change in TG concentration is affected by baseline TG concentration, regression to the mean and true change, we analysed TG concentrations as a continuous variable taking into account each of these factors separately. Subjects in the top quintile in whom plasma TG deteriorated were compared to subjects in the middle three quintiles in whom there was no change in triglyceride level. By logistic regression analysis the probability of deterioration was independently associated only with measures of obesity; baseline BMI (or WHR) and the increase in BMI (or WHR) over 4.5 years. (For baseline BMI; adjusted odds ratio 1.13 (95%CI 1.04-1.23, p=0.003 [men]; adjusted odds ratio 1.03 (95%CI 0.98-1.08, p=NS [women]. (For change in BMI with time adjusted odds ratio 1.22 (95%CI 1.02-1.46, p=0.028 [men]; adjusted odds ratio 1.17 (95%CI 1.04-1.33, p=0.01 [women]. These results show that increasing levels of obesity with time mediate adverse changes in triglyceride metabolism and suggest that a reduction in fat mass would improve the cardiovascular risk factor profile.

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THE RISK FACTORS IN ADULT PROGENY OF PATIENTS WITH NON-INSULIN-DEPENDENT DIABETES MELLITUS

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The major risk factors for coronary heart disease is amplified in diabetic patients and their families. To explore the occurrence of risk factors in the progeny of diabetes, 88 patients with non-insulin-dependent diabetes mellitus and 86 control subjects were recruited from a health center of northern Taiwan. Their adult progeny were invited to have the screening about the risk factors through a structured questionnaire and blood check-up to measure plasma glucose, cholesterol, triglyceride, HDL-cholesterol and HbAlC. Totally, there were 168 and 165 adult progeny in diabetic and control groups respectively. In univariate analyses, the diabetic progeny had higher percentage of parental hypertensive history than the control group. Besides, the male progeny of diabetes had higher mean BMI, blood pressure, HbAlC, serum cholesterol and triglyceride levels. Multiple regression analyses showed paternal diabetes was positively associated with HbAlC, systolic and diastolic blood pressure while maternal diabetes was associated with higher diastolic blood pressure and HbAlC in the male progeny. There was no risk factor associated with parental diabetes in the female progeny. In conclusion, our study demonstrated that the male progeny of both paternal and aternal diabetes were associated with more coronary risk factors including higher blood pressure and HbAlC.

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PREVALENCE OF ASYMPTOMATIC BACTERIURIA IN NIDDM FEMALES AND ASSOCIATION WITH RISK FACTORS.

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Aim of our study was estimating the prevalence of asymptomatic bacteriuria (AB), a well-known and described condition in NIDDM females, and associate it with any relevant parameters. We prospectively enrolled 363 patients (m.age 61.3±10.5 yrs range: 32-81, m.duration of Diabetes 10.8 yrs) followed in our Diabetes Clinic and screened them for the presence of AB and associated the results with their demographic, history, complications and glucaemic control data . Analysis was done via an IBM compatible computer using the EPI5-INFO (CDC/WHO) 1993 program. Statistical analysis was performed with Yates corrected chi square test. We found AB in 35 patients (9.7%) with *E.coli* (28), *Proteus* (3), *Klebsiella* (2) and *Citrobacter* and Enterococcus 1 each, been the isolated organisms. There was no difference found in the age and Diabetes duration of AB+ v no AB patients. (64.3 and 11.3 v 61.1 and 10.6 years respectively).or with the kind of Diabetes treatment or glycaemic control. The prevalence of AB by age group did not differ significantly, with the exception of the 70-79 yrs age group where AB was more prevalent (p=0.02). The presence of Diabetes complications was not associated with AB, including individual analysis for those with Diabetic nephropathy and neuropathy. The prevalence of AB among the sexually active women was 9/128 (7%) less than the overall percentage. The factor that was more strongly associated with AB was the history of previous urinary tract infections, whereAB was found in 73% of those with such history (p<0.001). In conclusion AB was found in a considerable percentage of NIDDM females, should be sought for in all age groups. It is also of critical importance that screening for AB must not be omitted in patients with previous urinary tract infection history.

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SEVERE HYPERGLYCEMIA AND MACROALBUMINURIA IS MORE PREVALENT IN THE RURAL AND AMONG THE POOR DIABETICS

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In the developing countries, compared with the urban and the rich, diabetes awareness is less in the rural people and least among the poor socio-economic class which leads to late detection of diabetes among them. To compare the glycemic status and macroalbuminuria (as one of the diabetic complications) between the rural and the urban subjects we investigated 8879 (M=5405, F=3474) non-insulin-dependent diabetes mellitus (NIDDM) subjects over 29 years of age. Among these newly registered subjects in 1995, the urban and rural diabetics were 70 and 30%, respectively. According to social class the rich, the middle and the poor were 29.9, 66.0 and 4.1%, respectively. Compared with the urban subjects, the mean (±SD) duration of hyperglycemic symptoms (typical: polyuria, polydipsia, weight loss) was significantly higher in the rural diabetics (21.6 ±15.6 vs 18.1 ±13.2 mo, p=0.03). The mean age (±SD) of rural subjects of either sex was also significantly higher (men: 50 ±11 vs 47 ±10yr, p<0.001; women: 49 ±11 vs 46 ±10, p<0.001). Rural subjects had also significantly higher fasting plasma glucose (FPG in mmol/l: men, 12.2 ±5.3 vs 11.0 ±4.3, p<0.001; women, 12.2 ±5.0 vs 10.9 ±4.2, p<0.001). Similarly, 2h after glucose load (2h-PG) was significantly higher among them (p<0.0001). In contrast, adjusting for sex, the rural subjects had significantly lower body mass index (BMI±SD) than their urban counterpart (men: 21.5 ±3.8 vs 23.3 ±3.3, p<0.001; women: 23.0 ±4.6 vs 25.2 ±4.1, p<0.001). Hyperglycemia (2h-PG) over 20.1 mmol/l was more frequent among the rural than the urban subjects (42.2 vs 34.4%: X^2 46.0, p<0.001) and also more frequent among the poor than the rich (47.3 vs 32.3%, p<0.001). In addition, compared with the urban and the rich, macroalbuminuria was significantly associated with the rural (11.4 vs 13.8%, p=0.01) and with the poor (11.5 vs 15.4%, p=0.03) diabetics. Therefore, this study suggests that regardless of diabetes prevalence in rural and urban population, severe hyperglycemia with typical symptoms and low BMI is more frequent in the rural area and among the poor social class. Similarly, macro-albuminuria, an indicator of advanced stage of diabetic nephropathy is also more prevalent in these subjects.

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Low Birthweight and Gestational Diabetes

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LOW BIRTHWEIGHT AND RAISED PLASMA CORTISOL CONCENTRATIONS IN ADULT LIFE

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Low birthweight in babies born at term is associated with insulin resistance, glucose intolerance and raised blood pressure in adult life. Because there is evidence from animal models that undernutrition in utero could lead to persisting alterations in the hypothalamic-pituitary-adrenal axis, we have investigated whether low rates of fetal growth are associated with altered plasma cortisol in adult life. In a follow-up study of 370 men born in Hertfordshire during 1920-30 whose birthweights were recorded and who attended a local clinic for oral glucose tolerance tests, fasting plasma cortisol concentrations rose with increasing age ($p<0.001$) and fell with increasing body mass index ($p=0.05$). Plasma cortisol fell progressively with increasing birthweight from 408nmol/l among those who weighed ≤2.5kg at birth to 309nmol/l among those who weighed ≥4.3kg ($p=0.007$), a trend that was independent of age, body mass and levels of cortisol binding globulin. Higher fasting plasma cortisol concentrations were associated with insulin resistance estimated by HOMA ($p=0.006$), raised systolic blood pressure ($p=0.02$), and higher 2-h plasma glucose ($p=0.04$). Low rates of fetal growth are associated with increased circulating concentrations of cortisol throughout life. This may be one mechanism underlying the association between low birthweight and diabetes and hypertension.

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GESTATIONAL DIABETES AND NEONATAL OUTCOME: EFFECTS OF PREPREGNANCY BMI

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This study investigates the clinical characteristics of gestational diabetes mellitus (GDM) and neonatal outcome in relaction to prepregnancy body mass index (BMI). Data were obtained using a computerized data system for all deliveries (n.4171) that occurred at the Departments of Obstetrics and Gynecology of the University of Pisa (Italy) from Jan.1, 1987 through to Dec. 31, 1992. 93 women with GDM (prevalence 2.3%) were found and divided into three groups in relation to their pre-pregnancy BMI: normalweight (Nw), overweight (Ow) and obese (Ob). 110 matched control subjects were also evaluated. GDM was diagnosed earlier in Ow and Ob than in Nw ($p<0.01$) and insulin treatment was used in 86% of Ob-GDM and 91% of Ow-GDM patients in respect to Nw-GDM women (77%, $p<0.001$). Preterm deliveries and cesarean sections resulted significantly increased in all B.M.I. categories of GDM patients in respect to normal controls. Prevalence of neonatal macrosomia was higher in GDM patients (44.6%) compared with normal controls (15.4%) and correlated ($p>0.01$) with prepregnancy BMI in both groups. The body weight increase during pregnancy was not associated with neonatal macrosomia. In conclusion, this study shows that the degree of overweight is related to an earlier diagnosis of GDM. Moreover prepregnancy BMI is more predictive of macrosomia than weight gain specially in women suffering from GDM.

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INTRAUTERINE GROWTH RETARDATION AND PRENATAL GLU COSE INFUSIONS: RISK FACTORS FOR NIDDM AND SYNDROME X ?

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Recent epidemiological studies in adult populations suggest that intrauterine growth retardation (IUGR) is a risk factor for developing NIDDM and syndrome X later in life. Two groups of children and adolescents (aged 9.6 to 20.3 years) with low birthweight were compared: an IUGR group (group1, n=50) and another group of low birth weight children (group2, n=50) whose mothers received a minimum of 5 i.v. infusions of 2000 ml 10% glucose during pregnancy to compensate a suspected fetal malnutrition because of placental insufficiency. All subjects underwent an OGTT and IVGTT.

In the OGTT we observed a high prevalence of IGT according to WHO criteria. In group1 16% and in group2 22% had IGT. Subjects with IGT showed significantly higher insulin levels at 60 min ($p=0.007$), 90 min ($p=0.0001$), 120 min ($p<0.0001$) and higher proinsulin levels at 0 min ($p=0.003$), 30 min ($p=0.01$), 60 min ($p=0.0002$), 90 min ($p<0.0001$), 120 min ($p<0.0001$). The birthweight correlated inversely with the diastolic blood pressure ($r= -0.25$; $p=0.01$) and the serum uric acid ($r= -0.25$; $p=0.015$). Systolic and diastolic blood pressure were correlated with serum creatinine ($r=0.51$ and $r=0.45$; $p<0.0001$) and serum uric acid ($r=0.60$).

The early phase of IVGTT did not reveal any abnormalities in our patients.

Our results suggest a higher risk of developing diabetes and syndrome X later in life in persons with IUGR because there is an association of low birthweight and impaired glucose tolerance, insulin resistance (hyper-insulinemia and hyperproinsulinemia) and high blood pressure. There were no significant differences in the glucose metabolism of the both test groups. We conclude therefore that the prenatal glucose infusion therapy did not have an additional diabetic effect on these children and adolescents.

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SERUM LIPID AND LIPOPROTEIN LEVELS IN WOMEN WITH GESTATIONAL IMPAIRED GLUCOSE TOLERANCE

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A progressive increase in serum lipids and lipoproteins has been observed in pregnant women. However, variable results in pregnancies complicated by pregestational diabetes mellitus (PGDM) and gestational diabetes mellitus (GDM) had been previously reported. The purpose of this study was to investigate the effect of pregnancy on serum concentrations of lipids and lipoproteins in gestation impaired glucose tolerance (GIGT). The WHO 75g glucose tolerance protocol test and criteria were used between 24 and 28 weeks of pregnancy to diagnose GIGT. Fasting serum concentrations of lipids and lipoproteins were measured. As a result 219 consecutive laboratory tests were conducted and evaluated on women in a public prenatal care center in Salvador-Bahia. Glucose tolerance tests were normal in 200 pregnant women and GIGT was observed in another 19. The mean concentrations of lipids and lipoproteins shown in the normal glucose tolerance pregnant group (NGT) and GIGT were as follows:

	TC (mmol/l)	TG (mmol/l)	LDL (mmol/l)	HDL (mmol/l)	VLDL (mmol/l)
NGT n=200	4.63	1.27	2.95	1.09	0.25
GIGT n=19	4.08	1.19	2.40	1.13	0.24
P value	0.07	0.44	0.05	0.38	0.47

Body mass index (BMI) was significantly higher in GIGT women as compared to the NGT. Maternal age ,weight gain during pregnancy and parity were similar in the two groups. LDL levels were significantly higher in NGT than in GIGT. No hypertriglyceridemia was present in the GIGT women. Serum lipids showed a trend towards lower levels in GIGT women and were especially significant in LDL levels The absence of an increase in triglyceride levels detected thus far during pregnancy was unexpected. Additional researches are needed to assess the effect of abnormal glucose tolerance on the metabolic regulation of lipids in pregnancy.

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Low birth weight does not predict metabolic syndrome in adult life.

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It has been proposed that there is an association between low birth weight and metabolic syndrome (MBS) in adult life. In order to test this hypothesis, we screened all subjects aged 36, 41 and 46 years (N=1008) in Pieksämäki, a town in central Finland, for MBS, and collected data on birth weight, and weight and height of seven years of age . MBS was defined to be present in those subjects having simultaneously dyslipidemia (hypertriglyceridemia≥ 1.70 mmol/l and/or low high density lipoprotein cholesterol <1.00 mmol/l in men and <1.20 mmol/l in women) and insulin-resistance (impaired glucose tolerance or non-insulin-diabetes or hyperinsulinemia≥ 13.0 mU/l). We determined the occurrence of MBS in adult life according to quintiles of birth weight, and to quintiles of body-mass index (BMI) at the age of seven years. Chi-squared test was used for analysis. In all,712 (71%) subjects participated in the screening. Data on birth weight and BMI at age of seven years were obtained for 428 (42%) subjects. In these, MBS was present in 31/210 (15%) men and in 23/218 (11%) women. The prevalence of MBS was 9% in both the lowest and the highest quintile of birth weight, and ranged from 14% to 17% in three middle quintiles without there being any statistically significant difference between quintiles (p=0.32).The prevalence of MBS was 8% in the lowest quintile of BMI at seven years of age and increased up to 21% in the highest quintile. This increasing trend according to quintiles was statistically significant (p=0.026). Conclusion: we could not identify any evidence that there would be on association between low birth weight and MBS in adult life. However,a high BMI at seven years of age predicted MBS in adulthood.

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LIPID PROFILE OF WOMEN WITH GESTATIONAL DIABETES AND IMPAIRED GLUCOSE TOLERANCE

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It is known that during pregnancy changes occur in the plasma lipid profile, in particular, increases in the levels of triglycerides (TG), cholesterol and phospholipids, with corresponding changes in the lipoproteins-mainly VLDL. The aim of this study was the evaluation of the level of lipids and lipoproteins in pregnant women with gestational diabetes or impaired glucose tolerance. A cohort of 136 women in the 28th to 32nd week of pregnancy were selected. Following a 12 hour fast and a 3-day measured intake of 150 g/day carbohydrate, each woman was subjected to a glucose tolerance test (OGTT) with 100 g glucose. Plasma glucose was measured colorimetrically at baseline and at 1, 2 and 3 hours after glucose load, and levels of CHOL, TG, HDL, LDL and VLDL were measured colorimetrically in the plasma prior to the OGTT. Using the diagnostic criteria of O'Sullivan and Mahan and according to the results of their OGTT, the women were divided into 3 categories: (i) 40 with confirmed gestational diabetes (GD). (ii) 63 with impaired glucose tolerance (IGT). (iii) 33 normal controls. Statistical analysis was by the student "t-test". It was confirmed that in relation to the normal controls, the women with GD (t=4.86, p<0.0001) and those with IGT (t=4.53, p<0.0005) had significantly increased levels of TG in their plasma, with parallel increases in the levels of VLDL, GD (t=3.72, p<0.001), IGT (t=3.72, p<0.001). Between the 2 groups of women with disturbed glucose metabolism (GD and PGT) there was not a significant difference in the levels of plasma TG and VLDL, as similary, there was not between these two groups and the normal controls when the plasma levels of CHOL, HDL and LDL were compared. In conclusion, the characteristic phenomenon of increased levels of plasma TG and VLDL in pregnant women with gestational diabetes or impaired glucose tolerance was confirmed.

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SIZE AT BIRTH AND CORONARY HEART DISEASE MORTALITY IN FINNS BORN DURING 1924-33.

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Diabetes is a major risk factor for coronary heart disease (CHD). Impaired fetal growth, as measured by low birthweight and low ponderal index (birthweight/length3, a measure of thinness at birth) has been associated with an increased risk of both NIDDM and CHD mortality. To study the impact of early life on CHD mortality we collected birth data from 11704 hospital births during 1924-33 in Helsinki. Of these 9578 subjects were identified in the population register, which was computerised in 1971. Birth weight could be measured in 9545 and ponderal index in 6987 subjects. During follow up since 1971 there have been 1463 deaths, of which 543 were due to CHD (448 male, 95 female). The impact of size at birth on CHD mortality was assessed using Cox's proportional hazards model. Risk of CHD death was 5.4 times higher in men (p=0.0001), decreased by 15.3 % (0.2%-28.1%, p=0.0479) for each additional kg of birthweight, and decreased by 3.5 % (1.2%-5.7%, p=0.003) for each additional unit (kg/m3) of ponderal index. Dividing ponderal index into equal fourths, risk of CHD death ranged from 2.0(1.4-2.9)- 1.6(1.1- 2.3) - 1.5(1.0- 2.2) -1.0(baseline) in men and from 1.3(0.6- 2.8) - 0.8(0.3- 2.0) -1.2(0.53- 2.669) -1.0(baseline) in women. Studies concerning the impact of impaired fetal growth on glucose metabolism are in preparation. Conclusion: thinness at birth predicts death from CHD in adult life.

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THE IMPORTANCE OF A FAMILY HISTORY OF DIABETES MELLITUS IN RELATION TO THE AGE OF THE WOMAN IN THE MANIFESTATION OF GESTATIONAL DIABETES MELLITUS

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The aim of this study was the appraisal of the importance of a family history of diabetes mellitus (DM), both independently and in correlation with age, in the manifestation of gestational diabetes mellitus (GDM). A cohort of 295 pregnant women were selected aged from 20 to 44 years, of whom 178 (60.3%) had a family history of DM, while 117 (39.7%) did not. Each woman was subjected to an OGTT using 100 g of glucose and plasma glucose was measured colorimetrically at baseline and 1, 2 and 3 hours after the load. According to the criteria of O'Sullivan and Mahan, out of the 178 women who had a family history, 21 (11.8%) had a normal OGTT, 83 (46.6%) had an impaired OGTT, while 74 (41.6%) had an abnormal OGTT. Out of the 117 women with no family history of DM, 23 (19.7%) had a normal OGTT, 52 (44.4%) had an impaired OGTT and 42 (35.9%) had an abnormal OGTT. The statistical comparison was obtained using the chi-squared test after the correction of Yates and the student "t-test" from which it was confirmed that the women with no family history of DM had a significantly increased frequency of normal OGTT (x^2=4.08, p<0.05). When this statistical exercise was applied to the ages of the women, it was found that only in this group of women (no history) was there a particularly significant correlation for the manifestation of gestational diabetes mellitus (t=2.68, p<0.01). We conclude that: 1) All pregnant women with a family history of DM, independent of age, should be subjected to an OGTT. 2) Pregnant women who have no family history of DM but are relatively advanced in years should be subjected to an OGTT.

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MATERNAL CARBOHYDRATE METABOLISM AND REDUCED FETAL GROWTH

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Our purpose was to determine the impact of maternal carbohydrate metabolism and anthropometric characteristics on fetal growth in pregnancies with small for gestational age. We studied 8 women with unexplained small for gestational age and 10 with normal pregnancies using an oral glucose tolerance test and hyperinsulinemic euglycemic clamp in the third trimester of pregnancy. These data and maternal anthropometric characteristics were subsequently related to neonatal birth weight and percentile. The women with small for gestational age were relatively more insulin sensitive compared to controls ($p<0.05$) and showed a reduced insulin and glycemic area ($p<0.01$ and $p<0.04$, respectively). Insulin sensitivity had the strongest correlation with neonatal birth weight and percentile in the small for gestational age ($r=-0.77$, $p<0.03$ and $r=-0.73$ $p<0.04$ respectively). In contrast, in control women the best correlation between birth weight and birth weight percentile was seen with maternal weight gain ($r=0.83$, $p<0.01$ and $r=0.71$, $p<0.03$ respectively). Including all variables in the stepwise model we found that insulin sensitivity, insulin area and fasting insulin together explained 80% of the variability of neonatal weight in women with small for gestational age. On the contrary, in the controls the independent predictor accepted in the model was maternal weight gain and parity that explained 75% of the variability of birth weight. We conclude that insulin sensitivity seems to be a determinant factor for fetal growth in women with small for gestational age.

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THE IMPORTANCE OF THE AGE OF THE WOMAN IN THE MANIFESTATION OF GESTATIONAL DIABETES MELLITUS

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The aim of this study was the appraisal of the importance of the age of the woman in the manifestation of gestational diabetes mellitus. A cohort of 556 pregnant women, aged from 18 to 44 years were selected and subjected to an OGTT with 100 g glucose following a 3-day diet including 150 g/day carbohydrates. Plasma glucose was measured colorimetrically at baseline and 1, 2 and 3 hours after the glucose load. The assessment of the results was according to the diagnostic criteria of O'Sullivan and Mahan. Results: Nine out of 65 women (13.8%) aged up to 24 years, 29 out of 165 (17.6%) aged 25-29 years, 66 out of 203 (32.5%) aged 30-34 years, 37 out of 102 (36.3%) aged 35-39 years and 21 out of 47 (44.7%) aged 40-44 years were diagnosed as having gestational diabetes mellitus. The statistical comparison was performed using the chi-squared test after the correction of Yates. It was subsequently proven that women aged 30-34 years had a significant difference from women aged 25-29 years ($x^2=11.4$, $p<0.01$). A still greater effect on the diagnostic outcome was observed in women aged 35-39 and 40-44 years. Overall, the women who were over 30 years of age had a significantly different and pathological OGTT from women aged under 30 years ($x^2=25.2$, $p<0.001$). We conclude that for women aged over 30 years extra vigilance for the onset of gestational diabetes mellitus is required during a pregnancy.

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MATERNAL SIZE, NUTRITION AND FOETAL GROWTH IN RURAL INDIA.

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Recently described association between poor intrauterine growth and adult diabetes may be particularly relevant in India. We studied maternal size, nutrition and foetal growth in 6 villages near Pune. 814 pregnant women (age 21.3 y, height 1.52 m, weight 41.7 kg and haemoglobin 119 g/L; mean) delivered 771 live babies (weight 2613g, length 47.4 cms, placenta 357g). Average maternal intake was 1790 kcal, 45 gm proteins and 18 mg iron. In addition to the length of gestation and sex, birthweight was related to maternal preconceptional weight ($r=0.23$, $p<0.001$) and height ($r=0.16$, $p<0.01$), and to weight gain ($r=0.15$,$p<0.01$), haemoglobin ($r=-0.12$, $p<0.05$) and fasting plasma glucose concentration ($r=0.09$, $p<0.05$) at 28 wks and to placental weight ($r=0.61$, $p<0.001$). It was, not related to maternal intake of calories, proteins and iron. Maternal size and weight gain though significant determinants, together made a relatively small contribution to the variability of birth weight (~5%).

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INSULIN SECRETION AND SENSITIVITY IN WOMEN WITH GESTATIONAL DIABETES MELLITUS AT FOLLOW-UP

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In women with gestational diabetes (GD), abnormal insulin secretion (SEC) and sensitivity (SI) have been described. We aim to assess the contribution of these factors to GD and to abnormal glucose tolerance postpartum. At postdelivery follow-up, a 75 gr oral glucose tolerance test (0'30'60'120' glucose and insulin) has been performed in 55 GD women and a control group (C) with negative GD screening. SEC has been estimated as the ratio of insulin to glucose incremental areas 0-30' and SI as the index described by Cederholm. Mann-Whitney test is used for comparison. GD women have a SI lower than controls (50.5±12.2 vs 69.0±4.1 $mg.l.l.mmol^{-1}.mU^{-1}.min^{-1}$, $p<0.05$) but a similar SEC (16.5±17.0 vs 20.7±16.4 mU/mmol, ns). According to NDDG criteria, glucose tolerance is abnormal in 12 GD women (GD_AGT) and normal in 43(GD_NGT). From C to GD_NGT to GD_AGT, there is a stepped decrement in SI (69.0±4.1 $mg.l.l.mmol^{-1}.mU^{-1}.min^{-1}$ vs 53.9±11.2 vs 38.3±6.6, C vs GD_NGT $p=0.05$, GD_NGT vs GD_AGT $p<0.001$). For SEC the only decrease is between GD_NGT and GD_AGT (20.7±16.4 mU/mmol vs 18.8±18.6 vs 8.5±3.3, C vs GD_NGT ns, GD_NGT vs GD_AGT $p<0.01$). We conclude that the main feature of GD is low SI whereas both low SI and SEC contribute to AGT in women with prior GD.

FIS 94/1538

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ASSOCIATION BETWEEN BIRTH WEIGHT AND ADULT METABOLIC PROFILE IN THE OFFSPRING OF NIDDM SUBJECTS

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Impaired and disproportionate fetal growth has been associated with an increased risk for NIDDM, coronary heart disease, and hypertension in large-scale epidemiological studies. To determine whether a similar risk profile occurs across a spectrum of birth weights (BWs) in individuals at high risk for NIDDM, we analyzed data from the FUSION study. 466 adult offspring of a diabetic parent who were products of singleton pregnancies delivered at term participated in a clinical examination with an oral glucose tolerance test (75 g glucose load) and a frequently-sampled intravenous glucose tolerance test (FSIGT) using the Minimal Model approach. The offspring were divided into two groups depending whether the affected parent was the mother (AFMO) (n=172) or the father (AFFA) (n=294). Birth weight did not differ between the two groups (AFMO 3606g, SD 582; AFFA 3548g, SD 442; p=0.26). The relationship between BW and systolic and diastolic blood pressure, fasting and 2-hour post-load plasma glucose and serum insulin, FSIGT measures (acute insulin response, insulin sensitivity, glucose effectiveness) and serum lipids (total and HDL-cholesterol, triglycerides) were tested using Generalized Estimating Equations, adjusting for gender and age of the offspring. In the AFMO group there was a positive nonlinear relationship between BW and acute insulin response (p=0.032). Furthermore, a negative nonlinear relationship between BW and fasting insulin was observed (p=0.0051). We found negative relationships between systolic (p=0.0010) and diastolic blood pressure (p=0.0042) and BW in the AFMO group. In the AFFA group, the study showed evidence for a positive relationship between birth weight and fasting insulin value (p=0.0234). All the other variables failed to show a significant relationship to BW. Thus, the offspring with diabetic mothers showed a stronger association between factors associated with fetal growth and adult metabolic risk factor profile than those with diabetic fathers. These findings suggest an important influence of intrauterine development on blood pressure and hyperinsulinemia in families with NIDDM.

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PREGESTATIONAL DIABETES : INSULIN MULTIPLES DOSES AND CONTINUOUS SUBCUTANEOUS INSULIN INFUSION

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OBJECTIVE. To compare the results between the usage of combination of intermediate and regular insulin multiple doses (IMD) and continuous subcutaneous insulin infusion with pumps (CSII) during pregnancy in intensified insulin therapy treated type I diabetic patients, in two medical centers of Argentina,

MATERIAL AND METHODS. From 1984 to 1996, 94 pregnancies in 68 women with IDD were evaluated. IMD was used in the course of 59 pregnancies (Mean and SD of: 1) the age at the time of pregnancy: 24.8 +/- 4.9 yr.; 2) the age at the beginning of IDD : 14.7 +/- 6.7 yr. 3) the IDD evolution time: 10.2 +/- 5.7 yr.) and CSII was used in 35 (Mean and SD of : 1) the age at the time of pregnancy: 25.8 +/- 4.1 yr., 2) the age at the beginning of IDD: 16.4 +/- 6.9 yr. and 3) the IDD evolution time : 9.3 +/- 5.6 yr.). In the study, 74.5% of the patients with IMD and 52.4% with CSII went through her first pregnancy. Statistical methods: Student, Fisher and Mantel-Haenszel Tests.

RESULTS. The mean newborn weight was 3508 +/- 562 g in the 37.4 weeks in the IMD group and 3586 +/- 401 g in the 38.4 weeks in the CSII group. Statistical difference between groups was not found to exist in the age at pregnancy, the age at the beginning of IDD, the IDD evolution time and the newborn weight, but there was difference at the moment of delivery. The following difficulties were observed:

	IMD	CSII	
Abortion or in uterus fetal death	15	3	p = 0.044
Newborn complications	18	4	p = 0.035
Congenital abnormalities	6	4	p = 0.550

CONCLUSIONS. In a study of comparable groups of pregnancies in IDD patients treated with IMD or CSII, there was not difference in the weight of the son at born, but there was a week of difference in the mean of the date of delivery. The frequency and severity of congenital abnormalities were similar in both groups, but in patients with CSII the number of abortions and in uterus fetal death and the complication of the newborn (hypoglycemia, polycythaemia,etc) were significantly less, maybe because the CSII would permit the extension of the pregnancy and would facilitate a more tightly glycemic control near the time of delivery.

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PARENTAL GLUCOSE TOLERANCE AND FOETAL GROWTH IN RURAL INDIA.

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We studied glucose tolerance (75g OGTT, WHO 1985) in 597 pregnant women (mean age 21.5 y, height 1.52 m, preconceptional weight 42.0 kg, BMI 18.1 kg/m^2) at 28 wks gestation, in 6 villages near Pune. Mean fasting plasma glucose concentration was 4.0 mmol/l and 2h post glucose 4.4 mmol/l, neither were related to age, SE status, weight, BMI, waist hip ratio and weight gain. Four women (0.07%) showed IGT, none diabetes; one woman had pregestational diabetes. Fasting but not 2h plasma glucose concentration was related to birth weight (r=0.09, p<0.05). In husbands (29y, 1.65 m, 52.9 kg, 19.5 kg/m^2), fasting and 2h plasma glucose concentration was 5.0 mmol/l and 5.1 mmol/l, 13 (2.3%) showed IGT and 1 diabetes. Paternal glucose was also related to birth weight (r=0.08, p<0.05). Our findings show a very low prevalence of gestational diabetes in rural India, probably because of low caloric intake and high level of physical exercise (farming). Maternal plasma glucose though a significant relation, is not a major determinant of birth weight in this population.

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FASTING GLUCOSE LEVELS AT DIAGNOSIS PREDICT INSULIN REQUIREMENTS IN GESTATIONAL DIABETES(GDM).

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Data on 330 women with GDM and a singleton fetus was analysed to determine the efficacy of the fasting plasma glucose level (FPG) at diagnosis for identifying women likely to require insulin therapy during pregnancy.The diagnosis of GDM was made on a FPG ≥5.5mmol/l and/or a two hour level ≥8.0mmol/l during a 75G oral glucose tolerance test.The women had a mean age of 34 years,BMI of 25.7 and parity of 2 (R 1-9).Seventy one percent of the women were Caucasian,11% Asian, 8% Mediterranean, 8% Indian, and 2% Maori. Fifteen percent had a previous history of GDM and 50% had a family history of diabetes. Diagnosis was made at a mean of 30 weeks gestation and insulin was commenced if fasting capillary blood glucose levels were repeatedly ≥5.0mmol/l or ≥6.5mmol/l two hours postprandial in spite of dietary intervention. Seventy two of these women required insulin with a mean dose of 34 IU per day, commencing at a mean of 31 weeks gestation. Mean birthweight was 3400G. Twelve percent of the babies were >4000G. A 50% probability for requiring insulin was reached with a FPG at diagnosis of 3.5 mmol/l if tested at 10 weeks gestation,5mmol/l at 20 weeks and 5.7 mmol/l at 30 weeks (p<.001). It is concluded that the FPG level at diagnosis may identify a subgroup of women more likely to require greater intervention during the pregnancy.

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SCREENING FOR GESTATIONAL DIABETES MELLITUS. RELATIONSHIP WITH THE GESTOSIS DEVELOPMENT. N.V.Trusova, A.S.Ametov, L.E.Mourashko Russian Academy of Advanced Med.Studies, Moscow, Russia.

Gestational Diabetes Mellitus(GDM) connected for mothers with an increased risk of subsequent overt diabetes mellitus and in addition to the well-known perinatal risk may be associated with an increased risk of childish obesity and NIDDM development in later life. That's why the early diagnostic of GDM is very important and provides not only a healthy baby, but a unique opportunity to identify individyals at high risk of NIDDM at the time when the change of lifestyle can reduce overall diabetic morbidity. But at the present time it's no international agreement regarding diagnostic criteria for GDM and its treatment. We screened 600 pregnant women with and without risk factors at 24-28 weeks of gestation. Our programme includes 50gGCT, 100g OGTT, self-monitoring blood glucose(glucometer One Touch II (LifeScan)). Another problem is that women with GDM have an increased rate of gestosis cases. The mechanism underlying the association is not comletely understood. We obtained all women undergoing glucose challenge test to determine connection between plasma glucose level in screening and frequency of gestosis development. Our study defines the importance of uniformity in screening, diagnosis and management programme. Also we marked increase of the GDM rate in Russia and developed the programme of this disease management adapted to our conditions.

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DIABETIC KETOACIDOSIS IN GESTATIONAL DIABETICS

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The diabetic ketoacidosis (DKA) is an uncommon complication of the pregestational diabetic (less than 1.5%) and is almost anecdotal in gestational diabetic mellitus (GDM). In a period of 3 years we had 4 cases of DKA in GDM. The principal data was:

	A	B	C	D
Age	19	17	22	29
Glycemia (mg/dl)	810	765	778	857
Arterial $_{p}H$	7.10	7.01	7.28	7.27
Bicarbonate mmol/l	7.4	2.0	9.0	8.0
I.C.A's	(-)	(-)	(-)	(-)
O.G.T.T.	(-)	(+)	(+)	(+)
Preeclampsia	(+)	(-)	(+)	(+)
B.M.I.	23	24	21	30

I.C.A's: Islet cell antibody; **O.G.T.T.:** Oral Glucose Tolerance Test; **B.I.M.:** Body Mass Intex

Because of ICA's were negative aloud us to classify these patients as NIDDM. After 6 month of delivery, 3 patients remain with diabetic OGTT according with the OMS criteria (1985), and are on insulin therapy. Only 1 patient is been treated for hypertension. One of them is prenant again (8^{th} month). Four patients had infection as the cause of diabetic derrangemet and have the DKA after week 28^{th} of pregnancy. Two had fetal death. The diagnosis of GDM is very easy to do and must be established in any health system. We do not have an explanation why these patients developed DKA. The DKA in GDM is a very important cause of fetal death and very dangerous for the mothers.

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SCREENING AND INTERVENTION IN GESTATIONAL DIABETES MELLITUS

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Referred incidence of gestational diabetes (GDM) in the country is lower than 1%. Current practice of screening for GDM is OGTT (WHO criteria) in 24-28 week in women with risk factors. The aim of the study is an evaluation of this approach.

Data from 60 of total 122 women with GDM or impaired glucose tolerance (IGT) in 24-28 pregnancy week was compared with a group of 60 randomly selected from 2000 referred in the same period with normal OGTT. Fifty percent of women were less than 24 weeks pregnant. Women with IGT or GDM were older (95%ci: 31-32 years) and heavier (prepregnancy body mass index (BMI): 95% ci 26-28) than controls (95% ci age: 22,9-25,1 years, 95% ci BMI: 29-32). There were no differences in family history or history of stillbirth and miscarriages. All women with abnormal OGTT were treated targeting mean blood glucose <6,1 mM. Birth weight was insignificantly higher in IGT and GDM group. There were more caesarean sections (14) and maternal complications (6) than in controls (3 and 2 res.). There were no stillbirths, one major malformation was present in IGT and GDM group.

Even with intervention, pregnancy outcome in GDM differs from controls. By the present approach around four- fold reduction in number of screened women is expected. However, a large number of gestational diabetes is missed and it seems better to screen all the pregnancies.

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GLOMERULAR AND TUBULAR DYSFUNCTION IN WOMEN WITH PREVIOUS GESTATIONAL DIABETES MELLITUS

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Our aim was to study the occurrence of late diabetic renal complications in two groups of women with previous gestational diabetes mellitus (GDM) after reclassifying their carbohydrate metabolism. Women first insulinized during pregnancy (GDM-I [n=75]; mean age 38 [range: 24-52]yrs, time elapsed since diagnosing GDM: 7.1 [3-14]yrs) were investigated. Patients on diet during gestation served as controls (GDM-D [n=29]: age 37 [26-52]yrs, follow up 6.9 [4-10]yrs). Serum creatinine, beta-2-microglobulin [b2m]; urinary albumin, creatinine and b2m were measured. To increase the sensitivity of microalbumin and urinary b2m measurements albumin/creatinine [UA/CR] and b2m/creatinine [B2M/CR] ratio were also calculated. None of the cases had elevated serum creatinine (mean [range]: GDM-I 81 [51-112]µmol/l; GDM-D 82 [69-93]). Microalbuminuria (MAU) [30-300 mg/day] was found in 15 GDM-I cases (one macroalbuminuria too): in 10/45 of verified diabetic patients (22.2%), 4/23 (17.4%) in non-diabetic persons (P=0.2, NS.) and in 1/6 IGT; in 2 GDM-D cases (1/6 diabetes, 1/6 IGT). UA/CR and B2M/CR ratio was significantly higher in GDM-I (average 6.8 ± 14.1 mg/mmol; and 0.014±0.018mg/mmol) than in GDM-D (1.9 ± 2.9 mg/mmol; and 0.007±0.004, resp); (P<0.05). However, the ratio of UA/CR and B2M/CR did not provide further information than measuring their concentration only, if the data were analized one by one. Urinary b2m concentration was significantly elevated but still in normal range in diabetic patients of GDM-I (0.068±0.07) compared to GDM-D (0.035±0.016) group; (P<0.05). One patient had pathological urinary b2m in GDM-I. Based on these data we suggest that similar prevalence of MAU in both diabetic and non diabetic patients with high frequency of hypertension may indicate that MAU could be an early cardiovascular risk marker rather than the sign of early renal impairment. On the other hand, slightly elevated urinary b2m among diabetic patients may reflect an early tubular dysfunction.

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SCREENING OF GESTATIONAL DIABETES MELLITUS

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To know the incidence of ***Gestational Diabetes Mellitus (GDM)*** in our population, 1000 pregnant women were screened for glucose intolerance by a ***Glucose Challenge Test (GCT)*** and ***Oral Glucose Tolerance Test (OGTT)***. Of them 67 (6.7%) were found to have GDM. The GDM mothers were significantly elderly ($p < 0.001$) and overweight ($p < 0.001$) than the non-diabetics. Family history of diabetes was significantly more among the GDM group. They also belong to comparatively higher socio-economic class than the non-diabetic group ($p < 0.001$). ***Bad Obstetric History (BOH)*** was more among GDM ($P < 0.01$) but the two groups did not differ by parity. Analysis documented overweight, diabetic family and BOH as individual risk factor for GDM. Mothers without any of these three risk factors had lowest rate of GDM (1.68%). Underweight or younger age were not protective for GDM; combination of risk factors increase the rate. A diabetic family history plus other risk factor(s) showed significant susceptibility than any other combination. After delivery 4.5% (n=3) became diabetic, 16.4% (n=11) IGT and rest 79% (n=53) normal.

Key Words: GDM, IGT, GCT, OGTT, Diabetic family, BOH.

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ACCURACY OF THE MEDISENSE PRECISION BLOOD GLUCOSE METER IN GESTATIONAL DIABETES MELLITUS (GDM)

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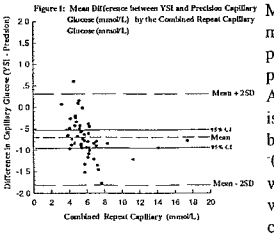
The accuracy of blood glucose meters used in GDM is of prime importance, especially when haematocrit changes in pregnancy may be a source of error. The aim of this study was to measure agreement of the Medisense Precision meter(MPM) versus the Yellow Spring Instrument (YSI) in women with GDM. Due to the controversy regarding whether whole blood or plasma glucose should be used to monitor GDM, this study also compared the MPM, which used whole blood, with plasma glucose derived from the YSI after adjustment for haematocrit [calculated plasma glucose = (capillary whole blood result)/[1.0-(2.4×10^{-3} x haematocrit%)]]. 200 μL of capillary blood was collected into a lithium heparin microlette CB 1000 from 50 women with GDM (gestation 10-39 weeks). Repeat blood glucose readings were obtained on both the MPM and YSI. Mean haematocrit was 36% (range 31-42%). The mean capillary blood glucose using the MPM and Microflo test strip was 6.5 ± 2.5 mmol/L compared to 5.8 ± 2.4 mmol/L ($r=0.98$; $p<0.0001$) or 6.3 ± 2.6 mmol/L for calculated plasma glucose ($r=0.98$; $p<0.0001$). Agreement/difference between the MPM and YSI is shown in Figure 1. The mean difference between the instruments was -0.7 (95% CI: -0.6 - -0.9) mmol/ L for whole blood. This difference was reduced to -0.2 (95% CI: -0.05-0.3) mmol/L when MPM whole blood was compared with calculated plasma glucose. This study showed a strong agreement between the MPM and YSI over a wide haematocrit range. The agreement was even stronger with calculated plasma glucose after adjustment for haematocrit, indicating that the MPM is an accurate instrument with which to monitor diabetic control in women with GDM.



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DIAGNOSTIC VALUE OF THE MULTIPLICATION OF THE FASTING LEVELS OF GLUCOSE AND TRIGLYCERIDE IN WOMEN WITH GESTATIONAL DIABETES

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In a previous paper we proved that the concentration of the fasting blood glucose (FBG) upto a level of 105 mg% does not confirm or exclude the diagnosis of gestational diabetes mellitus (GDM). In another research project we found that increased levels of triglyceride (TG) are a characteristic finding in gestational diabetes mellitus. Therefore the aim of this work was the evaluation of the diagnostic value of multiplying the FBG with the TG (mg/dl) in cases of GDM. To this end, 191 women in the 28th to 32nd week of pregnancy and without a family history of hypertriglycemia were subjected to an oral glucose tolerance test (OGTT) using 100 g of glucose after three days of carbohydrate intake (150g daily) and a 12 hour fast immediately prior to the test. Glucose by the glucose oxidase methods and triglyceride by a colorimetric method were measured in the plasma of the fasting blood with further samples for the OGTT being taken at 1, 2 and 3 hours after the administration of the glucose load. Using the diagnostic criteria of O'Sullivan and Mahan, the women were categorised into 3 groups: 1) 66 with overt GDM, 2) 84 with impaired glucose tolerance (IGT), 3) 41 with normal OGTT. For each woman the FBG X TG was calculated and a statistical relationship was determined using the student "t-test". It was found that in comparison with the women who had a normal OGTT, women with GDM had a significantly increased value when the FBG was multiplied by the TG ($t=5.77$, $p<0.0001$) and women with poor glucose control also had an increased value but to a slightly lesser extent ($t=4.7$, $p<0.0005$). Furthermore, betwen the women with GDM and those with IGT the values for FBG X TG were significantly different ($t=2.1$, $p<0.05$). Conclusion: The multiplication of the FBG by the TG in pregnant women has a high diagnostic value for gestational diabetes mellitus.

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SPECIFIC INSULIN ASSAY TO MEASURE INSULIN RESISTANCE AND GLUCOSE EFFECTIVENESS IN GESTATIONAL DIABETES MELLITUS (GDM)

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Insulin insensitivity and hyperinsulinaemia are recognised features of normal pregnancy and GDM. The proportion of biologically inactive insulins, detected by RAI, is increased in GDM. We therefore aimed to measure insulin secretion and insulin action in women with GDM using a specific insulin assay after performing an insulin modified frequent-sampling intravenous glucose tolerance test. Eight women with GDM were compared with 8 control pregnant women and 8 non-pregnant controls; body mass index was similar in the pregnant groups but lower in controls (median[IQR]) (non-preg 23.6[20.4-29.6] vs preg 28.0 (25.1-31.1] vs GDM 32.9 [26.4-35.2] kg $\cdot m^{-1}$, $p<0.05$), gestational age was similar in the pregnant groups (preg 32[30-34] v GDM 33[30-3]). A specific insulin ELISA and minimal modelling were used to assess insulin sensitivity (S_I) and glucose effectiveness (S_G). Fasting plasma glucose concentrations were similar in the three groups (non-preg 4.9 [4.7-5.2] vs, preg 4.7 [3.9-5.4], GDM 4.5 [3.9-4.7]mmol.l^{-1}). Fasting plasma insulin was raised in GDM compared to non-pregnant women (non-preg 3.4[1.1-5.6], preg 13.6 [10.1-30.4], GDM 13.5 [7.0-21.9]pmol/l), as was insulin area under the curve 2-24 minutes (non-preg 97[53-444], preg 393[237-885], GDM 394 [172-614] pmol per 24 min, $p<0.05$). Glucose tolerance (K_d) was decreased in pregnancy and further decreased in GDM (non-preg 2.4 [1.5-3.0], preg 1.8 [1.5-2.2], GDM 1.1 [0.7-1.3]%, $p<0.05$). S_I was decreased in pregnancy and further decreased in GDM (non-preg 18.3 [6.2-26.6], preg 4.2 [2.5-5.2], GDM 2.2 [1.3-3.3]$10^{-4} \cdot m^{-1}$ per $pM.l^{-1}$, $p<0.05$). S_G was decreased in GDM (non-preg 2.5 [1.7-3.0], preg 3.1 [2.1-4.6], GDM 1.4 [0.4-2.1]$10^{-2} \cdot min^{-1}$, $p<0.01$). Triglyceride concentrations were increased in pregnancy and further increased in GDM (non-preg 0.63 [0.57-0.67], preg 1.46 [1.14-2.16], GDM 2.21 [1.98-3.00]$mM.l^{-1}$,$p<0.01$). The use of a specific insulin assay with minimal modelling has demonstrated insulin resistance and reduced glucose effectiveness in women with gestational diabetes.

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PROGNOSTIC VALUE OF FASTING BLOOD GLUCOSE FOR GESTATIONAL DIABETES MELLITUS

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The aim of this study was the appraisal of the prognostic value of a fasting blood glucose (FBG) <130 mg% in the diagnosis of gestational diabetes mellitus, given that two separate measurements above this threshold is regarded as being diagnostic for the condition. A cohort of 728 pregnant women were studied aged from 20 to 42 years. Following 3 days of carbohydrate intake of 150 g/day, each woman was subjected to an OGTT using 100 g of glucose and measuring plasma glucose at baseline and 1, 2, and 3 hours after the load. According to the criteria of O'Sullivan and Mahan the results of the women were divided into the following categories: (i) 159 women (21.8%) with normal OGTT. (ii) 347 (47.7%) with impaired glucose tolerance. (iii) 222 (30.5%) with gestational diabetes mellitus. Also, the women were divided into groups according to their fasting blood glucose: (i) up to 89 mg%. (ii) 90-105 mg%. (iii) 106-130 mg%. Each group was then divided into 3 sub-groups depending on whether the OGTT was normal, impaired or abnormal. Results: There were 67 women (17.7%) with confirmed GDM in group 1, 84 women (32%) in group 2 and 71 (81.6%) in group 3. Statistical comparison was obtained using the chi-squared test after the correction of Yates. The women in group 3 had a statistically significant difference in the effect of confirmed GDM on their FBC from the women in group 2 (x^2=66.97, p<0.005) and an even greater difference from the women in group 1 (x^2=141.83, p<0.0001). Also, the results of the women in group 2 were statistically different from those of the women in group 1 (x^2=18.60, p<0.005). We conclude that: 1) A fasting blood glucose (FBG) of 106-130 mg% in a pregnant woman is indicative in a relatively high percentage of cases of gestational diabetes mellitus (GDM). 2) A FBG of 90-105% conceals the incidence of 1 in 3 cases of GDM. 3) A FBG less than 90 mg% does not exclude GDM, and for this reason, if it is to be aided by other tests for the diagnosis of impaired glucose tolerance, then an OGTT is the test of choice.

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SPECIFIC INSULIN ASSAY DEMONSTRATES INSULIN RESISTANCE IN WOMEN WHO HAVE HAD GESTATIONAL DIABETES MELLITUS

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NIDDM in women is often preceded by gestational diabetes (GDM). We investigated insulin action and secretion in healthy women with previous GDM (median months after pregnancy 17 (IQR 38)). An insulin-modified frequent-sampling intravenous glucose tolerance test was performed in 8 women with previous GDM and 9 control women with similar BMI (median 26.4 (IQR 7.9) vs 26.0 (8.1) kg $\cdot m^{-2}$) and age (35.5 (3.5) vs 40.0 (9.8) yrs). Specific insulin was measured by ELISA as opposed to the more conventional but less specific insulin RIA; minimal modelling was used to measure insulin sensitivity (Si) and glucose effectiveness (Sg). Fasting triglycerides were higher (1.1 (0.5) vs 0.7 (0.1) mM $\cdot L^{-1}$, p=0.0008) and HDL cholesterol lower (1.1 (0.3) vs 1.5 (0.3) mM $\cdot L^{-1}$, p=0.01) in previous GDM. Fasting insulin was also higher (14.0 (11.0) vs 4.8 (5.2) pM $\cdot L^{-1}$, p=0.008). The lower Si in women with previous GDM (4.2 (6.5) vs 14.7 (18.3) $10^{-4} \cdot min^{-1}$ per pM $\cdot L^{-1}$) was not statistically significant (p=0.07). Glucose tolerance (K_d) was not significantly decreased in previous GDM (1.4 (0.9) vs 2.4 (1.1) %, p=0.07). There was no difference in Sg (1.9 (1.0) vs 2.3 (1.1) $10^{-2} \cdot min^{-1}$) nor in the area under the curve for insulin. In conclusion women who have had gestational diabetes have fasting hyperinsulinaemia and suggest insulin resistance, using the specific insulin ELISA.

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GESTATIONAL DIABETES MELLITUS IN KOREA: PREDICTION OF SUBSEQUENT GLUCOSE INTOLERNACE AT EARLY POSTPARTUM

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To determine the prevalence of impaired glucose tolerance and diabetes, and identify clinical and metabolic parameters that can predict the risk of glucose intolerance at early postpartum in women with gestational diabetes mellitus (GDM), we performed 75 g oral glucose tolerance test between 6 and 8 weeks postpartum in 149 women with GDM. The recommendations from International Workshop-Conference on GDM were used for screening, diagnosing, and subclassifying GDM. National Diabetes Data Group criteria were used for classification of glucose tolerance postpartum. Among 149 women with GDM, 109 were GDM class A_1, 27 were GDM class A_2, 13 were GDM class B_1 in antepartum oral glucose tolerance test.

Thirty-eight (25.5%) of the patients had abnormal glucose tolerance in the early postpartum period; 16 (10.7%) had impaired glucose tolerance (IGT) and 22 (14.8%) had diabetes. Those who had IGT or diabetes at early postpartum had significantly higher antepartum glucose levels at 0, 1, 2, and 3 h compared with those who had normal glucose tolerance at postpartum. They had also significantly lower insulin levels at 1, 2, and 3 h than those with normal glucose tolerance at postpartum. However fasting insulin levels were not different between women with normal glucose tolerance and women with abnormal glucose tolerance at postpartum. The gestational age at diagnosis of GDM in women with diabetes postpartum was significantly lower than those with normal glucose tolerance at postpartum. Logistic regression analysis showed that abnormal glucose tolerance at early postpartum testing was independently associated with prepregnant BMI (P<.05), gestational age at diagnosis of GDM (P<.05), and 2 h glucose and 1 and 2 h insulin levels at antepartum glucose tolerance test (P<.05, P<.001, P< 5, respectively). Our results demonstrated that impaired β-cell function and obesity at diagnosis of GDM were associated with the abnormal glucose tolerance at early postpartum.

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MATERNAL AUTOIMMUNE THYROIDITIS DOES NOT ELEVATE STREPTOZOTOCIN SENSIBILITY IN OFFSPRING

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In order to evaluate the impact of autoimmune thyroiditis (AT) during gestation on streptozotocin (STZ)-reactivity in offspring the maternal (CBA/J x C57Bl/KsJ)F_1 mice with AT, gestational diabetes (GD), AT+GD, or controls and their progeny were used. AT was induced by s.c. injection of human thyroid antigen with Freund's adjuvant, the maternal mice were rendered diabetic by an i.p. injection of STZ (50 mg/kg) on the first day of pregnancy. To characterise the sensibility to STZ in offspring the i.p. GTT (2 g glucose/kg) was performed at 2 and 3 months of age (before - and a month after a single injection a nondiabetogenic dose STZ-35 mg/kg i.p.). The used dose of STZ did not induce any alterations of basal blood glucose levels (BGL) and tolerance to glucose 1 month after STZ-injection in control mothers offspring (n=7) compared to vehicle-injected ones (n=8). In offspring of diabetic mice (n=11) this dose of STZ resulted in an increase of BGL (8.3±0.2 mmol/l vs 5.7±0.1 mmol/l in controls, p<0.001) and a decrease of tolerance to glucose (integral glycemia during i.p.GTT at 0, 30, 60 and 120 min was 39.8±3.2 vs 25.6±1.6 mmol/l in controls, p<0.01). The single maternal AT did not increase offspring sensibility to STZ: integral glycemia in offspring of AT-mothers was like to controls (28.2±1.2mmol/l). Moreover, it was revealed significant decrease both basal and integral glycemia in offspring of (AT+GD) mothers (n=9) as compared with GD-mothers offspring (respectively, 7.3±0.4 mmol/l, p<0.05; 30.9±1.7 mmol/l, p<0.05). Thus the maternal AT during gestation does not enhance reactivity to STZ in offspring of mice with -and without GD.

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GLUCOSE TOLERANCE AFTER GESTATIONAL DIABETES IN SRI LANKAN WOMEN

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Gestational diabetes mellitus (GDM) has long term implications for the health of the mother. We assessed the rate of recurrence in GDM and the subsequent development of diabetes mellitus (DM) in a cohort of 220 women with GDM (group A) who were followed up for a period of 5 years and a cohort of 220 women who had no GDM in the index pregnancy (group B) matched for age and parity.

75g oral glucose tolerance tests were performed at 5 years. A second pregnancy occurred in 116 of A and 121 in B. GDM recurred in 31.8% in A and occured in 9% in B. (p=0.01). DM was diagnosed in 72 (32.7%) in A and14(6.3%) in B (p= 0.01) An increase in weight of 2.3Kg between pregnancies. maternal age 30 yrs and a higher parity were associated with a higher rate of recurrence in GDM weight gain =1.5Kg. age=30 were associated with a higher risk of developing DM.

GDM recurrs in one third of Sri Lankan Women and one third develop DM within 5 years of GDM in an index pregnancy.

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LEVELS OF IGF-1, IGFBP-1,INSULIN C-PEPTIDE IN UMBILICAL CORD SERUM: CORRELATION WITH BIRTH WEIGHT

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Our purpose was to the determine the correlation between birth weight , insulin, insulin-like growth factor-1 (IGF-1) and its binding protein (IGFBP-1) in neonates from 49 normal pregnancies (NW) and 21 complicated by pregestational (n.5) and gestational (n.16) diabetes (DW) . The two groups were similar as for age, BMI, weight gain during pregnancy and gestational age. Immediately after delivery umbilical venous blood was taken from clamped cord. Samples were collected for insulin, C-peptide, IGF-1 and IGFBP-1. DW showed higher glucose (104.4±29 vs NW 77.5±9.7 mg/dl, p<0.001) and higher HbA1 (5.58±1.6 % vs NW 4.5±0.38%, p<0.001) levels by 36-38 weeks. The neonatal weight was similar in DW (3448±485 gr.) and NW (3392±542 gr.), as the prevalence of macrosomia (LGA) (DW: 38.09% vs NW: 30.6%). Levels of insulin and C-peptide were higher in newborns from DW than in NW (28.8±60 vs 10.6±12.9 µU/ml, p<0.04;1.37±1.25 vs 0.9±0.3ng/ml, p<0.02, respectively). There were no differences in IGF-1 and IGFBP-1 concentrations in both groups. LGA newborns (n. 23) had higher levels of IGF-1 than normal size babies (79.5±22.9 vs 60.9±37.1 ng/ml, p<0.03). LGA newborns from DW had higher insulin levels (LGA-DW 19.3±8.6 vs LGA-NW 9.5±5.5 µU/ml, p< 0.005). Birth weight was positively correlated with cord serum IGF-1 (r=0.38, p>0.001), but negatively with IGFBP-1 (r=-0.28, p<0.01). Serum levels of IGFBP-1 in umbilical cords from all neonates were negatively correlated with cord serum IGF-1 (r=-0.55, p<0.0001), but no correlation was found with insulin.We conclude that cord serum IGF-1 is related to fetal growth in newborns from both NW and DW, while cord serum insulin is mainly related to diabetic macrosomia. Cord serum IGFBP-1 may be a growth inhibitor in the fetus and it seems to be insulin-independent in fetal life.

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GESTATIONAL DIABETES DETECTION PROGRAM - RAMOS MEJIA HOSPITAL

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Objective: to assess prevalence of Gestational Diabetes (GD) among pregnant women who attend the ambulatory consultation of Ramos Mejía Hospital and to relate it to the presence of Risk Factors (RF). Design and Method: from July 26, 1995 to September 9, 1996, 269 pregnant women were studied according to an algorhythm recommended by the Argentine Diabetes Society. Pregestational diabetic women were excluded from this study. Initial screening test (ST) (weeks 24-28) was considered positive if, 1 hour after an oral load of 50g glucose, glycemia was ≥140 mg/dl. Negative ST with positive history of RF was repeated at week 32. A 100g oral glucose tolerance test (OGTT) was done in all cases with positive ST. Results were considered positive when 2 or more values exceeded the following: basal 105, 1h: 190, 2hs: 165, 3hs: 145 mg/dl (O´Sullivan and Mahan). When initial ST was positive and OGTT negative, a second OGTT was done at week 32. All positive OGTT were considered as diagnostic of GD. Results: 218 women completed the full algorhythm. Out of 55 women with RF, 35 had positive ST; some patients showed 2 or more RF; these were: age ≥30 (26); body mass index (BMI) ≥26 (26); 1st. degree relatives with diabetes (25); personal history: macrosomic faetus (9), perinatal mortality (3), GD (2), gestosis (11). GD was diagnosed in 5 of 218 (2.29 %). Conclusion: in our Country it is difficult to perform an OGTT in all pregnant women; this is a major cause of motivation for doing the ST, which we consider an efficient method in programs of collective detection of GD applied to the general population of pregnant women, since searching for GD only in women with RF considerably decreases its efficacy. The ST is simple, cheap and useful in relation to a population study. Prevalence found in our initial program agrees with data published in our Country and abroad, thus encouraging us to proceed with this Program.

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CORD BLOOD C-PEPTIDE LEVEL IN NEWBORNS OF DIABETIC MOTHERS.

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Purpose of our study was to determine the correlation between mothers' glycemia and newborns weight and cord blood C-peptide level. 116 cord serum samples, taken from 80 patients with GDM and 36 with PGDM, were analyzed for C-peptide level and correlated with mothers' glycemia (MBG, HbA_{1C}, FA), pregnancy trimester at which the intensive care was started and newborns weight.

In the two analyzed subgroups (GDM and PGDM) the results of mother's metabolic control pertaining to all trimesters were as follows: GDM group-HbA_{1C} (normal range < 6,3%) in I trimester-5,08%, II- 5,5%, III-6,1%, FA (normal range < 2,7 mmol/l) in I trimester-2,8 mmol/l, II- 2,2 mmol/l, III 192 mmol/l. In the PGDM group HbA_{1C} concentration was: I trim. - 7,1%, II- 6,6%, III-6,9%, FA -in I trim.-2,5 mmol/l, II-2,5 mmol/l, III- 2,3 mmol/l. Mean mothers' diurnal glycemia in III trimester reached 86,5 mg%in the GDM group, but in the PGDM as much as 103,9 mg%. All intergroup differences were statistically significant. Newborns' body weight in the GDM group amounted to 3300g and in PGDM to 3380g (difference n.s.), but the newborns' glycemia in GDM-43 mg% and PGDM-34 mg% respectively differed significantly. C-peptide levels ranged from 0,79±o,54ng/ml in newborns with IUGR, over 1,06±1,38ng/ml in the AGA newborns, up to 1,41±1,87 in the LGA newborns. In newborns of the GDM group presenting with IUGR, c-peptide levels were 0,6±0,26 ng/ml, in those from AGA group 0,81±0,47ng/ml and in those from the LGA group 0,59±0,22 ng/ml. In the PGDM group c-peptide in IUGR newborns amounted to 1,09±0,8ng/ml, in those of the AGA group to 1,95±2,67ng/ml, and in those belonging to the LGA group to 1,87±2,27ng/ml. The analysis of results pertaining to C-peptide in cord blood in dependence of the onset and or duration of the mothers' treatment has shown a negative correlation between these parameters.

C-peptide in cord blood is a parameter correlating with diabetic mother's metabolic control and fetal birth weight but is mainly related to fetal IUGR.

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Reevaluation of Diabetes Mellitus or glucose tolerance in Women after gestacional diabetes.

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A study with (39) women who had diabetes mellitus (DM) during pregnancy, who were attended at a State Hospital in São Paulo City from 1983 to 1993, and reevaluated on average at a seven year time interval, allowed the conclusion that 53,8 of those who developed DM or impaired glucose tolerance (IGT) were over 35 years old, overweight or obese, and had a higher birthrate than the women from the control group, and higher number of caesarean operations, only the OGTT-2h and not the glicemia on fasting allowed the identification of the alterations in both instances; the family history of diabetes did not constitute a significant risk factor for the later development of diabetes did not constitute a significant risk factor for the later development of alterations of glucose tolerance (AGT), unlike insuline therapy during pregnancy; the values of the anti-insulin antibodies did not exceed the limits of normality, but were greater in the group with diabetes in repeated pregnancies with current AGT in relation to those with a single pregnancy and AGT. The HbA1 values were altered in all women with AGT on re-evaluation, whilst the insulin dosage did not differ from that of the control group. The recurrency of diabetes during pregnancy did not appear as a risk factor int the prevalence of the DM or IGT during the average seven year time interval.

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AMNIOTIC FLUID GLUCOSE AT 14-16 WEEKS PREDICTS SUBSEQUENT GESTATIONAL DIABETES

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Glucose, insulin and C-peptide have often been measured in amniotic fluid (AF) during late gestation, but little is known about their concentrations during early pregnancy. We examined the predictive value of amniotic fluid glucose (AFG) and maternal serum glucose (MSG) at 14-16 weeks gestation for subsequent gestational diabetes (NDDG criteria) and macrosomia in unselected 336 gravidas (mean age 34.5±4.7 years) with chromosomopathy risk. In a randomized subgroup of 73 cases amniotic fluid insulin (AFI) and maternal serum insulin (MSI) were also analyzed. They were classified as normal glucose tolerance (group-I, n=148), 1 abnormal value (g-II, n=120) and >1 abnormal value (g-III, n=68). Birth weight (BW) was expressed as % of the 50th percentile adjusted by sex and gestational age. Large for age (LGA) was defined when BW above 90th percentile for sex and gestational age. There was a significant correlation between AFG and glucemia post 50 g glucose test (r=0.32, p=0.0001) and between AFG and MSG (r=0.45, p=0,0001). The correlation between AFI and MSG and MSI was also signifcant (r=0.46, p=0.0001 and r=0.42, p=0.001 respectively), but not with AFG (r=0.19). The AFG and MSG values (95 Pct Con) between groups were progresively higher (AFG: g-I:44.6 to 47.6, g-II:47.2 to 50.7 and g-III:53.7 to 62.7 mg/dl, p=0.00001; MSG: g-I:74 to 80, g-II:79 to 88 and g-III:83 to 101 mg/dl, p=0.0004). BW was similar between groups (101.1%, 101,1% and 101.2%). LGA were detected in 41 cases (12%). No significant differences in AFG, AFI, MSG and MSI between LGA neonates and normal BW neonates were found. In summary, gestational diabetes is associated with increased AFG at 14-16 weeks, suggesting that altered glucose metabolism is present in early fetal period.

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PREVALENCE OF RISK FACTORS FOR GESTATIONAL DIABETES IN ZAIREAN WOMEN. EK. Sinamuli, N.F. Tandu-Umba and K. Kandjingu, University of Kinshasa, Kinshasa, Zaïre.

Gestational Diabetes Mellitus is still a worrying among pregnant women in Zaïre. To determine the prevalence of diabetic features in the high-risk population, a study has been realized from January 1984 to December 1993 at the Department of Obstetrics and Gynecology of University Clinics of Kinshasa. 58 gestational diabetic women have been compared with 32 insulino-dependent diabetes pregnant women and 84 normo-glycaemic pregnant ones with diabetic risk factors. Family and obstetrical stories and perinatal complications have been checked out. Statistical analysis (chi- square and Pearson tests) shows very significant differences of predictive values of family obesity, high blood pressure, perinatal mortality(p<0,005 respectively) and macrosomia (p<0,025) during previous pregnancies on the one hand and hydramnios, glycosuria, excessive weight gain and fasting glucose level (p< 0,005 respectively) on the other hand.

This finding suggests the possibility of intervention in high-risk groups.

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INSULIN-LIKE GROWTH FACTOR-1 IN AMNIOTIC FLUID OF WOMEN WITH AND WITHOUT GESTATIONAL DIABETES.

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Insulin-like growth factor-1 (IGF-1) is known as a potent mediator and regulator of cell growth and development, whereas diabetes and gestational diabetes are characterized by an increased incidence of intrauterine growth alterations. Thus the aim of this study was to examine IGF-1 in the amniotic fluid (reflecting fetal situation) of 20 women with gestational diabetes (GD) and compare them with 96 healthy control women (no pathological serological or cytogenetical screening results) representing different gestational ages (subgroups: 15/16.(C1); 17/18. (C2); 19/20. (C3); 21-25.wk (C4)). Both groups were matched in BMI before pregnancy (24.3±0.8(GD) vs. 23.0±0.4(C) kg/m^2; mean ± SE; p=0.36), parity (1,1±0.2 vs. 1,0±0.1 (C)), age (36.3±0.6 vs. 34.0±0.4 (C); p=0.06) and delivery on term (38-42 wk). IGF-1 was determined by a RIA method (Fa. Nichols, Cal., USA) after acid-ethanol-extraction. Results: We found an increase in amniotic IGF-1-content depending on gestational age (83.6(C1); 121.2(C2); 291.2 (C3); 355.4(C4) ng/mL; geometric means; p<0.0001), confirmed by regression analysis which showed a log-log-linear relationship (p<0.0001; r=0.61). Women with GDM showed also this time-dependent increase, but on a much lower level (30.5(GD1); 77.6(GD2) ng/mL; log-log-regression p=0.0082; GD1 vs. C1 p=0.019). Further analysis of obstetrical data showed similar mean values for birthweight (3690(GD) vs. 3411 g(C); p=0.25) and height of the infants with a higher incidence for SGA- and LGA-babies in the GD-group, whereas regression analysis could not show a relationship between IGF-1 content and obstetric measurements. Thus the impact of decreased IGF-1 in GDM remains unclear and further investigations are necessary to determine the role of IGF-1 in intrauterine growth alteration.

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PREVALENCE OF GESTATIONAL DIABETES MELLITUS: IMPACT ON FETAL MACROSSOMIA IN A BRAZILIAN POPULATION.

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Gestational Diabetes Mellitus (GDM) is defined as carbohidrate intolerance of variable severity first diagnosed during pregnancy. There is no agreement to the most appropriate way to diagnose GDM. This study (Brazilian Gestational Diabetes Study Group - BGDSG - Rio de Janeiro) considered the WHO criteria (oral glucose tolerance test - OGTT - 2h, 75g) for both GDM and gestational impaired glucose tolerance (GIGT). The purpose of this study was to evaluate the prevalence of GDM and GIGT, the influence of maternal age, obesity and alcohol intake to the severity of the disease, and the consequence of GDM and GIGT on birth weigth. The BGDSG - Rio de Janeiro enrolled a sample of 508 pregnant women (December 1992 to August 1994), aged 20 years or older, followed at the prenatal clinic amongst 1314 women. The selected women were tested with a 75g OGTT between the 24 th and 28 th gestational weeks. Prepregnancy body mass index (BMI) as ≥ 25 Kg/m² was used to define obesity. 450 deliveries during this study were analyzed for perinatal morbity, specially macrossomia. Fetal macrossomia was considered as a birth weight ≥ 4000 g. Significant differences were detected with Mann Whitney test, t-test, and Fisher test. Prevalence of gestational glucose abnormalities was 12.0% (CI 95%= 9.4%- 15,2%) (GDM = 0.4% and GIGT=11.6%) maternal age strongly influenced prevalence (12.5% to 31.8%, for 30-34 yrs and ≥ 40 yrs). Alcohol intake and pre pregnancy obesity also influenced prevalence significantly($p < 0.005$). All 450 pregancies delivered 24 macrossomic newborns. Prevalence of macrossomia was 5.3% (CI 95%= 3.5% - 7.9%). GIGT influenced the prevalence of macrossomia (11.4%) when compared with control group (4.3%) $p< 0.005$.These data support the importance of diagnose GDM and GIGT. These findings suggest that, given contemporary antenatal medical care, GDM and GIGT do not adversely affect their outcomes.

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Is There a Genetic Link between MODY and Gestational Diabetes Mellitus ?.

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MODY is defined as adult onset diabetes of the young ,associated with autosomal dominance inheritance and detected before age 25 years; Gestational Diabetes Mellitus (GDM) is usually detected in pregnant women older than 25years .MODY and GDM are classified as subtypes of NIDDM.

While screening patients for gestational diabetes at The Antenatal Clinic,University Hospital of the West Indies it was noticed that some patients who were diagnosed with gestational diabetes mellitus had unexplained hyperglycemia but not diabetes in previous pregnancies. The hyperglycemia was detected before age 25 years and treated by diet alone. All had family history of diabetes consecutive in three generations. Most of these patients fulfilled the criteria for MODY.

The patients with previous hyperglycemia later diagnosed as gestational diabetes showed varied heterogeneity; some had high fasting glucose and normal postprandial values ,others had both high fasting and high postprandial values.The insulin and c-peptide values ranged from subnormal to supranormal.Three patients had hypoinsulinemia with a 1 hour mean insulin value of 20±9 uUml which when compared with normal pregnant women (140±24 uU/ml)was statistically significant ($p< 0.0005$). Four patients showed marked hyperinsulinemia with mean insulin value of 325uU/ml ($p<0.002$). Regression analysis confirmed the heterogeneity of the metabolic response.

The genetic heterogeneity of MODY could possibly explain the metabolic heterogeneity of these patients with GDM as they fit the criteria for MODY.It has been shown that polymorphisms in the glucokinase(GCK) gene on chromosome 7 are tightly linked with hyperglycemia in a panel of French MODY family. MODY Patients with mutation in the GCK gene demonstrated a milder insulin secretory defect than MODY associated with polymorphic DNA markers on chromosome 20 which causes an inability to increase insulin secretion as the plasma glucose value rises above 7-8mmol/l.

Determining the genetic makeup of these special patients could help us in understanding the possible relationship between these two subtypes of NIDDM .

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RISK FACTORS ASSOCIATED WITH THE USE OF ORAL CONTRACEPTION AND VASCULAR EVENTS IN WOMEN WITH IDDM.

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Oral contraception (OC) ("pill") is rarely recommended for type I diabetic women (D). In D with microvascular complications, smoking and poor metabolic control OC are even considered to be contraindicated because in such women they are presumed to be associated with an excess risk of vascular events. However, so far, this assumption is not based upon valid data. The aim of our study was to assess for type I diabetic women actual and previous use of contraceptive methods, risk factors and their association with vascular events. 1028 consecutive diabetic women (age 16-46 yrs) attending a structured treatment programme for intensified insulin therapy from 1989-92 were mailed a multiple choice questionaire in 1993. 409 non-diabetic women served as a control group. 808 D (79%) answered the questionaire (age 31.5(7.2)yrs., duration of diabetes 14.4(8.2) yrs). Of all women who used any contraception in either group OC were used by 44% in the diabetic group and by 57 % in the control group. Of 202 diabetic OC-using women (age 27(5)yrs) 65% had at least one "contraindication" to OC-use (proteinuria, hypertension, retinopathy, smoking, poor metabolic control). During a total of 3945 diabetic women-years of OC-use (in 301 previous and 202 actual users) there had been 5 cases of deep vein thrombosis, i.e. a risk comparable to non-diabetic OC-users. 12 diabetic women had a history of major vascular events (7 myocardial infarctions, 5 cerebrovascular events), 11 of which had occured without actual OC-use. One event (thromboembolic stroke) had occured during actual OC-use in a 21 yr old smoker with nephropathy. In Germany, OC are a popular method of contraception in type I diabetic women paying little attention to traditional "contraindications" other than age. The data suggest that OC-use in young diabetic women without nephropathy is not associated with an excess rate of vascular events.

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GESTATIONAL DIABETES MELLITUS: AN IMMUNOLOGICAL AND METABOLIC FOLLOW UP FOUR YEARS AFTER PREGNANCY.

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Many studies have been performed to identify the presence of serological markers of pancreatic autoimmunity in gestational diabetes mellitus (GDM), but the results obtained are quite variable. We want to verify in GDM the presence of serological markers of pancreatic autoimmunity and HLA fenotype in an italian population. Islet cell antibodies (ICA), complement fixing-ICA (CF-ICA), insulin autoantibodies (IAA), other organ-and non organ specific autoantibodies, circulating lymphocyte subpopulations and HLA fenotype were evaluated in 68 women with GDM at 37th week of gestation and compared to 38 matched controls. ICA-IgG were found in 2/68 (2.9%) and IAA in 1/68 (1.5%) GDM patients. Other organ and non organ specific autoantibodies, lymphocyte subpopulations and HLA fenotype were not significantly different when compared to matched controls. 38 patients (the 3 with pancreatic autoimmunity and 35 negative) were followed up for 4 yrs after delivery by re-evaluating their immunological status and OGTT. During follow up, 8/38 patients (21%) became diabetics: 6/8 (75%) developed diabetes 1 year after pregnancy, 1/8 (12.5%) 2 years and 1/8 (12.5%) 3 years after pregnancy. Four of them required insulin therapy (basal C-peptide 0.8-1ng/ml), 1 was treated with oral hypoglycemic agents and 3 were on diet (basal C-peptide 2.3-3ng/ml); all patients were in fair good metabolic control. Furthermore 6/38 (16%) patients showed an impaired glucose tolerance (IGT) and 24/38 (63%) a normal glucose tolerance. During follow up all patients maintained unchanged their serological reactivity, but one of the two ICA-positive patients became diabetic (50%). In the 14 patients who developed IGT or diabetes the mean values of activated T-lymphocytes (HLA-Dr+CD3+) during pregnancy were significantly higher than those of pregnant control pregnant women (8.5+3.3 vs 2.4+0.5%; $p<0.05$). Moreover four years after delivery no significant differences were found in the number of activated T-lymphocytes. So GDM lacks of both immunological and genetic markers of type 1 diabetes, but progression to IDDM is more frequent in patients with serological signs of pancreatic autoimmunity. The high frequency of diabetes one year after pregnancy strongly support importance the importance of a "strict" post partum follow up in GDM patients.

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THE INCIDENCE OF GESTATIONAL DIABETES USING TWO DIFFERENT CRITERIA

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Until now there is no universally accepted criteria for the diagnosis of gestational diabetes.The mostly used screening procedures and criteria are the NDDG and the WHO. When we started our first screening we used the NDDG criteria and later on we changed to more simple criteria. We report the results of these two different methods.

Glucose challenged test with 50 gram glucose were performed in both methods.Those with challenged test positive were followed by OGTT. The NDDG used 3-hour OGTT and our criteria only 2-hour OGTT. The NDDG criteria needs two abnormal values for the diagnosis of gestational diabetes. In our new criteria , as a normal value for fasting < 100 mg/dl, 1-hour < 170 mg/dl and 2-hour < 140 mg/dl. Gestational diabetes is diagnosed if there is one abnormal value.

2074 pregnant women were screened using NDDG procedure, 42 women were positive for gestational diabetes or an incidence of 2,0%. Using our new criteria, from 1041 pregnant women , 27 were gestational diabetes or an incidence of 2,6%.. In the NDDG group cessarian section was 17,7%, and perinatal mortality 7,1%. From our new criteria, cessarian section was 22,2% and perinatal mortality 7,4%.

In conclusions, the incidence of gestational diabetes as well as perinatal mortality were not different between these two different methods of screening for the diagnosis of gestational diabetes.

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HLA-DR/DQ-HAPLOTYPES AND AUTOANTIBODIES IN PORTUGUESE GESTATIONAL DIABETIC WOMEN

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Aim: to assess HLA-DR/DQ-haplotypes and autoantibodies (AAs) ICA, IAA, GAD 65 and correlate them in GDM women. Material and Methods: a Caucasian population of 111 GDM, 240 healthy controls (C) for HLA-analysis and 54 for AAs and 32 IDDM, were studied. HLA-analysis was performed by PCR-Oligotyping, ICA by indirect immunofluorescence, IAA by RIA and GAD by IRMA-RSR. Results: GDM is strongly associated with DR8 *vs* C *vs* IDDM ($p<0.05$) and DR7 with C vs IDDM ($p<0.02$). A positive association of DQA1*0401-DQB1*0402 and DQA1*0501-DQB1*0201, was found in GDM *vs* C vs IDDM ($p<0.05$) and of DQA1*0101-DQB1*0501, DQA1*0201-DQB1*0201 and DQA1*0501-DQB1*0301 in GDM and C vs IDDM ($p<0.05$), being the DQA1*0300-DQB1*0302 the most common in IDDM *vs* GDM and C ($p<0.05$). The haplotype DR8-DQA1*0401-DQB1*0402 (DR8/DQ4) was present and positively associated only in GDM vs C and IDDM ($p<0.05$), DR11-DQA1*0501-DQB1*0301 (DR11-DQ7) was the more prevalent in GDM (20%) and C (28%) *vs* IDDM (1.8%) ($p<0.05$) and DR14-DQA1*0101-DQB1*0503 (DR14-DQ5) in C vs GDM and IDDM ($p<0.05$). DR4-DQA1*0300-DQB*0302 (DR4-DQ8) was the most frequent in IDDM (40%) vs GDM (14%) and C (15%) ($p<0.05$). Heterozigotia DR4-DQ8/DR17-DQ2 was found in GDM and C only in 4.5% and 3.9% *vs* 36% in IDDM ($p<0.02$). Susceptibility to IDDM determined by non-Asp57 and Arg52 in DQ molecules was weakly present in GDM and C and strongly associated with IDDM. The frequency of autoantibodies positive (AAs+) in GDM was 4.5 % and in C 1.8%: ICA 3.6 and 0%, GAD 1.8% and 1.8% and IAA 0/0%, respectively. One GDM with ICA and GAD+ was DR4-DQ8/DR9-DQ9. The others ICA+ were DR16-DQ5/DR17-DQ2, DR17-DQ2/DR4-DQ8 and DR7-DQ9/DR12-DQ7. The other GAD+ was DR16-DQ5/DR4-DQ8. At least one susceptibility factor was present in the phenotype Conclusion: GDM was similar to healthy controls in terms of immunogenetic markers except for the haplotype DR8/DQ4 that was significantly increased only in GDM. The susceptibility genes and haplotypes as well as AAs+ for IDDM were found in GDM in a small percentage of cases, what makes the disease as a whole heterogenic but not immunogenetic. In spite of the results these studies are very important to select the GDM women more prone to insulin needs during pregnancy and later IDDM.

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RISK FACTORS FOR GESTATIONAL GLUCOSE INTOLERANCE. THE BRAZILIAN STUDY OF GESTATIONAL DIABETES (EBDG).

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Risk factors for gestational glucose intolerance (GGI), as defined by WHO criteria, have been little investigated using multivariable techniques. To do so, we studied consecutive pregnant women, age 20 years or more, without history of diabetes mellitus outside pregnancy, in selected clinics of 6 major Brazilian cities, analyzing here the 4909 with complete data. Interview and anthropometric data were obtained and a standardized 75g oral glucose tolerance test was uniformly applied between gestational weeks 24 and 28. GGI was defined as a 2h plasma glucose $\geq$ 7.8 mmol/L, and obesity as the sum of 4 skinfold thicknesses. Odds ratios (OR) were obtained through multiple logistic regression. Independent positive associations ($p<0.05$) were found for increased age (4th vs. 1st quartile OR=3.3), increased skinfold thickness (4th vs. 1st quartile OR=2.7), a positive history of gestational diabetes (OR=2.9), and a positive family history of diabetes (OR=1.6). Independent protective associations were found for those with 4 or more previous pregnancies (compared to none: OR=0.61), for those with black skin color (OR=0.50), and for those with greater maternal height (4th vs. 1st quartile OR=0.66). In conclusion, greater maternal height, black skin color and greater gravidity were important independent factors associated with lesser prevalences of GGI in Brazilian women. The traditional risk factors of age, obesity, a family history of diabetes, and a personal history of previous gestational diabetes, were associated with increased GGI prevalences. This pattern of associations is similar to that seen for NIDDM in Brazil, highlighting the similar nature of these two conditions.

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MORPHOMETRIC EVALUATION OF THE ISLETS OF LANGERHANS IN AN EXPERIMENTAL MODEL OF GESTATIONAL DIABETES.

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Diabetes during pregnancy induces alterations in the fetus with persisting consequences for the glucose tolerance in the offspring, including gestational diabetes. The aim of the present study is to quantitate the mass and composition of the islets of Langerhans in adult offspring of streptozotocin-diabetic rats in order to look for a morphological origin for these alterations. Pancreatic biopsies were collected from non-pregnant and term-pregnant youngsters of mildly (MD) and severely (SD) diabetic mothers, stained immunologically for insulin, glucagon, somatostatin and pancreatic polypeptide, and examined morphometrically with an automatic image-analyzer. Total islet mass and islet size distribution were evaluated and related to islet neogenesis and replication of differentiated cells. The contribution of the different endocrine celltypes was measured and related to the metabolic condition of the animals. In the MD-offspring the amount of islet and B-cell mass and the islet size distribution are normal, and can not explain the deficiency in stimulated insulin output, which must be related to alterations in the islet composition: more glucagon cells (18% versus 13% in controls, $P = 0.004$) and less pancreatic polypeptide cells (2,1% versus 4,6%, $P = 0.0002$). In the SD-offspring the excessive insulin output is associated with an increase in total islet and B-cell mass (438.644 μ^2 versus 267.150 μ^2 for the islets, $P = 0.006$; 367.082 μ^2 versus 142.835 μ^2 for the B-cells, $P = 0.004$), mainly due to islet neogenesis (preponderance of small islets). During normal pregnancy islet and B-cell mass double theire size (550.230 μ^2 versus 267.150 μ^2 for the islets, $P = 0.001$; 301.060 μ^2 versus 142.835 μ^2 for the B-cells, $P = 0.001$), by a combination of islet neogenesis and replication of differentiated cells. Also the glucagon and somatostation-positive tissues increase in the same proportion, while the PP-cell mass does not change. In both MD- and SD-youngsters, the occurrence of gestational diabetes can not be attributed to a deficiency of these adaptations since islet size and composition are normal, but must be related to the factors that induce the increased B-cell function of pregnancy.

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COMPARISON OF SOMATIC DEVELOPMENT IN THE FIRST YEAR OF LIFE IN THE CHILDREN OF MOTHERS WITH EITHER GESTIATIONAL (GDM) OR INSULIN DEPENDENT (IDDM) DIABETES MELLITUS

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SUMMARY

The somatic development was assesed in the first year of life in the children of (GDM) and (IDDM) mothers. For each group , 26 children were assesed. All the children were full-term infants and norme suffered from illnesses with effects on somatic development. The social and economic conditions did not differ between groups. The BMI, assesed before pregnancy was higher for GDM mothers. The BMI for fathers did not differ significantly. The following somatic values were measured in the 1st, 6th and 12th month of the child's life: body mass, length, head circumference and chest circumference. For both groups the harmonic development and state of nutrition were assesed. 21 children (40.3%) were born with a birth mass above 90 centile (c) and this was disproportionately weighted towards children of GDM mothers. In the 12th month, 6 children (11.5%) were obesqe and they were all LGA children. The body lenght above 90c was more often observed for children of GDM mothers. The head circumference below 10c was statistically more often observed for children of IDDM mothers.

Basing on this study the fillowing conclusions were drawn:

1. For both GDM and IDDM children somatic development for the majority of children below the 12th month was within the broad range of the norme medical standard.
2. All the children showed a harmonic development.
3. In the 12th month 11.5% of the children were obesqe and the obesity was more often present in children born with macrosomatic features ($p<0.05$).
4. The type of mother's diabetes seems to have a significant influence on somatic development.

CONCLUSIONS

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Pregnancy

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PREGNANCY OUTCOMES OF WOMEN WITH PREGESTATIONAL DIABETES IN NORWAY AND NORTHRHINE (GERMANY) AND ORGANISATION OF CARE

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To eliminate excess perinatal complications of mothers with pregestational diabetes (PD) is feasible and a WHO target for diabetes care (St. Vincent declaration). The aim of our study was to compare the outcome of these pregnancies to nondiabetic (ND) mothers in two European countries and to identify factors in the health care systems that may explain differences. Data of the Perinatatalerhebung Northrhine (NRPE) and the Medical Birth Registry of Norway (MBRN) from 1987-1992 were analysed. NRPE and MBRN are quality assurance programmes of pre- and perinatal care covering 93-100% of all births (annual birthrate ≈100.000 (Northrhine) and ≈59.000 (Norway)). Both countries have similar, highly structured, free-of-charge standard prenatal care systems, but different systems of diabetes care (in Norway centralized (14 centres) and in Germany mostly with local physicians). PD was present in 0.41 (0.35-0.57) % (mean (range)) of all births in Norway, and in 0.39 (0.35-0.45) % in Northrhine. In Northrhine, perinatal mortality rate in births of PD mothers was twice as high as in Norway (2.4 vs. 1.2 %); in the ND it was only slightly higher (0.7 vs. 0.6 %) . The higher perinatal mortality in PD in Northrhine was mainly due to stillbirths. The rates of prematurity, macrosomia and caesarian section in Norway and Northrhine were similar (18 vs. 19 %, 25 vs 24 % and 45 vs 40 %) and 2-3 times higher than the respective rates in the ND. Only 40 % of PD in Northrhine but 90 % of those in Norway attended specialized diabetes and pregnancy centres. In Germany and Norway 1 of 200 to 250 births is complicated by PD. In Norway, the excess perinatal mortality has nearly been eliminated, probably due to centralisation of diabetes care and reaching out to noncompliant patients. Reduction of other perinatal complications will require further sophistication of specialized care.

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C-PEPTIDE AND INSULIN LEVEL IN WOMEN IN LABOUR AND UMBILICAL VEIN DEPENDING ON BIRTH WEIGHT AND LABOR PARITY.

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To study insulin secretion in non-diabetic women in labour and their infants in relation to the body weight of the newborns, fasting IRI and C- peptide levels were measured in 415 healthy non - diabetic women in labour aged 18-36 years (161-first delivary, 254 - repeated delivery; physiologic gestation 38-40 weeks). Umbilical vein blood of 192 normal weight (NW) neonates and 223 overweight newborns were tested immediately after birth. Body weight of 4000g and more we considered excessive. Infants of primapara were overweight in 30% of cases, while in repeated delivery this number increased to 66 %. Not depending on the delivery parity women with large newborns had apparently more excessive weight gain during the given pregnancy ($p<0.05$). IRI blood level in women in birth never exceeded that of the nonpregnant group (0.75 ± 0.04 ng/ml). C - peptide level, C- peptide/IRI ratio in primapara with NW infants were 2 - fold higher comparing to controls and to women with large newborns (4.4 ± 3.2 ng/ml;5.5+0.18; respectively $p<0.05$) IRI and C-peptide levels and IRI/C- peptide ratio were apparently higher in the umbilical blood of the large infants than in NW ones (0.47 ± 0.08 ng/mg; 5.3 ± 0.09ng/ml, respectively). Thus, pregnant women, especially those with repeated parity, showing considerable body gain during given pregnancy and lack of the fasting compensatory insulin hypersecretion, comprise the risk group of gestational DM, and in their healthy newborns together with excessive body weight, insulin hypersecretion, as in macrosomic infants of diabetic mothers, is observed.

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THE OUTCOME OF PREGNANCY IN A GROUP OF DIABETIC PATIENTS IN A RURAL HOSPITAL IN JAMAICA, 1993-96
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The aim of the study was to determine the outcome of pregnancy in a group of 60 diabetic patients admitted to the Obstetric Ward at the Cornwall Regional Hospital, 1993 - 1996. Patients were chosen randomly and followed through to delivery. They were matched with non-diabetic pregnant patients admitted to the Obstetric Ward with an acute episode of urinary tract infection. Controls were matched for race, same clinic attendance and time of admission. The mean age and gravidity in the diabetic group was 32.20 yrs. and 4.25 respectively while that in the control group was 23.70 yrs. and 3.02. There was a significant difference ($p < 0.01$) between the mean birth weights for the diabetic population (mean 3.85 kg ± 0.64 SD) and the control group (mean 2.92 kg ± 0.37 SD). In the diabetic group, 46.7% had lower segment caesarean section. Indications included macrosomia (57.14%) and previous caesarean section (14.29%). In the control group, 15% had lower segment caesarean section. Indications included fetal distress (44.44%) and previous caesarean section (33.33%). In the diabetic group, post partum haemorrhage was the only maternal complication (13.3%). Seventy-eight per cent of infants of diabetic mothers had complications; macrosomia 42.60%, hypoglycemia 27.66% and intrauterine death 14.89%.

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INSULIN RESPONSE TO GLUCOSE IS SELECTIVELY MAGNIFIED BY PARITY IN WOMEN WITH NORMAL GLUCOSE TOLERANCE.
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The importance of parity in modifying both glucose tolerance and insulin secretion during the pregnancy is a matter of debate. This longitudinal study was aimed to verify the hypothesis that parity leads to a progressive modification in glucose tolerance and/or insulin secretion or sensitivity in non diabetic women. In a cohort of 45 mothers without gestational diabetes, glucose tolerance, as well as basal and post-load plasma insulin and C peptide were tested by means after a 100-g OGTT in two consecutive pregnancies between the 24th and the 28th gestational week. On the second pregnancy (40±17(SD) months on average from the previous) the women had higher pre-pregnancy (61±11 *vs* 59±8 Kg, mean±SD) and final body weight (71±10 *vs* 69±9 Kg, p=.06 for both), and delivered larger babies (3,486±473 *vs* 3,242±506 g; p=.002). As compared to the previous pregnancy, there was a significant increase in basal plasma C peptide (1.6±0.7 *vs* 1.3±0.4 ng/ml; p=.004) and in the area under curve for insulin : AUCI, (52±13 *vs* 48±8 nmol l^{-1}2h; p=.04), but no significant change in the area under curve for glucose AUCG (0.78±0.18 *vs* 0.77±0.16 mol l^{-1} 2h). The percent increase in AUCI was not related to either maternal or neonatal weight, and was independent from difference in age, AUCG, increment in maternal weight, or parity. This finding is compatible with the concept that each additional pregnancy in mothers with normal glucose tolerance amplifies the insulin response after an oral glucose load by about the 8%, likewise inducing an additive stress to beta-cells. Furthermore, the parity-induced increment in neonatal weight appears unrelated to both modifications in glucose tolerance and to insulin response to a standardised oral glucose load.

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DIABETIC PREGNANCY: PREPREGNANCY CARE AND PREGNANCY OUTCOME
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In diabetic pregnancy, preconceptional clinics (PC) have shown a great impact in reducing the rate of congenital malformations (CM). Our aim is to summarize our experience in PC since its initiation in 1986. Up to 195 pregnancies of women with pregestational diabetes have been attended with 74 (37.9%) having enrolled in PC. Attenders had a longer diabetes duration (11.2±7.1 vs 8.6±6.6 years, $p<0.05$) and non-attenders had a higher first pregnancy HbA1c (in SD around the mean) (4.0±3.2 vs 2.7±2.6, $p<0.01$). The final HbA1c was in the normal range in both groups. Pregnancy outcome (abortion 14.9%, caesarean section 59.7%, preterm delivery 24%, hypertension 21.8%, perinatal mortality 1.8%, 1 min Apgar <7 15.7%, birthweight >P90 27.2%, obstetric trauma 5.6%, hypoglycemia 23.5%, hypocalcemia 0.6%, respiratory distress 11.1%, neonatal jaundice 19.8%, polycythemia 9.0%) was similar in both groups. The rate of CM was 8.9% in non-attenders vs 3.3% in attenders (OR 2.89, 95% CI 0.60-13.8). We conclude that: 1) A similarly fair glycemic control at the end of pregnancy allows for a similar outcome in terms of diabetic fetopathy 2) Even in women in moderate glycemic control at the beginning of pregnancy, PC seems to lower the rate of CM

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ALIMENTATION AND OBESITY DURING PREGNANCY AND MATERNAL INSULIN SECRETION
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The alimentation during pregnancy may be very important for the development of maternal metabolic abnormalities, including impaired secretion of insulin. The role of insulin in fetal development remains uncetain. The aim of this study is to evaluate the importance of obesity in development of insulin resistance and hyperinsulinemia during pregnancy. The study included 51 healthy pregnant women, mean age 27/5.2yr (22-37yr). Depending on increase in body weight (+ w) in pregnancy, all examined women were divided in three groups: A - n=18, +w<15 kg; B - n=19; +w 15-25kg; C - n=14, +w>25kg. Insulin levels (by RIA) in the sera of pregnant women and newborns (umbilical venous) were determined post partum. For statistical analysis Mann-Whitney, Spearman's rho i ANOVA tests were used. We found a significantly higher insulin levels in pregnant women in B i C groups than in group A (48.02/27.65 and 51.59/18.7 vs 36.87/15.27;p<0.05). Insulin levels were similar in all newborns (groups: A - 16.59/7.52; B - 15.05/6.92; C-17.00/8.57; p>0.05), but were not correlated with maternal insulin concentration. Birth weight (F=4.56;p<0.05) and lenght(F=4.95;p<0.05) were significantly increased in newborns of mothers from group C. We found the relationship between fetal insulin levels and: a) birth weight(R=0.73;p<0.05) and birth lenght(R=0.65;p<0.05) in these babies. Also, neonates with birth weight greater than 4000g were significantly higher in group C(50%) than in A(21%) and B(21%) groups of pregnant women. We conclude that obesity inrease insulin resistance and secretion in pregnant women.

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OUTCOME OF PREGNANCY WITH DIABETES IN A DEVELOPING COUNTRY. C. Munichoodappa and Ashok Gurudas The Bangalore Hospital, Bangalore, India.

We reviewed 253 patients having impaired gestational glucose tolerance (IGGT) 18, gestational diabetes mellitus (GDM) 112, pregestational diabetes (PGDM) - White Class A 66, Class B 46 and Class C (Insulin dependent) 11 having 270 pregnancy and 273 fetuses (3 had twins). Age ranged from 19 to 38 years. None had microangiopathy or macrovascular disease. Two with IGGT were on diet alone. One GDM chose to continue sulphoylurea (SU) throughout gestation. The rest were continued or switched over to mostly purified insulin. Glycemic control was excellent to good in 197 pregnancies (73%) and was unsatisfactory in 73 (27%) mainly PGDM, 250 fetuses survived and 23 (8.5%)were wasted. The latter due to 11 abortion, 3 congenital heart disease, cardiomyopathy and premature birth and death 2 each, respiratory distress, intrautrine infection and hypocalcemia, intrautrine death, still birth and multiple congenital anamoly one each. Despite good control there was a tendency to macrosomia in IGGT and GDM. Associated hypertension in 3 PGDM resulted in small for gestational age infants. Twenty eight with previous bad obstetrical events had successful outcome. The use of SU at conception in 22 and till term in one had no adverse effects. Delayed detection of IGGT and GDM, unplanned pregnancy in PGDM and late referals appear to have caused 8.5% fetal mortality

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SERUM LEPTIN CONCENTRATION IN WOMEN WITH NORMAL PREGNANCY AND PREGNANCY COMPLICATED WITH GDM.

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The aim of the study was to examine serum leptin levels in III trimester of normal pregnancy and pregnancy complicated with gestational diabetes mellitus (GDM) and compare them with normal non-pregnant women. We examined 19 healthy non-pregnant women (the blood samples were taken twice - in follicular and luteal phase), 25 healthy pregnant women (III trimester) and 14 women with GDM. Serum leptin and progesterone concentrations were measured with RIA. The mean serum leptin concentrations in non-pregnant women was 11.9 ± 5.8 and 16.6 ± 9.1 ng/ml in follicular and luteal phase, respectively. Pregnant women had serum leptin levels 25.1 ± 11.9 ng/ml. The mean serum leptin levels in women with GDM were slightly higher, 27.8 ± 11.4 ng/ml. The serum leptin concentration was positively correlated with plasma progesterone level.

In conclusion, results of the present study suggest the possible role of steroids in the regulation of leptin release.

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OUTCOME OF DIABETIC PREGNANCY IN MAURITIUS 1993-1996. S.Ramtoola ,B.H.Damry . Dr A.G.Jeetoo Hospital , Port-Louis, Mauritius.

Mauritius has prevalences of NIDDM and impaired glucose tolerance of 13.2% and 15.6% respectively. To evaluate the effect of such diabetes and IGT on pregnancy outcome , prospective data has been collected on all ongoing and new diabetic pregnancies since 01.10.93 at Dr A.G.Jeetoo Hospital, one of the four regional hospitals of Mauritius . 293 pregnancies were registered at 30.09.96. Outcome was analysed in 266 pregnancies , excluding patients not yet delivered and those lost to follow-up . 93 cases were of pregestational onset , including 5 women with IDDM. 85 of the 173 patients diagnosed during pregnancy had diabetes by classical WHO criteria . 17 pregnancies resulted in 1st or 2nd trimester abortion , including one therapeutic termination for anencephaly. There was one maternal death at 32 weeks' gestation preceded by intrauterine fetal death. Perinatal mortality rates in the remaining 248 pregnancies were 141.0/1000 for pregestational diabetes and 117.6/1000 for gestational diabetes , compared to whole island background rates of 26.8/1000 for 1995. There was no excess perinatal mortality in gestational IGT at 23.5/1000 . Intrauterine death in untreated or poorly controlled DM accounted for a significant part of the excess perinatal mortality in the 2 diabetes groups , with stillbirth rates of 76.9/1000 and 82.3/ 1000 for pregestational and gestational diabetes compared to 14.8/1000 background population rates . Prematurity with its complications was the commonest cause of neonatal mortality (50%) and congenital malformation accounted for 14.3% of the perinatal mortality . In conclusion , the exceedingly high perinatal mortality rate in pregestational and gestational diabetic pregnancy at 4.8 times the background rate calls for review of antenatal booking practices and referral patterns , and suggests the need for early screening for diabetes to ensure earlier institution of appropriate treatment .

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SERUM LEPTIN IN WOMEN AND *ob* GENE EXPRESSION IN RATS IN RELATION WITH INSULIN LEVELS DURING PREGNANCY.

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A long-term effect of insulin was demonstrated on serum leptin production and on *ob* gene expression in humans and in rodents. Furthermore, it has been suggested that insulin regulates *ob* gene expression and leptin production indirectly, probably through its trophic effect on adipocytes. Since the size of adipocytes during pregnancy is increased, this study was undertaken to further investigate the relationship between insulin and leptin levels.This was done under conditions of hyperinsulinemia, as it occurs during pregnancy, by measuring serum leptin levels in women and *ob* gene expression in female rats. In pregnant women (35-40 weeks) serum insulin levels were increased as previously shown: 2.17 ± 0.23 ng/ml (n = 80) versus 0.65 ± 0.07 (n = 10) ($p<0.02$) with no change in glycemia, and serum leptin levels were also found to be increased : 24.65 ± 1.82 ng/ml (n = 82) versus 13.21 ± 1.75 in controls (n = 12) ($p<0.02$). No correlation was observed between leptin and insulin levels. Compared to control female rats, at day 20 of gestation serum insulin was increased : 5.66 ± 1.01 ng/ml (n = 13) versus control rats : 3.3 ± 0.22 (n = 13) ($p<0.05$), and serum glucose was decreased: 0.94 ± 0.04 $g.l^{-1}$ versus 1.22 ± 0.03 ($p<0.001$), due to the increased uptake of glucose by the fetuses. However there was no significant change in *ob* mRNA levels in parametrial adipose tissue. These data suggest that the increased levels of insulin during pregnancy may regulate, probably indirectly, human leptin production but this increase had no effect on the expression of *ob* mRNA at day 20 of gestation in the rat.

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CONGENITAL MALFORMATIONS IN NEWBORNS OF PRE-PREGNANCY NON INSULIN-DEPENDENT DIABETIC MOTHERS.

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The aim was to determine if the use of sulphonylureas and/or biguanides during early pregnancy is associated with an increased risk of congenital anomalies in newborns of non insulin-dependent diabetic mothers before pregnancy. From 1980 to 1995 we followed prospectively 110 pregnancies in non insulin-dependent diabetic women before conception. Of these, 41 women were taking during early pregnancy oral hypoglycemic agents (21 sulphonylureas, 4 biguanides and 16 sulphonylureas+biguanides) and stopped these drugs at 8.89 ± 5.57 weeks of pregnancy. 69 non insulin-dependent pregnant women were treated with insulin or diet alone. At first examination all the patients started the diet and the insulin therapy and they were monitored by means of blood glycemic self-monitoring. Their newborns were examined at birth. At first examination glycosylated hemoglobin was 7.11 ± 1.66% and 8.0 ± 2.1% in oral hypoglycemic drugs treated patients and in insulin or diet treated patients respectively. In these series we observed 7 congenital anomalies: talipes equinovarus, syndactyly, microcephaly and microphtalmy in insulin/diet treated patients; right tibia agenesis, right diaphragm agenesis and pulmonar hypoplasia, and 2 interatrial defect in sulphonylureas+biguanides treated patients (interruption week range: 5th-21th week of gestation). Univariate logistic regression analysis and chi-square test were used for statistical analysis. The percentage of congenital malformations was 5% in offspring of non insulin-dependent diabetic mothers treated with insulin or diet, 0% in patients treated with sulphonylureas and 20% in patients treated with sulphonylureas+biguanides ($p< 0.01$). The higher percentage of congenital malformations in newborns of non insulin-dependent diabetic women treated with sulphonylureas+biguanides can be attributed partly to biguanides influence on embryogenesis period.

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TRIGLYCERIDEMIA IN DIABETIC PREGNANT WOMEN AND NEW BORN WEIGHT

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Uncontrolled metabolic situation and hyperglycemia in Diabetic pregnant women are considered for a long time a predominant factor of new born macrosomie. However, in spite of perfect metabolic control and normoglycemia, macrosomia is still observed in 25 % in our study. There by, other factors are incrimined in pathogenesis of macrosomia (genetic, dietetic factors and recently hypertriglyceridemia in pregnant women). Our prospective study suggest to know if macrosomia was correlated with hypertriglyceridemia in perfect metabolic control in pregnant diabetic patients. We have studied new born weight in tow perfect metabolic control pregnant diabetic patient groups : one group (n = 67) with hypertriglyceridemia , and the second group (n = 42) with normal triglyceridemia in 3rd trimester. Our results show that third trimester hypertriglyceridemia is not correlated with new born weight. Maternal triglyceridemia is not a determinant factor of new born macrosomia in diabetic women.

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FETAL MACROSOMIA IS ASSOCIATED WITH HIGH MATERNAL INSULIN SENSITIVITY AND GESTATIONAL WEIGHT GAIN.

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Increased perinatal morbidity-mortality are associated with gestational diabetes (GDM). We investigated glucose tolerance over the course of 69 pregnancies (by oral glucose tolerance test, OGTT, and hemoglobin A1c, HbA1c) as well as fetal intrauterine growth (by ultrasound) and pregnancy outcome.

In the 3rd trimester, 7 women had abnormal OGTT, but none of the 12 mothers of large babies (>3.9 kg) had GDM. Among fifteen pregnants with basal BMI>25 kg/m^2, 2 developed GDM, 5 had babies>3.9 kg, 8 had normal birthweight babies. In normal pregnancies (n=42), area under glycemic curve (AUGC, g min/dl) changed from 4.8±2.7 (1st trim) to 6.9±2.4 (3rd trim), area under insulinemic curve (AUIC, mU min/ml) from 6.6±3.8 to 9.3±4.9; in mothers of large babies (n=12), AUGC changed from 3.6±3.4 to 5.4±1.9, AUIC from 5.5±1.5 to 8.9±3.7; in mothers with GDM (n=7), AUGC increased from 6.6±3.4 to 12.3±1.6, AUIC from 8.3±5.3 to 9.7±5.5; in obese mothers of normal birthweight infants (n=8), AUGC changed from 6.0±3.1 to 7.3±3.0, AUIC from 8.1±2.7 to 10.6±2.5. Neonatal body weight was correlated ($p<0.001$) with maternal gestational weight gain, placental weight, 3rd trimester AUIC/AUGC ratio and 1st-2nd trimester HbA1c. Fetal growth indices (femur length, biparietal diameter and abdominal circumference) were correlated with both HbA1c and 2h OGTT glycemia.

Fetal growth rate is confirmed to be associated with maternal glycemic equilibrium, but one of the main determinants of high infant birthweight seems to be an enhanced maternal insulin sensitivity, accompanied with remarkable gestational weight gain, apart from glucose tolerance or the absolute level of serum insulin.

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PLASMA α-TOCOPHEROL LEVELS IN DIABETIC WOMEN DURING PREGNANCY.

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It has been clarified that administration of Vitamin E decreases the rate of embryo malformations in animal studies. To investigate the changes of α-Tocopherol in pregnancy which is a main component of Vitamin E, and elucidate the correlation between fetal anomaly and plasma α-Tocopherol level, we measured plasma α-Tocopherol levels in 52 diabetic pregnant women in the first, the second and the third trimester by using HPLC. Their glycemic controls were good (average level of HbA1c was 6.2% in the first, 5.1% in the second and 5.7% in the third trimester). The control group comprised 20 healthy non-pregnant women, 40 normal pregnant women and 239 diabetic non-pregnant women.

Non-pregnant state : The average level of plasma α-Tocopherol in 20 healthy non-pregnant controls was 0.7±0.1mg/dl. The average level of plasma α-Tocopherol in diabetic non-pregnant women was 0.9±0.3mg/dl ($p<0.005$ vs healthy non-pregnant controls).

Pregnant state : In the first trimester α-Tocopherol level was 0.8± 0.1mg/dl in normal pregnant women and 0.8±0.2mg/dl in diabetic pregnant women. No difference in plasma α-Tocopherol level was found in diabetic pregnant women when compared to normal pregnant women in the first, the second and the third trimester. There was a significant cooperative correlation between plasma α-Tocopherol level and gestational week in both normal and diabetic pregnant women. Two diabetic pregnant women had the baby with cardiovascular defects, but their plasma α-Tocopherol level was within normal range in the first trimester which is important period of organogenesis.

To elucidate the protective effect of Vitamin E against anomaly in human diabetic pregnancies, we have to establish α-Tocopherol level in the fetus.

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MATERNAL DIABETES AND FETAL MACROSOMIA IN MAURITIUS.

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Maternal diabetes and maternal obesity are associated with fetal macrosomia. There is evidence that all babies of diabetic mothers are macrosomic in that their weight is greater than predicted. We hypothesised that maternal obesity and triglyceride concentrations would predict macrosomia in Mauritius. Absolute weight and neonatal skinfolds were used to assess macrosomia. Nineteen diabetic pregnancies (11Asian Indian: 8 Europid) were compared with 67 control non-diabetic (43 Asian Indian: 24 Europid). Mothers with diabetes were older (dm31.0±7.2 v control 24.7±5.2 years, $p<0.05$) and more obese (BMI dm30.5±3.3 v control 27.3±4.1kg.m^{-2}, $p<0.05$). Birthweight was similar in the two groups (dm 3089±141 vs. control 3082±226 gm, NS) but mean 4 point neonatal skinfold was increased in infants of diabetic mothers (dm 5.71±1.14 vs. control 4.92±0.93mm, $p<0.001$). Models were constructed to predict birthweight or neonatal skinfold thickness on the basis of the presence of maternal diabetes, maternal age, maternal body mass index, glucose and triglyceride concentrations. Birthweight correlated with these variables ($r=0.48$ $p<0.01$); maternal BMI ($p<0.01$) and gestational age ($p<0.05$) contributed significantly. Likewise neonatal skinfold correlated with these variables ($r=0.44$ $p<0.01$) with maternal BMI ($p<0.05$) and maternal diabetes ($p<0.05$) contributing significantly. Maternal obesity therefore influeneces both birthweight and neonatal subcutaneous fat whereas maternal diabetes influences predominately subcutaneous fat in this population.

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ANTITHROMBIN III ACTIVITY IN IDDM AND NIDDM PREGNANTS

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The antithrombin III (AT III) - very important inhibitor of blood that inhibits thrombin, factor Xa, XIa, IXa, XIIa and VIIa. Data about AT III activity in diabetic pregnants are controversial. The aim of our study was to compare AT III activity in pregnants with IDDM and NIDDM. The study was carried out in: 31 pregnants with IDDM (17 without microangiopathy - mean age: 26.9±5.5 and 14 with it - mean age: 28.0±7.0); 10 with NIDDM (mean age:36.5±5.5) and in 24 healthy pregnants (mean age: 25.6±4.5). Blood was obtained in the first, second and third trimester, post-partum and after puerperium. Chromogenic substrats of Behring were used for determinations of AT III activity. The values of AT III activity during pregnancy, post-partum and after puerperium in healthy pregnants were in normal range (112.8-120.2%). The pattern of AT III activity in IDDM patients (without regerding vascular complications) was similar to that of healthy pregnants (115.5-127.5%). The AT III activity in IDDM diabetics without microangiopathy in the first trimester was significantly lower than in pregnants with microangiopathy (107.8±32.0vs142.9±35.2%, $p<0.05$). It increased in the second and third trimester of pregnancy and decraesd post-partum. AT III activity in IDDM diabetics with microangiopathic complications was very high in the first trimester and it was significantly decraesd in the second one (142.9±35.2 vs 101.0±23.4%, $p<0.05$). The level of AT III activity in the second trimester in group without microangiopathy was 139.2±52.1 and it was 101.0±23.4% in the group with microangiopathy. The difference was significant ($p<0.05$). AT III activity in NIDDM pregnants in the first and second trimester was significantly lower than in healthy and IDDM pregnants. In the first trimester: NIDDM-98.2±23.3 vs healthy-120.0±24.9%, $p<0.05$ and NIDDM-98.2±23.3 vs IDDM-123.4±37.1%, $p<0.05$; in the second trimester: NIDDM-97.4±18.0 vs healthy-115.4±28.1%, $p<0.05$ and NIDDM-97.4±18.0 vs IDDM-122.0±45.2%, $p<0.05$. AT III activity in IDDM pregnants with microangiopathy decreased in the second trimester and in NIDDM pregnants in the first and second trimester. It could indicat its consumption during increased fibrin formation in pregnancy.

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PERINATAL AND INFANT MORTALITY IN CHILDREN OF FINNISH WOMEN WITH TYPE I DIABETES.

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Perinatal mortality is increased in diabetic pregnancy but little is known on survival of the children later in life. From the Diabetes Drug Registrer of the Finnish Social Insurance Institution we identified all Finnish women born after 1940 that were diagnosed until the end of 1993 with insulin-treated diabetes when under 30 years of age. From the Finnish Population Register and the Finnish Central Statistical Office we identified all liveborn children and all stillbirths of these women from 1987 until 1994. We ascertained all deaths among these children until the age of one year. During the study period there were 1630 live births and 26 stillbirths in women with Type I diabetes diagnosed before pregnancy. Perinatal mortality was 2,2% (n=36). The relative risk (RR) compared to non-diabetic pregnancies was 2,9 (95% CI 2-4). Late neonatal mortality was 0,25% (n=4), RR=3,4 (95% CI 0,93-8,7). Post-neonatal infant mortality was 0,43% (n=7), RR=2,5 (95% CI 1,02-5,2). Perinantal mortality is elevated in pregnancies of women with pregestational diabetes and the children are at increased risk of dying also during infancy.

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PAI-1 ACTIVITY IN PREGNANTS WITH TYPE 1 AND TYPE 2 DIABETES.

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Plasminogen activator inhibitor type 1 (PAI-1) inhibits tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA). In the last decade it became evident that PAI-1 plays an important role in inhibition of fibrinolytic system. There are few data about the PAI-1 in diabetic pregnants. The aim of the study was the estimation of PAI-1 activity in type 1 and type 2 diabetics during pregnancy, post-partum and after puerperium. The study was carried out in: 31 pregnants with IDDM (17 without microangiopathy - mean age: 26.9±5.5 years and 14 with microangiopathy - mean age: 28.0±7.0 years); 10 with NIDDM (mean age:36.5±5.5 years) and in 24 healthy pregnants (mean age: 25.6±4.5 years). The patients were normoglycaemic ($HbA_{1c}<6.5\%$).

PAI-1 activity was determined in blood plasma at the end of the first, second and third trimester of pregnancy, post-partum and after puerperium. To determine of PAI-1 activity the amidolytic method of Bioopol (Sweden) was used. PAI-1 activity in healthy pregnants from the first trimester (5.1±4.7 IU/ml) significantly increased in second (12.0±11.6 IU/ml) and third ones (17.6±9.4 IU/ml), it decreased post-partum (7.9±7.6 IU/ml) and reached values of the first trimester after puerperium (6.5±6.7 IU/ml). PAI-1 activity in NIDDM patients at all time points of investigation except of values detected in the third trimester was significantly higher than in healthy group (10.0±4.4, 12.9±3.4, 22.2±8.2, 14.0±2.8, 13.6±4.0 IU/ml respectively) and in IDDM pregnants (4.0±3.9, 7.6±5.7, 15.9±11.4, 6,1±9.4, 7.6±8.7 IU/ml respectively). The PAI-1 activity in IDDM pregnants without microangiopathy was lower than in patients with microangiopathy, but the differences were not significant. *Conclusions* 1.The highest values of PAI-1 activity were observed in NIDDM pregnants. 2.In both healthy and diabetics pregnants the highest PAI-1 activity was found in the third trimester of pregnancy. 3.No significant differences of PAI-1 activity between IDDM pregnants without and with microangiopathy were observed.

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PREGNANCY OUTCOME IN WOMEN WITH GDM TREATED ACCORDING TO *STAGED DIABETES MANAGEMENT* IN ŁÓDŹ AND RADOM (POLAND).

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Aim: To compare the pregnancy outcome before and after *Staged Diabetes Management* (SDM) implementation. Research design and methods: In 1995-96 the SDM was introduced to screen pregnant women for gestational diabetes mellitus (GDM). It is based on a two-step diagnosis: 50 g GCT performed between the 24-28th gestation week (with the threshold 140 mg/dl) and - on GCT being positive - 75 g OGTT, according to WHO criteria. Previously the same criteria were recommended in Poland, but they were not widely used. Upon diagnosis of GDM all women were treated according to SDM protocol in two centers in Łódź and Radom. The study subjects were divided into three groups: I - non-screened GDM women (n=67, mean age 30±6.8); II - women with GDM found in active screening performed at our centers (n=205; mean age 30±4.7); and III - healthy pregnant women (n=104, mean age 29±4.6). Results: Mean (±SD) pregnancy time before GDM diagnosis was: I - 33.0±1.6; II - 30.1±5.2 wks (p<0.05). Insulin treatment was more frequently used in group I than in II (43.6% and 22%, respectively; p<0.05). There was no difference in pregnancy duration between the groups: I - 39.0±1.0; II - 39.2±1.1; III - 39.3±1.3 wks (p>0.05). Neonatal birth weight was significantly lower in groups II and III than in I: I - 3788±449; II - 3467±461; III - 3368±383 g (p<0.05). LGA features were found in 29.6% of children born in group I, in 12.5% of II, and 4.8% of III (p<0.05). Neonatal hypoglycemia occurred most frequently in group I: I - 48.1%; II - 20.8%; III - 1.9% (p<0.05). Conclusions: Upon SDM implementation, GDM was diagnosed earlier in pregnancy and the number of insulin-treated women decreased significantly. Both birth weight and macrosomia rate were lower in the GDM group, which had been screened, than in the non-screened population. The routine screening of pregnant women for GDM and proper treatment results in lowering pregnancy and neonatal complication rates.

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CHANGES IN SODIUM-HYDROGEN EXCHANGE ACTIVITY DURING NORMO-TENSIVE NON-DIABETIC PREGNANCY.

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Pregnancy is associated with a 30-50% rise in cardiac output and 50% increase in blood volume: the contribution of changes in the activity of primary and secondary active transporters to these hemodynamic adaptations remains unknown. For the first time, we measured sodium-hydrogen exchange activity over the course of normal pregnancy. Eighteen healthy pregnant women were studied at 14, 24 and 33 weeks of gestation and compared with 18 nonpregnant healthy women. No pregnancy was complicated by hypertension. At each antenatal visit, body weight and blood pressure were recorded; blood and 24 h-urine samples were taken to control renal function and metabolic equilibrium; maternal glucose tolerance was evaluated by oral glucose test and HbA1c; erythrocyte sodium-hydrogen antiport was also measured.

Erythrocyte antiport activity resulted 10.0±3.0, 9.6±2.9 and 8.4±3.5 mmol/L cells h in the three gestational trimesters, respectively, significantly higher at each trimester than in control women (6.8±2.5). The clearances of urea and creatinine were constantly elevated in pregnant women; at each trimester, their serum concentrations were lower than in nonpregnant women. Serum potassium significantly decreased during pregnancy. Serum total cholesterol and triglycerides, already above the normal range from the first trimester, further increased until the third one. Area under glycemic curve became larger during pregnancy, the area under insulinemic curve increased to a lesser extent. There was a significant association of antiport activity with serum triglycerides.

The observed hyperactivity of the transporter, peaking at the 14th week of gestation, may be a contributing factor to the hemodynamic adjustments attending upon normal pregnancy.

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OUTCOMES OF IDDM PREGNANCIES : SIGNIFICANT BENEFITS ASSOCIATED WITH CONCEPTION PLANNING.

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Whereas good metabolic control during early IDDM pregnancy is recommended to prevent congenital anomalies, utility of specific pre-pregnancy care to reduce perinatal morbidity has been poorly investigated. To assess the effects of conception planning of IDDM pregnancies on overall pathological pregnancy outcomes (PPO), we analyzed the cumulated occurrence of spontaneous/therapeutical abortions (S/TAB), perinatal and neonatal mortality (PN/NNMORT), congenital anomalies (CONAN), and perinatal morbidity (PNMORB) among 61 IDDM pregnancies. Group A included 21 planned pregnancies and group B, 40 unplanned pregnancies, with similar distribution of cases according to White's classification. Group A was characterized by earlier booking (6.7 ± 1.8 vs 11.1 ± 5.3 wks, p<0.01), lower GlyHb at conception (2.4 ± 2.6 vs 4.7 ± 4.2 SD above normal mean, p<0.05) and at 24th wk (1.0 ± 1.1 vs 1.9 ± 2.1 SD, p<0.05) and later delivery (36.8 ± 1.1 vs 35.8 ± 1.5 wks, p<0.05). Cumulated PPO were less frequent in group A : 7/21 (2 SAB and 5 cases of PNMORB) vs 25/40 (4 SAB, 1 TAB, 3 severe CON, 2 cases of PN/NNMORT, 19 cases of PNMORB) (p<0.05). Moreover, the comparison between the subgroups of group A (A', n=15) and B (B', n=16), both characterized by GlyHb<3SD above normal mean at conception, showed less frequent PPO in A' (1 SAB, 5 cases of PNMORB) than in B' (2 SAB, 1 case of PNMORT, 9 cases of PNMORB) (p<0.05). Lower occurrence of obstetrical complications (hypertension, premature labor, hydramnios) in group A (vs B, p<0.05) and A' (vs B', p<0.05) might be involved in the corresponding reduction of PNMORB. These data suggest that significant reductions of PPO, mainly related to PNMORB, can be expected from conception planning. Thus, providing specific pre-conceptional counseling to IDDM women can result in additional benefits to those obtained by good metabolic control in conceptional period and prenatal care.

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TRIMESTER-RELATED RANGES FOR FRUCTOSAMINE AND GLYCATED HAEMOGLOBIN IN CAUCASIAN NON-DIABETIC PREGNANT WOMEN

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This study was designed to determine trimester-related reference ranges for fructosamine and glycated haemoglobin (HbA1c) for a Caucasian non-diabetic population.The importance of strict glycaemic control during diabetic pregnancy is well recognised . Fructosamine and glycated haemoglobin are used as markers of control but quoted reference ranges are generally determined using a non-pregnant population . Between September - November 1996 , fructosamine and HbA1c was measured in 259 non-diabetic pregnant women attending routine ante-natal clinics.Of these 96 were < 12 weeks (T1) , 100 were 12-24 weeks (T2) and 63 were 25weeks-term (T3) . Only normally progressing singleton pregnancies were included.Women with known or suspected renal , cardiac ,liver or other metabolic diseases were excluded.Fructosamnine was determined colorimetrically . A latex enhanced turbidometric immunoassay (Roche Unimate 3) was used to measure HbA1c .In TI and T2 mean HbA1c was 5.1% (range 4.6-5.6 %). In T3 mean HbA1c was significantly higher at 5.3% (range 4.4-6.1%). These ranges are lower than the reference range presently quoted (range 3.5-6.5 %) , being that for a general non-diabetic population.Fructosamine levels decreased throughout pregnancy . In T1 , mean = 220 umol/lL(range 185-255 umol/L) , T2 mean = 212umol/L (range 172-251umol/L) and T3 mean = 188umol (range 158-218 umol/l). T1 versus T2 p<0.001 and T2 v T3 p<0.001 .These ranges are also lower than the quoted reference range (210-280umol/L). This study suggests that trimester-specific reference ranges should be established to enable truer monitoring of diabetic control throughout pregnancy.

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DIABETES AND PREGNANCY PROJECT IMPLEMENTATION IN GEORGIA.

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In 1996 a Project Diabetes and Pregnancy was started in Georgia. The Project is sponsored by Novo - Nordisk and is realized in accordance with the Izrael - Georgian Twinning Program. The aim of the Project is to develope a completely new conception, as during last 70 years young women with diabetes were discriminated. Treatment and education in pre-gesational DM were organized.14 women with IDDM (aged 21-32 yrs) were supervised. All of them were educated at the Diabetes School, and intensive preconception treatment for minimum 2 months prior to a planned pregnancy was carried out. Patients received multiple insulin injections (4 - 6 daily) to achieve fasting, preprandial and postprandial euglycaemia (<105mg/dl and <120mg/dl, respectively). HbA1c levels prior to the planned pregnancy were 7.5±0,3%. Throughout the pregnancy all women were examined once a week at the Center. HbA1c, blood pressure, body weight and renal parameters were determined, and insulin doses were adjusted. Insulin doses were based on home blood glucose monitoring data. The fetus was assessed by serial iltrasound imaging, fetal growth and anomalies. Target metabic control was achieved in12 out of 14 women (12%) Sever hypoglycaemia occured in 3 patients. The gestational age at delivary was 38,0±1.6 weeks, with an average birth weight of 3.200 ± 660g (2.600-3.800g) No cases of respiratory distress were observed. Cesarean section was used in one case. Thus, the Project, including patient education, preconceptional treatmen and care throughout the pregnancy gave positive results. The project is being continued.

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THYROID PEROXIDASE AUTOANTIBODIES :METABOLIC CONTROL AND NEED FOR THYROID TREATMENT IN PREGNANT IDDM WOMEN

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OBJETIVE: To study whether the presence of antithyroid peroxidase antibody (TPO-Ab) before gestation in IDDM affects thyroid function and metabolic control during pregnancy and early pospartum as well as neonatal outcome.

RESEARCH DESIGN AND METHODS: A prospective study at an outpatient Endocrine-Obstetric Unit was carried out in twenty pregnant IDDM women. Free T4, TSH, TPO-Ab and HbA_{1c} were assayed before gestation, during the first, second and third trimester of pregnancy and 3 months postpartum.

RESULTS: HbA_{1c} was significantly higher in TPO-Ab positive women than in those who were TPO-Ab negative during the second ($p<0.01$) and third trimesters ($p<0.05$). HbA_{1c} levels significantly decreased in TPO-Ab negative patients when the second ($p<0.01$) and the third trimester ($p<0.05$) were compared with before pregnancy and the first trimester. There was a significant increase in the dosage of insulin for TPO-Ab positive vs negative patients during the second ($p<0.05$) and third trimester ($p<0.01$) and three months postpartum ($p<0.05$). TSH was significantly increased in the second ($p<0.001$) and third trimester ($p<0.05$) and 3 months postpartum ($p<0.01$) when compared to TPO-Ab negative patients. 7.6% of the TPO-Ab negative group and 29% in the TPO-Ab positive group presented postpartum thyroid dysfunction and 42% of the TPO-Ab positive women required thyroid treatment.CONCLUSIONS: Pregnant women with IDDM who have a positive test for TPO-Ab before gestation have poorer glucose control and a high prevalence of hypothyroidism. Therefore we recommend that pre-pregnant IDDM be screened for anti TPO-Ab. Those with a positive result should be followed with serial monitoring of free T4 and TSH levels during each trimester as well as the postpartum period.

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RESULTS OF IMPROVED CARE FOR PREGNANT DIABETIC WOMEN IN YEARS 1970-94 IN A DISTRICT OF SLOVAKIA

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Aims:A proof of effectivity of intensive care for pregnant diabetic women on neonatal mortality in accordance with aims of SVD in comparison with older methods.In yeers 1970-94 we followed 164 diabetic mothers with Type I and II diabetes who gave birth in single or repeated pregnancies to 197 newborns.In 1970-74 19 diabetic mothers delivered 19 newborns with 15.7% perinatal mortality(PM).In1975-1980,with increased care,32 diabetic mothers delivered 33 newborns and PM dropped to 9%.In years 1980-84 from 59 newborns died two only,i.e.3.4%.In 1985-94,due to education,preconception care,screening of GDM,intensified MC-HM insulin therapy and coperation between diabetologist and gyneco-obstetrician we have lost only one from 86 newborns,1.15%. Moreover,there was also improvement with respect of P.White classification,because mothers classified as C and D,and even with moderate diabetic complications gave birth to normal newborns with low PM. Conclusion:Our 25 years lasting well documented study proves that a good education beginning with preconception and intrapregnancy care for diabetic mother complying with diabetologist and gyneco-obstetrician can assure to-day normal pregnancy and delivery,newborn with normal birthweight and nearly normal perinatal mortality of newborns of diabetic mothers-even with moderate complications in accordance with aims of SVD.

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GLUTATHIONE-DEPENDENT ANTIOXIDANT SYSTEM IN DIABETES-INDUCED EMBRYOPATHY

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We have recently shown that the mechanisms of hyperglycemia-induced embryonic malformations are mediated through increased free radical formation and depletion of intracellular glutathione (GSH) in embryonic tissues during organogenesis(Diabetes 44:992,1995). In this study, we investigated the role of glutathione-dependent antioxidant system and GSH on diabetes-related embryonic malformations. Embryos from streptozotocin-induced diabetic rats on gestational days 11 showed a significantly high frequency of embryonic malformations (neural lesions 21.5 vs. 2.8%, $P<0.001$) and growth retardation compared with those of normal mothers. The formation of intracellular free oxygen radical species increased in isolated embryonic cells of diabetic rats on days11. The concentration of intracellular GSH in embryonic tissues of diabetic pregnant rats on day 11 was significantly low compared with those of normal rats. The activity of γ-glutamylcysteine synthetase (γ-GCS), the rate limiting GSH synthesizing enzyme, in embryos of diabetic rat was significantly low, associated with reduced expression of γ-GCS mRNA. Administration of buthionine sulfoxamine (BSO), a specific inhibitor of γ-GCS, during the period of maximal teratogenic susceptibility (6 to 11 day of gestation) to diabetic rats reduced GSH by 46.7% and increased the frequency of neural lesions (62.1 vs. 21.5%, $P<0.01$). Administration of GSH ester to diabetic rats restored GSH concentration in the embryos and reduced the formation of free oxygen radicals leading to normalization of dysmorphogenesis (1.9 vs.21.5%) and improvement in growth retardation. Administration of insulin in another group of pregnant rats during the same period resulted in complete normalization of dysmorphogenesis (4.3 vs.21.5%) and growth retardation. Our results indicate that GSH depletion and impaired responsiveness of GSH-synthesizing enzyme to oxidative stress during organogenesis, are critical in development of embryonic malformations in diabetes.

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FIRST TRIMESTER METABOLIC CONTROL AND PERINATAL OUTCOME IN WOMEN WITH IDDM

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Metabolic control in IDDM women during pregnancy has greatly lowered maternal and perinatal mortality. Increasing evidence has strengthened the importance of metabolic control for adequate foetal development. Aim of the study was to evaluate foetal outcome in 93 consecutive pregnancies in women with IDDM referring to our Institute within 12 weeks after last menstrual period. Four patients were excluded because of nephropathy. Patients were allocated into 3 groups with 1) HbA1c ≤ 6% (n=24), 2) HbA1c >6.1≤7 (n=30), 3) HbA1c >7 (n=35) (normal <6%) determined at 12 week corresponding to the organogenic period. Mean HbA1c decreased in all groups by the third trimester to 5.5%±0.7, 6.0%±0.6 and 6.6%±0.9 in groups 1, 2 and 3 respectively but remained statistically different in the 3 groups. Age and diabetes duration did not differ among groups whereas BMI was higher in group 3. Patients of group 2 and 3 delivered earlier than those of group 1 and % of delivery before week 35 was 0% in group 1 vs 15.3% in groups 2 and 3 ($p<0.05$). The % of macrosomia was 16.7%, 36.7% and 51.4% in group 1, 2 and 3 respectively, $p=0.025$). Similarly the rate of caesarean sections was increased in group 2 and 3 (55% vs 29% in group 1 $p<0.05$). Pathological NST where more frequent in group 2 and 3 than in 1 (24.6% vs 4.2%, $p<0.05$) and hypertension or preeclampsia were observed only in groups 2 and 3. This data suggest that metabolic control early during gestation is associated with increased rate of macrosomia, earlier and preterm delivery, increased rate of caesarean sections and gestational hypertension/preeclampsia also in the group of patients in whom a value below 7% is reached by the 12th week.

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SELF-MONITORING BLOOD KETONE WITH PAPERSTRIPS AND REFLECTANCE METER IN DIABETIC PREGNANCY AND GESTATIONAL DIABETES MELLITUS.

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Controlled diet is important in the management of pregnancy with impaired glucose tolerance. However, such calorie restriction during pregnancy could produce maternal ketonemia that cause central nervous function depression and endangers the fetal well-being. Recently, a paper-strip sheet (ketofilm) and reflectance meter (ketometer) for blood ketone (3-OHBA) have been developed . In this study, we have evaluated the clinical usefulness of paper strips and reflectance meter in monitoring the blood ketone levels in diabetic pregnancy and GDM. Ten pregnant diabetic woman and three GDM, 8 of them were obese (BMI>25) and 5 were non-obese were the subjects of this study. All the subjects followed a strict diet of 1200-1600 kcal/day. The blood ketone, measured using the ketofilm/ketometer system, and the urinacy ketone were measured at the same time.

The normal blood ketone level is less than 100μmol/L. Before breakfast, the 5 non-obese diabetic subjects showed blood ketone levels of less than 100μmol/L and negative for urinary ketone body, whereas the 8 obese patients showed higher blood ketone levels (150-380μmol/L), 4 of them were negative for urinary ketone body. Addition of 160-240 kcal to the basic meal maintain the normal blood ketone level and negative urinacy ketone. Our results also showed that daily profile lowered the blood ketone level.

Our findings suggest that self-monitoring of blood ketone levels would motivate the patients to maintain the normal range and control their diet, especially the obese, thus reducing fetal risks in diabetic pregnancy and GDM.

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PROBLEMS OF PREMATURE DELIVERY IN DIABETIC PREGNANCY

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A population of 535 diabetic pregnancies treated in our department over a period ranging from 1.01.1990 to 29.02.1996, was analyzed with respect to the incidence, reasons and consequences for the newborns of premature deliveries. The analyzed group consisted of: 205 (38,3%) pregnat women classified as G_1DM (treated with diet), 116 (21,6%)- as G_2DM (treated with nsulin), 90 (16,8%)- as cl. B, 61 (11,4%) - as cl. C, 28 (5,2%) - as cl. D and 35 (5,5%)- as cl. R/F. Premature deliveries occurred in 89 instances, that is in 16,6% of the analyzed diabetic pregnancies. In classes G_1- C, the percentage of premature deliveries ranged from 12,2% - 14,7%, in class D it amounted to 25%, while in cl. R/F to as much as 54%. Spontaneous premature deliveries occurred in 25 (27%) cases and 65 (73%) had to be delivered by cesarean section. Fetal distress was the most frequent indication for operative delivery. The mortality rate in the premature group of newborns reached 7,9% and that in the mature group of newborns rated 3,2%. We want to stress our findings that malformations (3 cases) and RDS (3 cases) were the most frequent reasons for the death of prematurely delivered newborns.

Pregnancy in diabetic women is still a high risk for premature delivery, especially in the group with vascular complications.

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Blood Pressure Monitoring in Diabetic Women: Their Pregnancy Outcome

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Pregnancy induced hypertension (PIH) which is more frequently found in pregnancies complicated by diabetes mellitus, still worsens the pregnancy outcome. Our previous observations in 124 diabetic pregnant women, showed a relationship between the blood pressure (conventionally investigated) and microalbuminuria. Prospectively, we observed the blood pressure (ABP) profile for a 24-hour period (by using an automatic blood pressure monitoring system: TAKEDA Medical TM2421) in 54 out-patients (age 30.4±5.2) (WC: 7GDM, 19B, 10C, 18 D/R) (Type: 7GDM, 13 type 2, 34 type 1) consecutively enrolled, during pregnancy (<13, 20-22, 33-35 week of gestation). We investigated the mean of 24hour Systolic and Diastolic BP as well as Day/Night BP. A slight decrease of blood pressure was observed at the second trimester of pregnancy both in diabetic and non diabetic women (n=40), followed by an increase at the third trimester when the values reached those recorded in early gestation. In diabetic pregnant women we recorded higher ABP levels than in non diabetic women, even if they did not reach the level of significance. Among the diabetic patients, significantly higher values were observed in type 1 in comparison with gestational (GDM) or GD+type 2 diabetic women (III trim Diastolic ABP: 67; 4±2 vs 58; 1±2; 7 or 60; 3±2; 1 mmHg). In type 1 diabetic women a strong correlation was found with the diabetes duration whereas, in type 2 diabetic women with patients'age. Furthermore, several significant increases of ABP (Systo/Diastolic) were seen throughout the pregnancy, in the women whose diabetic duration had lasted for over 10 years as opposed to the patients of less than 10 year duration (1st Trim Total ABP: 115.5±4.6/73.2±2.5 vs 103.8±4.1/64.9±2.4 mmHg; 3rd Trim Total ABP: 115.9±3.2/70.9±1.9 vs 104.1±2.2/64.6±1.4 mmHg). Finally, early in pregnancy (<13 week of gestation), we noticed higher ABP values in those subjects who developed PIH. This finding could have a prognostic clinical meaning also because it was associated with an evident alteration of BP circadian biorhythm.

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SOCIAL STRESS DURING EARLY PREGNANCY INDUCES CHANGE OF GLUCOSE TOLERANCE AND SEXUAL BEHAVIOUR IN MALE OFFSPRING

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The aim of the study was to assay the influence of social stress during early pregnancy on glucose tolerance and sexual behaviour in male offspring. Pregnant Wistar rats were exposed social stress – every day rat was placed in another rat's association during the first 7 days of pregnancy. In 3-month-old offspring the i.p. GTT and sexual behaviour were examined. Then, the offspring were decapitated and blood plasma corticosterone levels were measured by fluorimetric method. After i.p. GTT the offspring have been divided into 2 groups. Group 1 had fasting plasma glucose levels and glycaemic pattern like to control rats (from intact mothers). Group 2 showed higher fasting plasma glucose levels (6.2±0.3 vs 3.9±0.2 mmol/l in control, $p<0.001$) and "plane" glycaemic pattern. Sexual behaviour was evaluated on the results of the 4th test. Group 1 had sexual activity, but number of mountings was elevated by 3-fold ($p<0.001$ vs control). Latency of intromissions and ejaculations was significantly lengthened ($p<0.001$ vs control), that resulted in the decrease of ejaculation frequency ($p<0.001$ vs control). Group 2 showed a loss of sexual activity during test. Maternal stress provoked the increase of corticosterone content both Group 1 and Group 2 (1.19±0.06 and 1.84±0.05 mmol/l, respectively vs 0.64±0.04 mmol/l in control, $p<0.001$). We conclude, that social stress during early pregnancy induces of glucose tolerance modification and sexual behaviour suppression in offspring and corticosterone may be one of the possible factors mediating these disorders.

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EFFECT OF STRICT DIETARY CONTROL OF DIABETES MELLITUS IN PREGNANCY.

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Seventy diabetic pregnant women were followed during pregnancy with strict dietary control (1800 - 2500 KCal/day according to ideal body weight). All received human insulin in a mixture of short and intermediate acting forms in morning and evening doses. Measurement of plasma glucose was done every two weeks by glucose oxidase method. During the last three months of pregnancy, the mean plasma glucose averaged 5.21 mmol/l. Patients were allowed to go as close to term as possible, with 50% being delivered at or beyond 38 weeks. 21.4% were delivered by C.S., Forceps or vacuum extraction deliveries were performed in 11.4% of cases. Perinatal mortality rate was 1.28%. Only 2 babies (2.8%) were delivered because of deterioration of feto-placental function tests. 17.9% of live born babies had neonatal hypoglycemia. 71.4% of live born babies had congenital anomalies mainly renal, cardiac or caudal regression syndrome. Our results suggested that strict metabolic control during pregnancy may decreases the incidence of perinatal morbidity and mortality, fetoplacental function abnormalities and neonatal hypoglycemia.

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FACTORS WHICH INFLUENCE ATTENDANCE AT A PREPREGNANCY CLINIC

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Because optimal glycaemic control in early pregnancy is of proven benefit to improve outcome in diabetes, educational strategies should be developed to encourage attendance at prepregnancy clinics. Methods: In a single geographic region 325 consecutive pregnancies in 194 women with established IDDM were investigated to assess registration at a well advertised pre pregnancy clinic(PPC). Availability of this clinic was emphasized in the educational program for newly diagnosed women with IDDM, teenage girls transferring to adult clinics and at the post natal multidisciplinary clinic. Results: Out of 325 pregnancies, 121(37.2%) were preceded by registration at the PPC. No significant relationships were found between registration and age at diagnosis(16.3±7.5vs15.2±7.3 yr), parity, pregnancy number , previous adverse outcome or prepregnancy weight (65.9±8.6 vs 64.6±8.9kg). Registrants were older (28.2±3.4 vs 26.4±4.4yr. $p<0.0001$) and had fewer smokers (20% vs30% $p<0.002$). Few women with advanced complications attended (Class F 0%,R 23%). Conclusion: Women of reproductive age with complications of diabetes should be particularly targeted for prepregnancy care.

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THE IMPACT OF PRECONCEPTUAL CARE IN WOMEN WITH INSULIN DEPENDENT DIABETES MELLITUS (IDDM)?

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Between April 1994-96, 47 women with IDDM attended our antenatal clinic, 12(26%) of whom attended the preconceptual clinic(PC). We analysed the latter by comparing pregnancy outcomes between the 12 attenders (PC) and the 35 non attenders (NA).The groups were similar for age, ethnicity, duration and complications of diabetes. Marital status was different between groups (100% (PC) v 54%(NA) married. There were less smokers (8% v 28%) and mean booking HbA_1C was lower (8.1% v 10%) in the PC group. 58% of babies were delivered at term (≥37weeks) and 42% preterm (30-36 weeks)(PC group) compared to 57% and 43% respectively (NA group), although 17% of the latter were between 26-30 weeks. The incidence of macrosomic babies was lower in the PC group (25% v 39%) and there were no major congenital anomalies.There were no neonatal deaths and 17% required neonatal unit care (NNU)(PC group) compared with two neonatal deaths and 30% requiring NNU care (NA group). This clinical service has improved periconceptual control (HbA_1C 8.1% v 10%), reduced neonatal morbidity(17% v 30%)(NNU) and mortality (0% v 5.7%) and decreased the number of macrosomic infants (25% v 39%) and deliveries before 30 weeks (0% v 17%). In view of these beneficial effects all women with IDDM should be encouraged to attend.

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GLYCAEMIC RESPONSE TO A STANDARDISED TEST MEAL IN PREGNANCY

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Two groups of pregnant women with (Group A n=982) and without (Group B n=192) risk factors for gestational diabetes mellitus had timed post prandial glycaemic responses measured following a standardised test meal. The meal(453kcal, cho 61.3%, prot 15.6%, fat 17.8%) was well tolerated. Dietary advice was given to group A when fasting or 120min glucose was >97%ile of group B. When dietary management failed to normalise blood glucose insulin was prescribed. Results:The differences between mean fasting and mean 120min glucose were A 1.0mmol/l and B 0.7mmol/l. Group B were younger (27.3±3.9 vs 28.6±5.1yr), had a lower BMI (23.0±3.4 vs 26.8±6.6), fasting (4.3±0.37 vs 4.7±0.66mmol/l) and post prandial glucose(5.0±0.5 vs 5.7±1.1mmol/l) than group A. 49%(451) of women in group A had dietary advice and 4%(37) insulin. Offspring of mothers in group A were heavier (3602.3 ±586 vs 3365± 410g, centile 53.9±29.1 vs 44.3±25.5), had more neonatal hypoglycaemia (7% vs 3%) and were more often admitted to the SCBU.

Conclusion: This novel approach to the detection of hyperglycaemia in pregnancy provides a physiological basis for intervention.

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THE COMPARISON OF THE THERAPEUTIC PROCEDURES IN DIABETIC CARE OF PREGNANT WOMEN IN THE LAST DECADE.

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The aim of the work was the comparison of the procedures used in diabetic and obstetric care of pregnant women in the last decade. Study was performed on the group of 246 patients (127 with diabetes mellitus type I [DM] and 119 with gestational diabetes mellitus [GDM]). Pregnant women were divided into 3 groups depending on the date of delivery. To group I - 30 patients were included (who delivered within period 1985-88) - before establishing the central system of obstetric and diabetic care and intensive insulin therapy. To group II - 129 patients were included (who delivered within 1989-93). It was a transitory period when centralization of care and intensive monitoring (NST, Doppler test, profile of glycemia, HbA_{1c}) were successively included. Group III - 87 patients delivering within 1994-95. At this time our regional center was totally organized. During the period of our investigations we achieved decrease in the mean fasting blood glucose concentration between groups I and III in I^{st}, II^{nd} and III^{rd} trimester from 200.3 to 122.0 mg/dl, from 173.9 to 110.0 mg/dl and from 157.3 to 95.7 mg/dl respectively. The rate of preterm deliveries decreased from 54% to 21%; macrosomia from 50% to 20%. The mean duration of gestation increased from 35.5 to 38.7 weeks. The amount of neonates with Apgar score higher than 7 increased from 46% to 90%.

Hospitalization in specialized center with adequate equipment and with experienced staff improved prognosis and results in treatment of gestation complicated with DM and GDM.

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Determinants of Glucose Intolerance during Pregnancy in Barbadian Women.

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A cohort of 342 pregnant Barbadian women was studied to evaluate determinants of glucose intolerance.Nutritional status of participants was assessed by measurement of plasma glucose and serum insulin in response to a 75 gm oral glucose load at 18 and 28 weeks gestation.Multivariate analyses indicated that age ($p< 0.0001$) and body mass index (BMI)($p= 0.0034$)were independent determinants of glycaemic status at 18 weeks gestation.Similar associations were evident at 28 weeks gestation for both age ($p< 0.0001$) and BMI($p= 0.0067$).Change in glucose curve area between 18 and 28 weeks gestation was independently determined by weight change adjusted for age,BMI and parity ($p= 0.028$).Fat mass gain over this period did not predict hyperglycaemia,but independently predicted increased insulin secretion($p= 0.016$).Thus age and BMI were determinants of hyperglycaemia.Weight change determined changes in glycaemic status between visits whereas changes in fat mass determined changes in insulin secretion.

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EMBRYONIC CONCENTRATION OF PHOSPHATIDYLINOSITOL IS NOT ALTERED BY MATERNAL DIABETES.

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Children of mothers with diabetes are at risk of being born with malformations. Embryos cultured in high glucose concentration have a decreased *myo*-inositol uptake into the lipid fraction and are protected by *myo*-inositol supplementation. Hence, an involvement of decreased concentration of phosphatidylinositol (PI) in the etiology of congenital malformation have been suggested. This study aimed to test the validity of this hypothesis by direct measurements of PI in offspring of diabetic rats.

Phosphatidylcholin, phosphatidylethanolamin+sphingomyelin and PI were measured with HPLC in rat embryos on gestational day 11 and in fetal liver, brain and placenta on gestational day 20. The fraction of PI was calculated as a percentage of all phospholipids.

In general, embryos had less phospholipids per wet weight than fetal tissue, and these levels were not affected by maternal diabetes. Furthermore, embryonic tissue had the highest fraction of PI (11.7±0.5%) compared to fetal liver (6.7±0.6%), fetal brain (5.6±0.9%) and placenta (0.7±0.%). Diabetes in the mother slightly decreased the PI fraction in fetal liver (5.4±0.3%) but not in embryos (12.3±0.2) or any other of the tissues examined.

In conclusion, embryos have a higher concentration of PI compared to fetal tissue. The data suggest that diabetes does not reduce the concentration of PI in embryonic tissue. Therefore, maternal diabetes probably interferes with inositol metabolism rather than the absolute concentration of PI in embryos of diabetic rats.

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CONTRACEPTIVE PRACTICE IN 93 INSULIN DEPENDENT DIABETES MELLITUS WOMEN.

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The aim of this study was to determine the contraceptive practice of IDDM women. From december 1995 to august 1996, we consecutively interwiewed 104 IDDM women 15-50 year-old. Four of them have had hysterectomy and 7 were pregnant. Among the 93 remaining patients, 17 did not use any contraceptive method (18%) and 76 used a contraceptive method (82%): hormonal compounds (HC) in 39 cases (51.3%), Intra-Uterin Device (IUD) in 19 cases (25%), sterilization in 11 cases(14.5%), and local contraception in 7 cases (9.2%). The 39 HC users took low dose progestogen only pills (POP) in 25 cases (64%), Oral Combined Pills (OCP) in 9 cases (23%), and high dose progestogen pills in 5 cases (12.8%). Age of patients and duration of IDDM were significantly higher in patients using non hormonal contraceptives methods, compared to those using HC (age: 36.8 +/- 9.52 vs 28.2 +/- 8.41 years, $p<0,0003$; duration of diabetes: 16.8 +/- 10.82 vs 10.4 +/- 7.17 years, $p<0,003$). HbA1c, BMI, daily insulin requirement were similar between these two groups. All of the women using IUD or who were sterilizated had >1 child, whereas 46% of the HC users had > 1 child. Microangiopathy was present in 10/39 HC users and in 18/37 non hormonal contraceptive users. Macroangiopathy was present in 2 patients (one using IUD, one sterilizated woman). Among the 9 OCP users, 1 had a diabetic retinopathy, and 5 had 1 or 2 other cardiovascular risk factor(s) associated with IDDM. A variable cycle length was present in 30/76 patients, more frequently in POP users ($p<0,05$). In this study, 58 patients were satisfied with their contraceptive method (63%), OCP administration was unadapted in 5 patients, and POP was the less comfortable contraceptive method. These results confirm that the contraceptive practice of IDDM women must be frequently reevaluated according to its safety, efficacy and acceptability.

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DISTURBED PROSTAGLANDIN METABOLISM IN RAT EMBRYOS CULTURED IN A DIABETIC ENVIRONMENT

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Previous studies have suggested that disturbed arachidonic acid metabolism may be associated with embryonic dysmorphogenesis in diabetic pregnancy. The aim of this study was to investigate the relationship between morphologic development and prostaglandin metabolism in day-9 embryos subjected *in vitro* to high concentration of glucose, arachidonic acid, PGE2, or cyclooxygenase inhibitors for 48 hours. Increasing the glucose concentration from 10 to 30 mmol/l caused embryonic dysmorphogenesis (from 2 % to 86 % major malformation). Addition of either 20 µmol/l arachidonic acid or 28 nmol/l PGE2 protected from the glucose-induced maldevelopment (12 % or 21 % major malformation). Culture in the presence of the cyclooxygenase inhibitors indomethacin (100 µmol/l) or acetylsalicylic acid (1 mmol/l) in low glucose yielded embryonic dysmorphogenesis of a comparable degree to that caused by high glucose alone (82 % or 100 % major malformation). Addition of either arachidonic acid or PGE2 to the low glucose culture medium with cyclooxygenase inhibitors normalized the embryonic development (0-6 % major malformation). In contrast, only PGE2 addition could normalize the development of embryos cultured in high glucose with cyclooxygenase inhibitors (4-21 % with PGE2, 93-100 % major malformation with arachidonic acid).

In conclusion, a diabetic environment disturbs both embryonic development and arachidonic acid metabolism *in vitro*. The results suggest a causal relationship, and that the prostaglandin disturbance includes an inhibition of the enzymatic activity of embryonic cyclooxygenase.

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FETAL INSULINISATION IN RELATION TO MATERNAL ANTHROPOMETRY AND BODY COMPOSITION

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Insulin functions as the major anabolic factor in the regulation of intrauterine growth. Factors affecting fetal insulinisation are not fully characterised. This prospective study was designed to examine the relationship of maternal body composition on fetal metabolism. Cord blood samples were collected from 60 infants of non-diabetic, healthy women. The samples were centrifuged and the plasma was pipetted and stored at -20°C. Where haemolysis had occurred only C-peptide was measured. In non-haemolysed specimens (n=48) both C-peptide and insulin were measured. Maternal weight, height and body composition (by Skinfold thickness measurements and Bio-electrical Impedance) were assessed at 13-15 weeks gestation. A positive correlation was observed with both cord insulin and/or C-peptide levels and maternal early pregnancy body mass index ($r=0.44$, $p=0.002$ and $r=0.33$, $p=0.008$ respectively). The result of multiple regression analysis (forward method) revealed that maternal fat mass in early pregnancy would explain the variation in cord insulin (0.002) and C-peptide levels (0.02) rather than the lean mass. We did not see a positive correlation between cord insulin/C-peptide levels and birth weight or birth centile. However, dividing the infants to three groups of SGA, AGA and LGA (small, average and large for gestational age) based on birth centiles, a significantly higher values of insulin was observed in LGA infants than the second group ((mean; µU/l): 4.46 >1.31, $p<0.05$). In conclusion, reducing maternal BMI (in particular maternal fat mass) at prepregnancy, might have an impact on the care of overweight diabetic women, in the sense that it might reduce the risk of fetal hyperinsulinemia and macrosomia. Further investigation on this specific group is required.

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HIGH 3-DEOXYGLUCOSONE (3-DG) CONCENTRATION IN MALFORMED RAT EMBRYOS *IN VITRO*

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Diabetic pregnancy is associated with increased risk of fetal malformation. High ambient glucose concentration has been identified as a teratologic agent in experimental studies, but possible effects of embryonic protein glycation have not been investigated. We measured the concentration of the glycating agent 3-deoxyglucosone (3-DG) in rat embryos after 48 h *in vitro* culture in 10, 30 or 50 mM glucose. These glucose concentrations produce a graded response of embryonic dysmorphogenesis. 3-DG is formed from the degradation of fructosamines and fructose-3-phosphate and reacts with proteins to form cysteinyl hemithioacetal adducts and advanced glycation endproducts. After *in vitro* culture, the embryonic content of 3-DG was: 0.3 nmol (10 mM glucose), 1.7 nmol (30 mM glucose), and 5.5 nmol (50 mM glucose). Furthermore, addition of 3-DG to culture medium with 10 mM glucose caused disturbed development. Embryos cultured in 10 mM glucose with no 3-DG had 29.6 somites and a malformation score (MS) of 0.1. (None or major malformation scored MS 0 or MS 10, respectively, therefore $0 \leq MS \leq 10$). Addition of 100 µM 3-DG yielded embryos with 24.3 somites and MS 4.0, addition of 500 µM 3-DG gave 23.1 somites and MS 6.2 whereas 1 mM 3-DG produced embryos with 18.8 somites and MS 7.7.

In conclusion, we have shown that increased ambient glucose concentration leads to increased embryonic concentration of the glycating agent, 3-DG. We also find 3-DG to be teratogenic *in vitro*, and suggest that protein glycation may have an important role in diabetic embryopathy.

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HYPERANDROGENISM AND HYPERINSULINEMIA AFTER PRE-ECLAMPTIC PREGNANCY

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Pre-eclampsia is known to be accompanied by metabolic changes similar to those in insulin resistance syndrome. We have recently demonstrated that some of the changes such as hyperinsulinemia is detectable 17 years after pre-eclamptic pregnancy. As hyperandrogenism is often related to insulin resistance we studied further this same study population and examined the serum concentrations of testosterone, estradiol (E2), dehydroepiandrosterone sulfate (DHEAS),androstenedione (ADIONI), calculated serum free testosterone and free androgen index (FAI), sex hormone binding glubulin (SHBG), insulin-like growth factor binding protein 1 (IGFBP), plasma endothelin-1 , insulin related binding proteins as well as the production of vasoactive endothelin.
Twenty-one women who had had previous pre-eclamptic first pregnancy and 20 BMI matched control women were studied. Women with prior pre-eclampsia had significantly elevated fasting serum insulin (7.1±0.6 vs 5.3±0.4 mU/l, mean ±SE, P=0.02) free testosterone levels (20.1±2.2 vs 15.1±1.2 pmol/l, P=0.03) and FAI (3.1 ±0.5 vs 2.0±0.2, P=0.04) whereas other parameters did not differ between the study groups. We conclude that a history of pre-eclampsia is associated with hyperadrogenism and hyperinsulinemia.

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INSULIN SENSITIVITY OF MUSCLE GLYCOGEN SYNTHASE DURING LATE PREGNANCY IN THE RAT

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During late pregnancy, maternal metabolism adapts to maintain an optimum fuel mix for presentation to the developing foetus, and a major adaptation involves the development of maternal insulin resistance in skeletal muscle with respect to glucose transport and phosphorylation. The present study examined the effects of pregnancy on skeletal-muscle glycogen synthase (GS) activation by insulin in vivo. The aim was to evaluate whether maternal glycogen storage is, or is not, resistant to the action of insulin in pregnancy through effects at the level of muscle GS. GS activity was measured in the absence (GSa) or presence (GSa+b) of 10 mM glucose 6-phosphate (G6P). Results are expressed as activity ratios (±G6P). The euglycaemic-hyperinsulinaemic clamp technique was used to produce a sustained elevation of the insulin concentration in the absence of any change in glycaemia. Muscles were sampled at 15 min after steady state had been reached. GSa and GSa+b activities were measured in three skeletal muscles containing predominantly fast-twitch fibres and in two skeletal muscles containing predominantly slow-twitch fibres. At 130-170 μU/ml insulin, mean %GSa for the three fast-twitch skeletal muscles were 46.7 ± 2.5 (n=15) for unmated rats and 51.0 ± 2.5 (n=18) for pregnant rats (n.s). The trend towards higher %GSa activities after insulin stimulation in the pregnant group achieved statistical significance for the slow-twitch muscles (unmated rats, 33.7 ± 2.9 [n=10]; pregnant rats, 55.7 ± 3.3 [n=12]; P<0.001). Insulin resistance at the level of GS activation is therefore not a feature of skeletal-muscle metabolism in late pregnancy at insulin concentrations in the high-physiological range. The findings are consistent with a lower EC50 for activation of GS than for stimulation of glucose transport by insulin. The possibility is raised that GS activation may play a role in carbohydrate homeostasis even when insulin concentrations are suboptimal for glucose clearance, possibly through limiting net glycogenolysis. By giving priority to maternal glycogen storage, loss of carbon is minimised and, under conditions where the carbohydrate supply is restricted and glucose oxidation suppressed, lactate and alanine derived from muscle glycogenolysis will be made available for gluconeogenesis.

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EVIDENCE AGAINST A ROLE FOR HYPERINSULINAEMIA IN THE GENESIS OF GESTATIONAL INSULIN RESISTANCE

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During pregnancy hyperinsulinaemia is associated with diminished insulin action. It has often been suggested that hyperinsulinaemia may cause insulin resistance. This hypothesis was tested in pregnant rats, which were treated with insulin (4.8 IU/day) from day 8 to 14 of gestation while normoglycaemia (5.0 mmol/l) was maintained by i.v. infusion of D-glucose. Despite constant elevated plasma insulin levels, the amount of glucose infused daily to maintain normoglycaemia increased from 12 ± 1 g/day on day 8 to 20 ± 1 g/day on day 14, suggesting improvement of insulin action. Saline-infused controls showed increased insulin responses to an i.v. glucose load (0.5 g/kg body weight; area under curve [AUC]: 243 ± 29 vs 57 ± 10 ng/ml min, $p<0.05$) accompanied by unchanged glucose disappearance on day 15 of pregnancy compared to virgin controls, suggesting decreased insulin action. This was associated with reduced 2-^{18}Fluor-2-deoxy-D-glucose (FDG) uptake, as measured by PET scanning, in heart (0.48 ± 0.10 vs 1.81 ± 0.20 mmol/l min, $p<0.05$) and brown adipose tissue (BAT: 0.11 ± 0.02 vs 0.30 ± 0.08 mmol/l min, $p<0.05$), but not in liver, brain and skeletal muscle. Furthermore, the relative GLUT4-protein content was diminished in heart (112 ± 23 vs 209 ± 25 %, $p<0.05$), BAT (114 ± 11 vs 175 ± 6 %, $p<0.05$) and white adipose tissue (WAT: 10 ± 4 vs 56 ± 21 %, $p<0.05$), but not in white gastrocnemius, red gastrocnemius and soleus muscle. 24 h after cessation of the combined insulin/glucose treatment, glucose-stimulated insulin secretion (AUC: 158 ± 16 vs 243 ± 29 ng/ml min, $p<0.05$) was diminished while glucose disappearance remained unaffected when compared to saline-infused day 15-pregnant controls, suggesting improved insulin action. This was associated with increased FDG uptake in BAT (0.28 ± 0.03 vs 0.11 ± 0.02 mmol/l min, $p<0.05$), and increased GLUT4 content of WAT (54 ± 10 vs 10 ± 4 %, $p<0.05$). Thus, prolonged hyperinsulinaemia exerts beneficial effects on glucose uptake and insulin action during pregnancy, presumably mediated by actions on (brown and white) adipose tissue. This argues against a causative role of hyperinsulinaemia in the impairment of insulin action during pregnancy.

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VITAMIN E TREATMENT INCREASE CuZn-DISMUTHASE ACTIVITY IN DIABETIC RAT PREGNANCY.

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The damage of antioxidant system in diabetic pregnancy is postulated as one of the main mechanisms that play an important role in the disturbances of embryonic development in human pathology as well as in the experimental animal models of the diabetic pregnancy. Tocopherol in a dose of 40 mg/kg/day acts as prophylaxis providing the metabolis changes in the experimental diabetic pregnancy. The aim of the study was to establish, if moderate, physiological doses of vitamin E could play a role in the prevention of antioxidant system disturbances in a rat diabetic pregnancy. In 30 female Wistar rats, diabetes were induced 7-12 days before conception using streptozootocin in one dose 40 mg/kg. The mean plasma glucose level in this group was 16 +- 8 mmol/l. These animals were divided into two gruops of 15 each. The first group was treated with vitamine E oil solution orally in a dose of 3 mg/kg of chow, and the second group was given only standard diet. Control groups consisted of 10 healthy pregnant animals treated with the same dose of vitamin E, 10 pregnant rats fed standard diet as well as 10 non pregnant rats. In both experimental and control groups the activity. of CuZn-dismuthase was estimated according to Misra and Fridovitch in tissues of uterus and liver in mother rats and in liver and lungs in neonates in the first day of live. The mean weight of neonates in diabetic pregnancy was found to be higher in comparison to control groups. The activity of dismuthase in animals treated with vitamin E was significantly higher in comparison to diabetic pregnancy without vitamin E - in the mothers liver 1.11 +- o.45 ng/mg of protein vs 1.90 +- 0.39 ng/mg of protein ($p<0.01$); mothers uterus 0.59 +- 0.21 ng/mg of protein vs 0.93 +- 0.21 ng/mg of protein ($p<0.05$); neonatal liver 0.74 ng/mg of protein vs 0.96 +- 0.14 ng/mg of protein ($p<0.05$); neonatal lungs 0.50 +- 0.09 ng/mg of protein vs 0.74 +- 0.13 ng/mg of protein ($p<0.05$). In both groups of diabetic animals, activity of estimated enzymes was lower in comparison to the control groups. In conclusion, the study shows that administration of moderate doses of vitamin E to the diet of diabetic pregnant rats elevated the activity of antioxidant system and diminished the complications of diabetic pregnancy - the fetal macrosomia and hypoglycemia in the rats neonates.

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INCREASE IN SERUM PROINSULIN CONCENTRATION AND PROINSULIN/INSULIN RATIO IN PREGNANT WOMEN

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Serum proinsulin is disproportionately elevated both at fasting and after an oral glucose load in non-insulin dependent diabetes mellitus. We investigated the effect of pregnancy on serum proinsulin level and the proinsulin/insulin ratio (PI/IRI) during an oral glucose tolerance test in pregnant women. Forty-six pregnant women with normal glucose tolerance (NGT), 60 pregnant women with borderline glucose intolerance (BGI), and 10 nonpregnant healthy controls were examined by 75g-oral glucose tolerance test(OGTT). Serum proinsulin was measured by radioimmunoassay using specific antiserum for human proinsulin. Fasting proinsulin levels in nonpregnant controls, NGT in early gestational period, NGT in late gestational, BGI in early gestational and BGI in late gestational were 5.2±1.2, 6.1±5.9, 8.9± 4.5, 8.6±5.5 and 10.9±4.4 pmol/l(mean±SD), respectively. Fasting PI/IRI ratios in these groups were 0.12±0.04, 0.12±0.09, 0.19±0.10, 0.19±0.13 and 0.27±0.14, respectively. In both NGT and BGI, fasting proinsulin levels and the PI/IRI ratio in late gestational period were significantly higher than those in early period. In 27 cases with BGI , OGTT was repeated in the late gestational period. Amelioration of glucose tolerance was observed in 6 cases (group A), but not in 21 cases (group B). Summed values of the serum PI/IRI ratio during OGTT in early gestational period in group A was lower than group B (0.08±0.02 vs 0.15±0.07, $p<0.05$). The results suggest that serum PI/IRI increases during pregnancy and that serum PI/IRI in early gestational period in BGI case serve as a predictor of glucose tolerance in late gestational period.

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INFLUENCE OF VITAMINE E TREATMENT ON ANTIOXIDANTS SYSTEM ACTIVITY IN THE RAT DIABETIC PREGNANCY.

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Diabetes mellitus decreases the activity of antioxidant systems in both, human and experimental diabetes animals. It is concomitant with dysmorphogenesis observed in diabetic pregnancy. The aim of the study was to estimate the influence of the vitamin E treatment on the antioxidant systems in the rats diabetic pregnancy. 30 pregnant Wistar rats were divided into two different groups of 15 each. The first group was treated with vitamine E oil solution orally in a dose of 40 mg/kg /day. The second group of animals was given only oil with the stanndard diet. On the 7th day of pregnancy streptozootocin in a dose of 40 mg/kg/day was given to induce diabetic conditions. The mean plasma glucose level in this group of animals in the day of delivery was 15 mmol/l +- 4 mmol/l and there was no difference between diabetic rats recived vitamin E and that not recived it. A control group consisting of 10 healthy pregnant animals treated with this same dose of vitamin E, 10 pregnant rats fed with standard diet and 10 non pregnant female rats. In all groups of animals the activity of glutatione peroxidase was estimated accdording to Paglia and Valentine and CuZn-dismuthase was estimated according to Misra and Fridovitch in tissues of mother and neonatal liver, mothers liver and neonatal lungs. The mean weight of neonates born to diabetic mother was higher then the control group. The mean activity of dismuthase and glutathione-peroxidase in animals treated with vitamin E was significantly higher. For example in the mother liver 1.55 +- 0.31 IU/mg of protein in comparison to diabetic pregnancy without vitamin E treatment 1.82+-0.33 IU/mg of protein ($p<0.05$) and adequately glutathione peroxidase 9.34 +- 2.21 ng/mg of protein vs 12.12 +- 2.12 ng/mg of protein ($p<0.05$). In both groups of diabetic animals, the activity of estimated enzymes was lower in comparison to control groups. We conclude, that the administration of vitamin E to the rats with diabetic pregnancy is effective and increase the activity of antioxidant enzyme system and decreased the complication of diabetic pregnancy.

PS 20
Hypoglycemia

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EFFECT OF DURATION OF RECENT, ANTECEDENT HYPOGLYCAEMIA ON RESPONSES TO SUBSEQUENT HYPOGLYCAEMIA IN HUMANS.

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It is generally accepted that recurrent hypoglycaemia (hypo) may induce hypo unawareness, but the number and/or duration of antecedent hypo required to exert the effect are not known. To assess the effect of duration of recent, antecedent hypo on responses of counterregulatory hormones (CR-H) and symptoms (Symp) to, and onset of cognitive dysfunction (CogDys) during, hypo, 8 normal volunteers were studied with the hyperinsulinaemic-hypo clamp (plasma glucose, PG, was decreased stepwise from 5.0 to 2.2 mmol/l) on 4 different occasions at 1 month intervals (Studies 1-4). On the day prior to studies, either euglycaemia (S1), or 1 insulin-induced hypo (PG 2.7 mmol/l, 21.00-23.00 h) (S2), or 2 hypo (13.00-15.00 h and 21.00-23.00 h) (S3), or 3 hypo (08.00-10.00, 13.00-15.00 and 21.00-2300 h) (S4) were performed. In S2, increase in CR-H initiated at lower PG (i.e. thresholds were higher) and maximal responses of adrenaline, glucagon, growth hormone and cortisol were all lower ($p<0.05$), but neither autonomic nor neuroglycopenic Symp and CogDys (battery of 12 different tests) were affected vs S1 (p=NS). In S3, and to larger extent in S4, not only CR-H, but also Symp (both autonomic and neuroglycopenic) and CogDys had thresholds greater and maximal responses lower than in S1 and S2 ($p<0.05$). Conclusions: the duration of recent, antecedent hypo (i.e. the number of hypo multiplied the duration of single hypo) is the main determinant of loss of responses of CR-H, Symp and CogDys to subsequent hypo. A single episode of hypo does not induce hypo unawareness, but only mild impairment in CR-H responses. Multiple, recent episodes are required to induce hypo unawareness. There is a hierarchy of loss of responses to hypo after multiple episodes of recent, antecedent hypo, i.e. responses of CR-H are lost first, and responses of Symp and CogDys second.

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SYMPATHETIC AND PARASYMPATHETIC ACTIVITY DURING HYPOGLYCAEMIA IN OBESE AND LEAN SUBJECTS

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Abnormalities of the sympathetic nervous system has been hypothesized in the aetiology or maintenance of obesity. However conflicting results, describing either a reduced or an increased sympathetic activity, are reported in obesity. Since hypoglycaemia is a powerful stimulus of both sympathetic and parasympathetic nervous systems, our study was designed to examine the effects of insulin-induced two-stepped hypoglycaemia on norepinephrine (NA), epinephrine (A) plasma levels and on the neuroautonomic function, evaluated by power spectral analysis (PSA) of heart rate variability in obese subjects and healthy lean controls. PSA comprised two frequency domain components: high (HF) and low (LF) frequency power, reflecting respectively parasympathetic and sympathetic activity; the LF:HF ratio reflects the sympatho-vagal balance. A modified glucose clamp technique (insulin infusion rate 40mU/min/m^2bs) was used to produce a standardized fall in glycaemia in 10 obese and 8 lean subjects. NA and A response and PSA were collected at baseline and during the two steps of hypoglycemia. Obese patients had a decreased response to hypoglycaemia regarding plasma NA and, in particular, A in respect to controls. In the lean group an increase in heart rate was showed ($p < 0.005$), but this finding was not observed in the obese group. The LF:HF ratio was increased in both groups ($p< 0.01$), in particular in the controls. While HF values decreased at the same extent (b= - 8.9 vs b= - 8.7, p=0.02 vs 0.04), LF values increased only in normal subjects ($p<0.05$). In conclusion, obese subjects seem to have a similar vagal withdrawal, but a decreased sympatho-adrenal function and a depressed cardiac sympathetic responsiveness to insulin-induced hypoglycaemia.

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EFFECTS OF LACTATE INFUSION ON HEPATIC AND MUSCLE GLUCOSE METABOLISM UNDER HYPOGLYCAEMIA.

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Hypoglycaemia is characterized by several metabolic alterations involving glucose metabolism in skeletal muscle and liver. Moreover several hormonal and cognitive responses to hypoglycaemia are reduced by administration of lactate in humans and animals. Little is known about the metabolic interactions between lactate and glucose under hypoglycaemia. In order to clarify the effect of hypoglycaemia and lactate on glucose metabolism we studied anesthetized normal rats under euglycaemic and hypoglycaemic clamp and infused with either sodium lactate or buffer solution. 3 experimental designs were carried out: 1) euglycaemic hyperinsulinemic clamp combined with the 3-^{3}H-glucose technique. 2) hypoglycaemic hyperinsulinemic clamp combined with the 3-^{3}H-glucose technique. 3) hypoglycaemic hyperinsulinemic clamp combined with the 2-deoxyglucose technique. In study 1 and 2 we calculated glucose rate of disappearance, hepatic glucose release, glucolytic flux and glycogen storage. In study 3 glucose utilization index was evaluated in individual skeletal muscles.
Results from our experiments show that hypoglycaemia per se reduced glucose rate of disappearance (24.5±0.5 vs 12.4±0.9 mg•min^{-1}•kg^{-1}; p<0.01) reducing to a similar extent both the glycolytic flux and glycogen storage. Also muscle glycogen content was reduced at the end of hypoglycaemic compared to euglycaemic clamp. Lactate infusion under hypoglycaemia did not alter overall glucose rate of disappearance (12.4±0.9 vs 13.9±0.8 mg•min^{-1}•kg^{-1}) while inhibited the suppression of hepatic glucose release by hyperinsulinemia (-1.0±0.9 vs 9.3±1.6 mg•min^{-1}•kg^{-1}; p<0.01). No differences were observed in glucose utilization index neither in individual skeletal muscles.
In conclusion our results show that hypoglycaemia inhibits both glycolysis and glycogen storage in skeletal muscle. Moreover lactate may influence hepatic glucose metabolism under insulin-induced hypoglycaemia by enhancing glucose release without affecting overall glucose disposal. We speculate that lactate may potentially be a protective mechanism against hypoglycaemia providing 3 carbons substrates for hepatic gluconeogenesis thus enhancing hepatic glucose production.

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EFFECT OF HYPOGLYCAEMIA ON BETA ADRENERGIC SENSITIVITY

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Reduced peripheral tissue sensitivity to catecholamines is suggested to be one of the mechanisms contributing to hypoglycaemia unawareness. It has been shown that a single episode of hypoglycaemia reduces awareness of hypoglycaemia. Therefore we tested the hypothesis that a single episode of hypoglycaemia reduces hypoglycaemia awareness by reducing beta adrenergic sensitivity. 10 healthy subjects (7 male, 25±4 years old) were studied twice using an isoproterenol test (IT) (0.25, 0.5, 0.75, 1.0, 1.5, 2.0 and 2,5 µg isoproterenol i.v. every 20 minutes) at 7:00: once after a hyperinsulinaemic, euglycaemic clamp (E) and once after a hyperinsulinaemic, hypoglycaemic (blood glucose 3 mmol/l) clamp (H) with 1 mU/kg/min insulin performed between 19:00 and 22:00. Heart rate and blood pressure were recorded continuously with computerized devices, blood glucose and free fatty acids were measured 5 minutes before and after isoproterenol injection. Increment of heart rate during IT was significantly higher after H (p=0.002, MANOVA), the dose required to increase heart rate by 25 beats per minute was 0.83±0.22µg after H and 1.13±0.21µg after E (p= 0.036). Systolic and diastolic blood pressure during IT increased (p=0.02) but showed no difference concerning preceeding H or E. Blood glucose levels didn't change during IT and showed no difference between H and E whereas overall fatty acids during IT were higher after H (0.054±0.02mmol/l) compared to E (0.45±0.01 mmol/l, p<0.001). We conclude that in normal subjects antecedent hypoglycaemia does not impair but increases sensitivity to catecholamines of heart and adipose tissue. This positive feedback is probably one factor preventing hypoglycaemia unawareness in normal subjects.

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LACTATE AND HYPOGLYCAEMIA INHIBIT INSULIN-MEDIATED GLUCOSE UTILIZATION BY HEART MUSCLE.

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Heart muscle can oxidize different energy-providing substrates to meet energy requirements. Several pathological conditions including diabetes, are characterized by an increased concentration of plasma lactate. The aim of this study was to investigate the role of increased lactate availability on glucose utilization in heart muscle under different plasma glucose concentrations ranging from mild hypoglycaemia (2.8 mmol/l) to hyperglycaemia (13 mmol/l). Sodium lactate or sodium bicarbonate were infused in anesthetized rats and animals underwent a euglycaemic, hypoglycaemic or hyperglycaemic clamp study. At steady state glucose concentration, a bolus of 2-deoxy-(1-^{3}H)-glucose was administered accumulation in of 2-deoxy-(1-^{3}H)-glucose-6-phosphate in heart muscle was measured. Results show that lactate inhibits glucose uptake in all the three conditions studied (euglycaemia: 17.5±4.5 vs 45.9±6.5 ng•mg^{-1}•min^{-1}, p<0.01, hypoglycaemia: 11.2±2.6 vs 32.6±3.0 ng•mg^{-1}•min^{-1}, p<0.01, hyperglycaemia: 15.7±1.6 vs 30.6±3.6 ng•mg^{-1}•min^{-1}, p<0.01). Moreover hypoglycaemia *per se* significantly reduced glucose utilization by 30% compared to euglycaemia. These results show that lactate may reduce heart glucose utilization regardless of glucose concentration and support the role of lactate as the preferred substrate for myocardial metabolism. Moreover they show that glucose utilization by heart muscle is reduced under hypoglycaemia. Taken together these results suggest that competition between lactate and glucose may reduce insulin sensitivity in heart muscle and may possibly play a pathogenetic role in the impaired glucose utilization in the diabetic heart.

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The effect of nocturnal hypoglycaemia on susceptibility to fatigue the following day

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This study assessed the effect of nocturnal hypoglycaemia on physical fatigue the next day using 10 subjects with Insulin Dependent diabetes. After an initial visit to determine workloads corresponding to 30 and 60% V_{O2} max (estimated from heart rate using bicycle ergometry), subjects were studied twice 4 weeks apart. One night blood glucose was lowered to 2.3-2.7mmol/l for 1 hour and during the other hypoglycaemia was avoided. Subjects underwent bicycle ergometry the next day initially for 30 minutes at the predetermined workload corresponding to 30% V_{O2} max, and then at 60% V_{O2} max until exhaustion. Fatigue was assessed every 10 minutes using the Borg scale. Although there was no difference in exercise capacity between visits (mean time 35.3±1.2min hypoglycaemic vs 38.2±1.8min control night, p>0.05), higher Borg scores were obtained after the hypoglycaemic night (p<0.05, ANOVA). There was no difference in glucose, potassium or catecholamine concentrations between visits either before exercise (mean glucose 14.9±0.84mmol/l vs 14.31±1.0mmol/l; potassium 4.5±0.14mmol/l vs 4.2±0.13mmol/l; adrenaline 0.17±0.04nmol/l vs 0.3± 0.03nmol/l; noradrenaline 1.7±0.26nmol/l vs 1.46±0.25nmol/l for hypoglycaemic and control nights respectively, all p>0.05) or at any point during exercise (p>0.05 ANOVA). Thus although 1 hour of nocturnal hypoglycaemia has no effect on exercise capacity, subjects feel more fatigued. This cannot be explained by differences in glucose, potassium or catecholamine concentrations.

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FATTY ACID UTILIZATION INFLUENCES THE COUNTERREGULATORY RESPONSE TO INSULIN-INDUCED HYPOGLYCEMIA IN RATS.

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Changes in the availability of energy substrates such as glucose and fatty acids (NEFA) lead to counterregulatory responses (CRs) to maintain energy homeostasis. The aim of the present study was 1) to characterize the counterregulatory response to insulin-induced hypoglycemia, and 2) to investigate the effects of changes in NEFA utilization on this CR. In the first set of experiments, four different concentrations of insulin (0.25, 0.5, 1.0 and 2.0 IU/h Velosulin® respectively) were infused for 90 minutes. In the second set of experiments, NEFA utilization was reduced by injection of the NEFA oxydation blocker mercaptoacetate (600 μmol/kg) during insulin-induced hypoglycemia (0.5 IU/h insulin). In control studies saline was administered. All studies were performed in permanently cannulated rats. Blood samples were withdrawn for determination of glucose, insulin, glucagon, adrenaline, noradrenaline and corticosterone levels. Hypoglycemia induced a dose-dependent increase in glucagon and adrenaline (peak values of glucagon 89 ± 7, 73 ± 9, 110 ± 13 and 151 ± 18 pg/ml and of adrenaline 38 ± 16, 5 ± 4, 105 ± 64 and 400 ± 134 pg/ml respectively (average ± SEM; Mann-Whitney U p-values all < 0.05)). Glucose levels were not different for the different concentrations of insulin (nadir levels 3.4 ± 0.2, 3.4 ± 0.1, 3.2 ± 0.2 and 3.0 ± 0.1 mmol/l, $p > 0.05$). Blockade of NEFA utilization during hypoglycemia significantly increased the glucagon response (peak values 93 ± 9 vs. 182 ± 13 pg/ml, $p < 0.05$) and the adrenaline response (peak values 5 ± 4 vs. 756 ± 108 pg/ml, $p < 0.05$). Blood glucose levels were slightly higher than in the control experiment. The data reveal that the counterregulatory response to insulin-induced hypoglycemia depends on the availability not only of glucose but also of fatty acids as energy substrates.

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HIGH PREVALENCE OF NOCTURNAL HYPOGLYCAEMIA IN CHILDREN WITH TYPE 1 DIABETES : ANALYSIS OF SLEEP PHYSIOLOGY

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Nocturnal hypoglycaemia in young children with IDDM may disturb sleep and subsequent cognitive function. In order to determine the prevalence of hypoglycaemia in prepubertal children and to examine its effects on sleep physiology and cognitive function two metabolic profiles were performed at home, 20:00 - 08:00 hours, in children aged 8.7 (5 9-12.9) years, duration of diabetes of 4.1(1.2-10.2) years. Blood was taken for glucose every 15minutes, every 60 minutes for insulin and metabolites and sleep EEG was recorded during the profile. Uncooked cornstarch was administered to those hypoglycaemic on night 1 to avoid hypoglycaemia on night 2 17 out of 23 were hypoglycaemic on one night (70%) The hypoglycaemic episodes were both profound, median glucose nadir 2 2(1.1-3.4) mmol/l and of long duration, median duration 210(15-630) minutes, despite waning insulin levels. Sleep recordings were analysed in 12 subjects (8 hypoglycaemic on night 1 and 4 on night 2). There were no significant differences, using Wilcoxon matched-pairs signed rank test (two-tailed), in total sleep time, 8.4(7.1 - 9.2) vs 8.6(6 8 - 9.7)hours, $p=0.784$ [median (10th -90th centile), hypoglycaemic vs non-hypoglycaemic nights, respectively]. There was a trend for reduced percentage of REM sleep, 11.6 (5.7 - 26.3) vs 15.2 (8.9 - 27.5)%, $p=0.158$, and increased number of arousals 6.5 (2 - 12.8) vs 3 (0 - 10.1)%, $p=0$ 136, on the night of hypoglycaemia. There were no associations between severity of the hypoglycaemic episodes and any of the sleep variables, perhaps reflecting apparent loss of adequate glucose counterregulation. However, overall the diabetic children had altered distribution of sleep with increased slow wave sleep ($p<0$ 0001) and increased number of arousals ($p<0.005$) compared to laboratory controls which could relate to recovery from previous hypoglycaemia This is being verified with controls undergoing home recordings.

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EFFECT OF HIGH GLUCOSE ON COUNTER_REGULATION HORMONE RESPONSE IN NIDDM VERSUS DMID.

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Diabetes mellitus induces defects in counterregulatory responses to reductions in glucose plasma levels.The important of these response has only been recently appreciated. We have study in 13 DMNID with BMI 38.7±7% (mean±ds) age 49.7±10 years and 8 DMID with BMI 23.6±5.1 age 31±10 years the response of glucagon,GH and cortisol to the infusion of 0.1 U/Kg insulin bolus after an overnight fast, basal glucose level DMNID vs DMID (226±65 vs 273 ± 67mg/dl, pns).We extraction sample at - 15 ,0,15,30,45,60,90 and 120 min. Basal plasma insulin level was higher in DMNID vs DID (33±16 vs 8±6.5, $p<0.05$), after insulin infusion insulin increased in DMNID vs DID to (563±158 vs 446±22, pns) at 15 min. and declined to basal level at 90 min.The lowering of blood glucose were not significantly between both group. During the hypoglycemic period glucagon level lower from the basal level (187±96 vs 163±80 pg/ml, pns) and was significantly lower at 120 min DID vs DMNID (102±69 vs 168±70 ,$p<0.05$). An absence of increase of cortisol and GH levels from basal levels was observed in both group DMNID and DID.In conclusion: insulin in hyperglycemic patients led to a decrease of glucose and glucagon with absence of cortisol and GH response.This observation provide new insights in the counter-regulatory response to hypoglycemia in diabetic patients.

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A POSITIVE CORRELATION BETWEEN THE MAGNITUDE OF THE SOMOGYI EFFECT AND THE DOSAGE OF INTERMEDIATE ACTING INSULIN.

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Somogyi suggested that insulin-induced hypoglycaemia can lead to hyperglycaemia in insulin-dependent diabetes, and the main aim of our study was to analyse the temporal relation between spontaneous episodes of hypoglycaemia and hyperglycaemia. There is little support, however, from clinical studies for significant hyperglycaemic 'counter-regulations' following hypoglycaemia. Using a physiologically based compartment model of the carbohydrate metabolism, we have analysed data on measured blood glucose, insulin injections, and meals from 20 inpatients with insulin-dependent diabetes and found evidence to support the occurrence of counter-regulations typically beginning 6-8 hours following hypoglycaemia and lasting for 16-18 hours with a magnitude of 4-10 mmol/l above expected blood glucose levels. We found a significant correlation between the dose of intermediate-acting insulin (as a fraction of total daily dose, F) and the magnitude of the counter-regulations (C, in mmol/l) according to the equation $C = 0.73 + 9.79 \times F$ ($r = 0.67$, $p < 0.001$). Other studies have suggested that elevated blood glucose levels following hypoglycaemia could be explained by reduced insulin absorption from subcutaneous depots in patients with insulin-dependent diabetes. Our data indicate that counter-regulations are related to the type of insulin treatment. We suggest that reduced absorption or subcutaneous inactivation of injected intermediate-acting insulin, rather than a direct effect of counter-regulatory hormones, may be a cause for the post-hypoglycaemic counter-regulatory response of elevated blood glucose concentrations.

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DIVERSITY OF HYPOGLYCEMIC THRESHOLDS FOR EPINEPHRINE SECRETION IN VARIOUSLY CONTROLLED DIABETES

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In daily diabetes practice, we often observe hypoglycemic symptoms due to epinephrine secretion to occur at wide hypoglycemic ranges. Some patients may perceive them at 90 mg/dl, while others don't at even as low as 40 mg/dl. To elucidate this diversity, we determined glycemic thresholds for counterregulatory hormone(epinephrine, GH, cortisol, glucagon) secretion, autonomic and neuroglycopenic symptoms by using artificial pancreas. Nineteen diabetics, aged 17–58 y.o., were analyzed. They were divided into 3 groups; high(H),low(L) and ordinary(O) threshold groups for epinephrine secretion. H was defined as having thresholds more than mean+2 S.D.(61.6 mg/dl) of normal controls, L less than mean−2 S.D.(40.6 mg/dl) and O in between H and L. H had significantly higher HbA1c level, 12.0 ± 3.5%, than O, 8.2 ± 0.6%. The HbA1c of L, 7.8 ± 1.5%,was not different from that of H, however had more hypoglycemic episodes(less than 40mg/dl in SMBG, 6.2 ± 2.9 times/month) than H, 0.5 ± 1.2 times/month. In general glycemic thresholds for epinephrine secretion pararell glycemic control, hypoglycemic episodes change this tendency. Therefore, those with poorly controlled diabetes usually having high thresholds could have normal or lowered thresholds for epinephrine secretion if they experienced recent hypoglycemia attacks. By knowing these facts we could develope better therapeutic means to avoid excess epinephrine secretion to minimize worsenig of diabetic angiopathies.

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THE BLOOD GLUCOSE ESTIMATION ACCURACY TRIAL PROVIDES A RELEVANT MEASURE OF HYPOGLYCAEMIA UNAWARENESS

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In several studies hypoglycaemia unawareness (HU) has been identified as the main cause of severe hypoglycaemia (SH). Investigation of HU is complicated by the wide range of definitions and assessment methods used. Often patient self-reports are used, providing a subjective measure of HU. Possibly a more reliable measure of HU can be derived from the "blood glucose estimation accuracy trial" (BGET), developed by Cox et al. The aim of this study was to determine the relationship of the BGET-derived measure of HU and of the self-reported degree of HU with the number of SH in the preceding year (as a clinically important consequence of HU) and with the level of fear of hypoglycaemia. So far we studied 11 insulin-dependent diabetes mellitus patients (6 men, 5 women, age (average) 32.5 (SD: 7.5) years, duration of diabetes 15.6 (6.1) years, HbA1c 7.8 (0.6) %, number of SH 1.8 (2.3), range 0-7) .For the BGET patients estimate and subsequently measure their blood glucose level 4 to 5 times a day during a period of 2 to 3 weeks. Both estimate and measurement are entered in a hand-held computer. The percentage of accurately estimated hypoglycaemic readings (% A-zone hypos) are used as a measure of hypoglycaemia awareness. During the initial visit patients filled in the worry scale of the HFS-95 and were asked to rate their ability to recognize hypoglycaemia on a scale from 0 to 5. They were given instructions for the BGET and made 70 estimates during the following 2 to 3 weeks. Data from two patients could not be analyzed because of early drop-out and lack of hypoglycaemic readings. A preliminary data analysis shows a highly significant correlation between % A-zone hypos and number of SH ($r = -.9$, $p < 0.0001$), but no significant correlation between self-reported hypo awareness and number of SH. Furthermore, a significant correlation was found between the HFS-95 worry score and self-reported hypo unawareness ($r = .9$, $p = 0.001$). The worry score did not correlate significantly with % A-zone hypos. These data suggest that the BGET may provide a clinically more relevant measure of HU than patient self-reporting. Furthermore, results so far suggest that BGET is applicable in clinical practice.

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COUNTERREGULATORY HORMONE AND SYMPTOM RESPONSES TO HYPOGLYCAEMIA IN DIABETIC CHILDREN

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We investigated hormonal and symptom responses to hypoglycaemia in 19 diabetic (age 14.2±1.4 years; mean±SD) and 16 nondiabetic children (14.4±1.0 years) with the glucose clamp technique. The hypoglycaemic thresholds for adrenaline, and for autonomic and total symptoms, were similar in the diabetic and nondiabetic groups, and were found at plasma glucose levels between 3.4 and 3.7 mmol/l. An increase of cortisol, growth hormone and glucagon was triggered at lower ($p<0.01$) glucose levels in the diabetic than in the nondiabetic children. Adrenaline, cortisol and glucagon increased less in the diabetic than in the nondiabetic group. In the diabetic children, BMI was positively correlated with the glycaemic thresholds for autonomic and total symptoms ($r=0.64$, $p<0.01$ and $r=0.72$, $p=0.001$, respectively). We conclude that hormone responses to hypoglycaemia are attenuated in diabetic as compared to nondiabetic children, whereas symptom recognition mainly is unaffected in the diabetic group. Diabetic children with a high BMI seem to have increased awareness of a declining plasma glucose level.

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EFFECT OF BLOOD GLUCOSE AWARENESS TRAINING ON EPINEPHRINE RESPONSES TO HYPOGLYCEMIA IN INTENSIVELY TREATED DIABETES

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To determine the effect of Blood Glucose Awareness Training (BGAT) on epinephrine (EPI) responses to hypoglycemia (HYPO) in IDDM during Intensive Diabetes Therapy (IDT), 36 subjects with uncomplicated IDDM (duration 3-15 yr; HbA1c = 9.02±0.22) were enrolled in a 4 month outpatient IDT program. Subjects were randomized to classes in BGAT or cholesterol awareness (CONTROL). All subjects underwent stepped hypoglycemic clamp studies prior to (Clamp 1) and at completion of IDT (Clamp 2). BG was lowered from 6.7 mmol/L (baseline) to 4.4, 3.9, 3.3, 2.8 and 2.2 mmol/L over 190 minutes. 18 subjects reduced their HbA1c by ≥ 1% with IDT. In this subgroup the mean HbA1c fell from 9.44 ± 0.49 to 7.50 ± 0.23 ($p<0.001$) for BGAT (n=9) and 9.73 ± 0.48 to 7.7 ± 0.38 ($p<0.001$) for CONTROLS (n=9) (p=NS between groups). EPI responses to HYPO were lower in the CONTROL group following IDT and this difference was most marked at the 3.3 mmol/L level ($p=0.05$). EPI responses to HYPO did not differ in the BGAT group.

Epinephrine pmol/L

Glucose	Clamp 1	Clamp 2	
	Controls		
3.3 mmol/L	1334 ± 538	787 ± 349	p=0.05
2.8 mmol/L	1512 ± 421	922 ± 210	p=0.08
2.2 mmol/L	3012 ± 670	2073± 395	p=0.2
	BGAT		
3.3 mmol/L	840 ± 218	802 ± 143	p=0.8
2.8 mmol/L	2075 ± 607	1890± 454	p=0.7
2.2 mmol/L	2367 ± 437	2923± 702	p=0.3

BGAT is associated with preservation of the EPI response to HYPO in IDDM subjects who achieved substantial improvement in glycemic control with IDT. BGAT may be useful in reducing the risk of iatrogenic hypoglycemia and blunted counterregulatory responses associated with strict glycemic control of IDDM.

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GLUCOSE INTOLERANCE IN HAMSTERS IS ASSOCIATED WITH INCREASED TURNOVER OF SEROTONIN AND NORADRENALINE IN THE VENTROMEDIAL HYPOTHALAMUS (VMH).

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The VMH is known to have a role in regulation of feeding, and of carbohydrate and lipid metabolism. Increases in noradrenergic activity within the VMH have been associated with increased hepatic glucose output and increased lipolysis, which may potentiate increased insulin resistance. Moreover, increases in central serotonergic activity has been shown to increase substantially both hypothalamic noradrenaline turnover and blood glucose concentrations. The present study employed in vivo microdialysis to investigate whether there are differences between the monoamine profiles in the VMH of freely moving naturally glucose-tolerant and glucose-intolerant Syrian hamsters. Male Syrian hamsters (ave BW 190 ± 10g) held on 14 hr daily photoperiods were divided into these two groups based on the results of glucose tolerance tests. Microdialysis cannula were implanted in the right VMH of each hamster. Dialysate samples were collected every hour for 24 hr through the microdialysis probe (perfused with Ringer's solution at 0.12 ul/min), and analyzed by HPLC with electrochemical detection. In glucose intolerant hamsters, the VMH extracellular levels of the metabolites of serotonin (5-hydroxyindolacetic acid) and noradrenaline (3-methoxy-4-hydroxy-phenylglycol) were 43% and 25% higher, respectively, than in glucose tolerant hamsters ($p<0.05$). There was no significant difference in VMH extracellular levels of a metabolite of dopamine (homovanillic acid). These findings demonstrate that increases in both serotonergic and noradrenergic activity within the VMH are associated with and may promote glucose intolerance. This conclusion is further substantiated by our recent study wherein treatment with bromocriptine, a dopamine agonist, improved glucose tolerance in obese hamsters while also reducing VMH serotonin and noradrenaline turnover.

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INSULIN INDUCED HYPOGLYCEMIA IN IDDM PATIENTS ADMITTED TO AN EMERGENY ROOM

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The aim was (1) to describe hormone responses in iatrogenic hypoglycemia and (2) to investigate if a combined treatment of insulin induced hypoglycemia with intravenous dextrose and intramuscular glucagon (A) would improve glucose recovery as compared to treatment with intravenous dextrose alone (B). Twenty adult patients with IDDM (age 47±4 y, BMI 23±1, diabetes duration 20±4 y, Hemoglobin A1c 7.8±0.5) admitted to the Emergency Room were randomized to one of the above treatments and plasma glucose and counterregulatory hormones were measured before and 30-120 min after treatment. Plasma glucose was 1.18±0.03 mmol/l on admission. Pretreatment counterregulatory hormone concentrations were significantly lower than concentrations during hypoglycemia in healthy control subjects (age 30±3 y, BMI 22±1) yet pre-treatment counterregulatory hormone concentrations were significantly above fasting concentrations in healthy control subjects for plasma epinephrine ($p=0.00024$), glucagon ($p=0.0012$), growth hormone ($p=0.00052$) and for cortisol ($p<0.00001$). Thirty min after treatment A plasma glucose had risen to 5.44±1.02 mmol/l and after treatment B 8.12±1.12 mmol/l ($p=0.042$). Despite access to food two of six patients in group A and one of five patients in group B had plasma glucose below 4.0 mmol/l after 120 min. In conclusion (1) the present study confirms low, yet significantly elevated concentrations of epinephrine and glucagon in diabetic patients admitted with hypoglycemia to an emergency room. (2) Plasma glucose concentrations were higher 30 min after a combined treatment of insulin induced hypoglycemia with both intramuscular glucagon and intravenous dextrose as compared to treatment with intravenous dextrose alone. (3) Regardless the initial treatment regimen a strict food regimen must be advised to prevent late recurrent hypoglycemia.

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EFFECT OF NICOTINOYL-GABA ON BRAIN METABOLIC STATE AND NEUROTRANSMISSION IN STREPTOZOTOCIN-INDUCED DIABETES

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We previously reported that serotonin-, dopamine- and GABAergic system functions deranged by diabetes. The aim of the study was to assess the mechanism of neurotropic action of nicotinoyl-GABA(pycamilon,P) in CNS-disfunction in diabetes.Four rat groups were studied: control and three groups of rats 1 month after induction of diabetes by STZ(70 mg/kg of body weight), two treated by P (20 and 200 mg/kg daily, 14 days) the forth untreated. The blood glucose level of diabetic rats was 312 mg/dl. The findings showed that both low- and high-dose P administration was accompanied by restoration of [U-^{14}C]GABA release by brain cortex synaptosomes, while for dopamine-and-serotoninergic systems his effect was absent. It was demonstrated that as a result of 6 hours injections of NA, GABA and P in low-dose brain NAD level increased to 0.204±0.014, 0.181±0.013, 0.220±0,015 vs 0.170±0.012 μmol/g, $p<0.05$ in diabetes respectively,while GABA level was almost unchanged. Brain ATP level in control was 2.07±0.07μmol/g. The P increasead this level to 1.62± 0.05 vs. 1.41±0.02 μmol/g, $p<0.05$ in diabetes. However, the P effect on cytosolic redox imbalance free NAD(P)-couples was negligible. It was shown that P inhibited NAD specific binding by diabetic synaptic membranes by 27%, besides NAD(1mM) inhibited P specific binding by 32%, as compared with diabetes. These results suggest that P was able to increase the NAD permeability into synaptosomes of diabetic rats. Our study suppouse that P plays an important role in improving the GABA-release mechanism and brain functional state in diabetic rats. Thus P is involved in the regulation of the processes in the brain streptozotocin-induced diabetes.

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DRUG INDUCED HYPOGLYCAEMIA IN DIABETIC PATIENTS-A REVIEW OF 114 HOSPITALISED PATIENTS

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We retrospectively evaluated 114 diabetic patients admitted to hospital with hypoglycaemia during the period Jan 95 to Mar 96 as defined by plasma glucose concentration ≤ 3.0mmol/l together with hypoglycaemic symptoms. It accounts for 0.5% of all admissions to the department of Medicine of Prince of Wales Hospital. All data were expressed as mean±SEM. The laboratory glucose on admission was 1.94±0.07mmol/l compared to 1.91±0.08mmol/l for capillary blood testing using a reagent strip. Sixteen patients wwere recieving OHA-insulin combination therapy. The remaining were divided into two groups for analysis-62 on oral hypoglycaemic agents (sulphonylurea± metformin,OHA) and 36 on insulin(INS). In the OHA group, 37 were on sulphonylurea alone and 25 on sulphonylurea plus metformin. These patients were older(71.4±1.56 vs 63.0±2.39years,$p<0.001$), mostly female(67.8%), and had a poorer premorbid state with multiple complications. Contrary to previous experience, the newer agents, gliclazide(n=13) and glipizide(n=10) accounted for 39% of hypoglycaemic episodes among the OHA group, while the remainder were receiving glibenclamide. These shorter-acting sulphonylureas were believed to be safer and hence selected for patients who were older and more complicated. Nevertheless, considerable hypoglycaemia still occurred. Restricted carbohydrate intake(64.04%), concurrent infection(32.2%), and other illness(45.7%) were commonly found predisposing factors. More severe clinical presentation in terms of duration of dextrose infusion was associated with plasma creatinine > 150μmol/l($p=0.06$), low albumin < 30g/dl($p=0.03$) and dependent premorbid state($p=0.01$). Drug interactions were not common, but ACEI therapy coexisted in 10%. We conclude that short acting sulphonylureas carry a significant risk of severe hypoglycaemia especially when used in elderly or complicated patients.

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FUNCTIONAL MRI CHANGES IN THE HYPOTHALAMUS OF STREPTOZOTOCIN-TREATED RATS AFTER GLUCOSE LOADING

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Functional MRI changes in the brain of streptozotocin(STZ)-treated diabetic rats was reported as follows; the hippocampus 20min. the paraventricular nucleus 40min., the dorso-medial hypothalamus(H). 60min. and the ventromedial H 100min. after insulin i.p. Overnight fasted male Sprague-Dawley strain rats, weighing 150g, treated with or without STZ(30 and 60mg/kgBW) i.p., were used, settled in a functional MRI(4.7 T). The metabolic change of each particular brain area was visualized chronologically by the magnetization, prepared rapid gradient echo pulse sequence method, following 2ml isotonic glucose solution injected, i.p., through the catheter. From 20 to 30min after glucose treatment, quite vivid metabolic change as the decrease of intensity was observed at the area between the hypothalamus and thalamus, especially the subfornical organ in case of rats injected with low dose or without STZ(control). These changes were recovered at 45min after glucose treatment. When rats were treated with high dose STZ, glucose treatment never caused any metabolic change in the same area of brain. These data suggested that fasted rats with glucose hunger could elicit certain metabolic change in the hypothalamus during glucose uptake into the brain and the neuronal activity of nuclei in these areas were declined due to recognition of sufficient glucose intake concurrently with insulin release, but STZ-induced diabetic rats never did because of insufficient glucose utilization peripherally.

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LONG-TERM ADMINISTRATION OF THEOPHYLLINE AND GLUCOSE RECOVERY AFTER HYPOGLYCEMIA IN IDDM.

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We tested the hypothesis that long term administration of theophylline augments glucose recovery after insulin induced hypoglycemia. The metylxanthine theophylline increases intrahepatic c-AMP. The major glucose counterregulatory hormones epinephrine and glucagon use c-AMP as second messenger when increasing hepatic glucose production. We have previously demonstrated an increase in glucose recovery when intravenous theophylline was administered during insulin induced hypoglycemia. Eleven healthy subjects (mean (range): BMI 24 (20-27), age 28 (22-34) y) and eight insulin dependent diabetes patients (BMI 24 (21-30) age 31 (22-37) y duration of diabetes 3 (1-10) y hemoglobin A1c 8.2 (5.8-12.1)) participated in two hypoglycemia experiments (intravenous insulin infusion for 60 min) preceeded by (in a randomized, placebo controlled double blinded design) the ingestion of theophylline or placebo for two weeks. The dose of Teophylline was adjusted to maintain serum levels of 55-85microM. We found an increase in plasma glucose area under the curve (calculated from the start of the insulin infusion to 150 min thereafter) after theophylline treatment ($p=0.03$ and $p=0.02$, healthy subjects and diabetes patients respectively). However, there were no significant concomitant increases in incremental area under the curve for plasma c-AMP ($p=0.60$ and $p=0.32$), for glucose production rate ($p=0.16$ and $p=0.71$) or for glucose disappearance rate ($p=0.20$ and $p=0.21$). As regards counterregulatory hormones no significant changes were observed except for a decrease in incremental area under the curve for plasma glucagon after theophylline in healthy subjects ($p=0.0007$). In conclusion: the biological significance of long term phosphodiesterase inhibition is limited in the counterregulation of hypoglycemia. Thus theophylline is not suitable for the prevention of insulin induced hypoglycemia.

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HYPOGLYCAEMIA DETECTION BY ECG RECORDING: REPRODUCIBLE EFFECTS ON T AND R WAVES

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ECG changes induced by hypoglycaemia might be used for hypoglycaemia detection. We compared reproducibility and magnitude of these changes between the 12 leads of a conventional ECG. On two study days 8 healthy volunteers (age 23±3 years, BMI 21.6±1.4 kg/m^2) received a s.c. injection of 0.15 U/kg regular insulin. During the subsequent 60 min blood glucose, insulin, potassium, and ECG changes (CS 3000, Picker-Schwarzer, Munich, Germany; amplitudes of R- and T-waves and QT-interval) were measured in 15 min intervalls. 60 min after insulin injection, blood glucose had declined from baseline values of 4.5 to 2.8 mmol/l, insulinaemia had increased from 41 to 193 pmol/l, while potassium levels fell from 3.9 to 3.7 mmol/l (all $p<0.05$). The ratio of R/T amplitudes showed a significant increase predominantly in the left precordial leads: 58 % in lead I ($p<0.01$), 55 % in lead V3 ($p<0.02$) and 63% in lead V5 ($p<0.005$). Repetition of the hypoglycaemic episode on study day 2 resulted in comparable changes in all measured parameters (R/T amplitude changes: 27 % in lead I, 70% in lead V3 and 135% in lead V5 ($p<0.05$)). The frequency-corrected QTc-intervals did not change in comparison to the initial values during the hypoglycaemic epsiodes. In conclusion, insulin-induced hypoglycaemic episodes resulted in reproducible ECG-changes in healthy subjects. These changes may be useful for a hypoglycaemia detection system.

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RESPONSE DIFFERENCES FOR RECOMBINANT GLUCAGON IN DOGS.

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A study of recombinant DNA-derived (rDNA) glucagon was conducted in beagle dogs for 2 weeks. This study was a placebo-controlled dose-ranging trial, with 3 dogs of each gender receiving daily subcutaneous doses of 0, 0.02, 0.06, and 0.2 mg/kg. Blood samples were collected for 2 hours after dosing for determination of serum glucose concentrations on study days 0 and 13. We calculated the maximum absolute blood glucose excursion from baseline (MAE), time to MAE (T_{MAE}), and the glucose excursion area under the curve (AUC_{ex}). Statistical comparisons were made across dose, gender, and day of measurement. Mean data are summarized below:

		Day 0			Day 13		
Dose, mg/kg	Sex	MAE, mg/dL	T_{MAE}, min	AUC_{ex}, mg•min/dL	MAE, mg/dL	T_{MAE}, min	AUC_{ex}, mg•min/dL
0	F	-3.0±19	50±61	-17±334	26±7	90±30	---
0.02	F	50±22	13±6	1038±966	109±16	23±12	5721±1578
0.06	F	44±4	10±0	593±208	76±6	10±0	2926±2001
0.2	F	103±34	23±12	5281±1145	115±28	27±6	8127±2669
0	M	-9.3±19	38±26	-144±301	8.3±20	87±58	---
0.02	M	40±18	10±0	542±219	41±13	17±6	1663±1151
0.06	M	58±4	10±0	627±214	76±9	10±0	2972±2028
0.2	M	74±25	37±21	971±613	82±4	10±0	3108±1010

Serum glucose concentrations peaked rapidly after subcutaneous injection of rDNA glucagon. Even though the glucodynamic response lasted for up to 2 hours, the response was greater on study day 13. A gender difference was detected with a greater response in females. In conclusion, rDNA glucagon is active and exhibits a dose-response in beagle dogs following a single subcutaneous injection.

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Well being and cerebral function the day after nocturnal hypoglycaemia

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This study assessed the effect of nocturnal hypoglycaemia on well being and cerebral function the next day. Ten subjects with Insulin Dependent Diabetes were studied twice 4 weeks apart. One night blood glucose was lowered to 2.3-2.7mmol/l for one hour, and at the control visit, hypoglycaemia was avoided. The following morning, well being was assessed using the Minor Symptom Evaluation Profile (MSEP) and cerebral function with PASAT, Digit Symbol Substitution Test (DSST), Trail Making B, Four Choice Reaction Time (RT) and Auditory P_{300} latency. All 3 components of the MSEP scored higher after the hypoglycaemic compared with the control night (mean scores 226±37.8 vs 168±24.7 contentment, $p<0.01$; 182±26.2 vs 126±18.9 vitality, $p<0.001$; 183±14.7 vs 135±24.4 sleep, $p<0.05$ for hypoglycaemic and control nights respectively). None of the cerebral function tests performed the next day were affected by nocturnal hypoglycaemia (mean Trail Making 55.9±6s vs 54.9±7.5s; DSST score 66.5±3.4 vs 69.2±4; PASAT 2.56±0.1s vs 2.57±0.19s; RT 414±17.7ms and 411±15.6ms ; P_{300} 309±5.34ms and 321±9.1ms for hypoglycaemic and control nights repectively, all $p>0.05$). Thus 1 hour of hypoglycaemia at night affects well being but not cerebral function the next day.

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INTRA-NASAL AND INTRA-MUSCULAR GLUCAGON IN HYPOGLYCAEMIC INSULIN-DEPENDENT DIABETIC SUBJECTS

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Glucagon treatment of severe hypoglycaemia could be facilitated if injection were obviated. Intra-nasal insufflation of a dry powder human glucagon (2.5 mg) formulation (NG) was compared to intra-muscular (IM) human glucagon (1 mg) in an open-label, random sequence study in 10 adult subjects with IDDM. Subjects were made hypoglycaemic (median glucose nadir 2.0 mmol/l (NG), 2.4 mmol/l (IM), NS (Wilcoxon)) by continuous intravenous insulin infusion, discontinued at the time of glucagon administration. A rise in plasma glucose was seen after glucagon in all subjects (median increment 1.5 mmol/l (NG), 1.2 mmol/l (IM), NS), the peak glucose occurring after 30 minutes in both groups. Peak plasma glucagon levels (median 1626 mg/l (NG), 1753 mg/l (IM), NS) occurred 15-20 minutes after administration by both routes. Mild-moderate nasal irritation of short duration was noted by 8 subjects immediately after nasal administration with mild erythema at rhinoscopy in 3 subjects. Intra-nasal and intra-muscular glucagon show equivalent metabolic responses and patient acceptability in hypoglycaemic adults. Easier administration by intra-nasal insufflation could extend the availability of this treatment option.

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MILD HYPOGLYCEMIA-INDUCED COGNITIVE DYSFUNCTION IS INDEPENDENT OF ANXIETY AND IS LIMITED TO DIABETIC SUBJECTS.

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To examine the relationship between anxiety, autonomic nervous system (ANS) responsivity and cognitive functioning during mild hypoglycemiain in adolescents and adults, we studied in 25 individuals with IDDM (age = 18.6±5.4 yrs (range 12-28yrs); duration = 8.9±9.5 yrs; M ± SD) and 16 age-matched nondiabetic controls. Each subject participated in a euglycemic (Eu) and a Hypoglycemic (Hypo) clamp, in random order, 2 months apart. During the Hypo, serum glucose was clamped at 5.6mm/L for 90 mins, dropped to 3.3mm/L and maintained for 60 min. Plasma epinephrine (Epi), norepinephrine(Nor) and pancreatic polypeptide (HPP) were measured every 15 min. A battery of cognitive function tests including Letter Rotation Reaction Time Test (LR), Digit Vigilance (DV) and Trail making B (Trail B) and self-reported anxiety (Spielberger State Anxiety Inventory) were performed during the last 30 mins of both hypo and eu clamps. The increment in anxiety levels during hypo was significant in both groups, but greater in controls. However significant declines on cognitive function were limited to IDDM subjects. In neither group was cognitive change correlated with age or anxiety. The mean nor during this time was significantly correlated with anxiety levels in both groups. Cognitive function deterioration correlated with ANS hormones only within the control group.

The table represents correlations with mean epi, nor and HPP levels during the 30min testing period.

	HPP : r (p)		EPI : r (p)		NOR: r (p)	
	control	IDDM	control	IDDM	control	IDDM
Anxiety	.2(.2)	-.2(.2)	.3(.1)	.1(.4)	6(.004)	.2(.2)
LR	.5 (.02)	.2(.2)	6 (.008)	.1(.4)	.5 (.02)	-1(.4)
DV	-.7 (.001)	-.1(.3)	-.1(.3)	-.1(.3)	-.3(.1)	-.03(5)
Trail B	.5 (.03)	-.2(.2)	.3 (.1)	-.04(4)	3 (.1)	.1(.3)

Conclusion: Only IDDM subjects showed significant cognitive deterioration which was independent of their level of anxiety and age.We speculate that the differences in cognitive function during hypoglycemia between subjects with and without IDDM may relate to differences in adaptation of glucose transport into the brain.

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HELICOBACTER PYLORI-INDUCED GASRITIS MAY CONTRIBUTE TO THE OCCURRENCE OF POSTPRANDIAL SYMPTOMATIC HYPOGLYCEMIA

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We have previously shown that *Helicobacter pylori* (*HP*) gastritis enhances glucose and meal-stimulated insulin release. Furthermore, in our clinical experience, postprandial symptomatic hypoglycemic (PSH) patients with *HP* gastritis showed a substantial improvement in their hypoglycemic symptoms after the eradication of *HP*. Therefore, in this study we have investigated whether *HP* gastritis may contribute to the occurrence of PSH. For this purpose, we have evaluated below parameters in 10 PSH patients with *HP* gastritis before and one month after the eradication therapy: a) the number of PSH attacks occurred in a one month period using 30 day diary, b) the total symptom score following a mixed meal using visuel scale analogue questionnaire (VSAQ), and c) the glucose and insulin responses to the mixed-meal. Before the eradication, all the patients had repetitive symptoms, 2-4 hours after their regular meals, consistent with chemical hypoglycemia (glucose values less than 60 mg/dL in at least three different symptomatic periods). After the eradication of *HP*, the areas under the curve for serum insulin decreased by 14.2% ($p<0.001$) whereas for plasma glucose increased by 9.4% ($p<0.001$) following the mixed meal. The mean±SD number of PSH attacks occurred in one month period decreased significantly from 35.6±7 to 16.5±3.8 ($p<0.0001$) and the mean±SD of the total symptom score in VSAQ decreased significantly from 5.8±1.7 to 2.9±1.2 ($p<0.0001$). In conclusion, our data suggest that *HP* gastritis may contribute to the occurrence of PSH.

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Plasma endothelin response to acute hypoglycaemia in insulin-dependent diabetes mellitus (IDDM).

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Acute hyypoglycaemia induces major haemodynamic changes mediated principally through activation of the sympatho-adrenal system and secretion of catecholamines and vasopressin. The endothelins are the most potent vasoconstrictor agents known, and have a central role in cardiovascular regulation.

The aim of the study was to determine whether acute insulin-induced hypoglycaemic provokes detectable alterations in peripheral plasma endothelin concentrations (ET) in patients with IDDM.

Plasma immunoreactive ET concentrations were determined in 20 patients with IDDM during insulin-induced hypoglycaemia, at baseline, at the onset of the autonomic reaction to hypoglycaemia (R) and at 15, 30, and 60 minutes thereafter. A significant increase in plasma ET concentrations was observed : from 3.81 ± 0.32 pg ml^{-1} at baseline to 6.72 ± 1.47 pg ml^{-1} at 60 minutes after the onset of the hypoglycaemic reaction ($p<0.05$).

Acute insulin-induced hypoglycaemia induced a rise in plasma ET concentrations in patients with IDDM occurring after the onset of the acute autonomic activation. This finding is consistent with the hypothesis of a putative role for ET in the mediation of hypoglycaemia-induced vasoconstriction, and possible precipitation of macrovascular or microvascular events.

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COMPLEX CARBOHYDRATES CAN PREVENT NOCTURNAL HYPOGLYCEMIA IN DIABETIC CHILDREN AND ADOLESCENTS.

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Common risks for hypoglycemia in children with type 1 diabetes mellitus are exercise, decreased food intake or excess insulin administration. In order to prevent nocturnal hypoglycemia in patients with type 1 diabetes mellitus with complex carbohydrates, a pilot-study was designed with 8 children 9.1-17.4 years old; the patients were selected from a group of diabetics with personal history of frequent symptomatic nocturnal hypoglycemia. We studied the patients twice, once for test and once for control study. On the test day the standard evening snack, was replaced by a test "snack" not containing mono- or disaccharides. Both the snacks, standard and test, were equivalent in energy and total carbohydrate content was similar. Blood samples were collected and when the patient had blood glucose < 60 mg/dl or symptoms of impending hypoglycemia, intervention with extra carbohydrates occurred. Six out of 8 children needed intervention after the standard snack. After the test snack this intervention was necessary in 3 out of 8 children. The time of intervention ranged from 11 p.m. to 4 a.m. and 10 p.m. to 12 a.m., respectively, on the day of the standard and test snack. Raw cornstark, as a source of complex carbohydrates, can be useful in the prevention of nocturnal hypoglycemia; moreover, blood glucose levels dropped more slowly than those after the standard snack. A combination of complex and semi-complex carbohydrates in the last evening snack or meal might be effective for the prevention of nocturnal hypoglycemia.

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MICROCIRCULATION DURING INSULIN INDUCED HYPOGLYCEMIA - THE IMPACT OF ENDOTHELIN

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In order to investigate whether hypoglycemia might influence microcirculation, and if so, whether the endothelium derived vasoconstrictor endothelin [ET] might participate in these mechanisms 8 male healthy volunteers (age 21 - 34, mean 25 $\pm$ 4 years, body mass index 22.4 $\pm$ 0.6 kg x m^{-2}) were studied. Subjects received in random order either 0.1 IU/kg body weight regular insulin (hypoglycemia) or 0.1 IU/kg body weight regular insulin plus glucose 10 % (glucose clamp) or 1 ml sodium chloride 0,9 % (placebo) intravenously on three separate days according to a double-blind, randomized study protocol. For the following 60 minutes we determinined in 10-minutes intervals blood glucose, adrenocorticotropin [ACTH], cortisol and ET as well as heart rate and blood pressure. Microcirculation was assessed at the same time points by measuring capillary blood cell velocity at rest using nailfold capillaroscopy of the left hand. Thirty minutes after insulin injection ischemia (200 mmHg for 2 minutes using a cuff around the hand ankle) was performed as a provocative test in which the time to peak capillary blood cell velocity [tpCBV] during reperfusion was measured. Hypoglycemia (blood glucose 38 $\pm$ 4 mg/dl, $p < 0.001$ vs placebo and glucose clamp) resulted in a significant increase in the tpCBV as compared to glucose clamp or placebo (23 $\pm$ 4 s vs 8 $\pm$ 2 and 10 $\pm$ 2 s respectively, both $p < 0.01$) as a parameter of an impaired capillary perfusion. In the hypoglycemia experiments only there was a subsequent rise in ET [$p < 0.05$ vs before insulin injection] as well as ACTH and cortisol [both $p < 0.01$ vs before insulin injection] as a sign of an activated pituitary-adrenal axis. Capillary blood cell velocity at rest as well as blood pressure and heart rate remained unchanged in all experiments. These results demonstrate that not insulin per se but the hormonal response to hypoglycemia is the cause for the impaired microcirculation. An increased release of the potent vasoconstrictor ET might participate in the mechanisms of the decreased capillary perfusion.

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THE HYPOGLYCEMIC / HYPERGLYCEMIC EFFECTS OF ANACARDIUM OCCIDENTALE (CASHEW)

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The use of herbal medicines in diabetes treatment is still an ongoing practice in Third World Countries. The Bark of the Cashew tree is widely used as an oral hypoglycemic agent. Preliminary studies by a group at the University of the West Indies , showed that a crude extract from the bark of the Cashew plant exhibited the hypogycemic principle. This investigation sought to isolate, purify and elucidate the structures of compounds found to show activity. Three compounds have been isolated with hypo/hypergycemic activities and the identity of these will be discussed. The isolation method involved solvent extraction while purification was performed using various chromatographic techniques. The structures will be confirmed by spectral and chemical methods, using High Resolution Fourier Transformation (FT-NMR), Heteronuclear Multiple Bond Correlation (HMBC), Heteronuclear Multiple Quantum Coherence (HMQC) and Total Correlation Spectroscopy (TOCSY) etc. The activities were assayed by the use of Oral Glucose Tolerance Test, using the glucose oxidase method to determine the blood sugar concentrations. The compounds were administered intravenously in dimethyl sulfoxide to the dogs (n=10) in a concentration of 1.0-3.5mg/kg body weight. In all experiments,each dog was used as its own control where only dimethyl sulfoxide was administered intravenously. When compared to the controls, two compounds showed significant blood sugar lowering effects where all the values obtained for the extract curve , from FBS II to 2 hours inclusive are significantly less ($p < 0.05$ at 95% confidence limit). However, the third compound displayed significant increase in blood sugar ($p < 0.01$) at FBS I. The combined effect of the two hypoactive compounds thus produced an overall hypoglycemic effect.

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REGULATION OF PRO- AND CONTRAINFLAMMATORY CYTOKINES DURING HYPOGLYCEMIA.

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Hypoglycemia can be regarded as a situation of stress inducing several humoral rescue mechanisms to prevent damage from the organism. We were interested whether hypoglycemia causes activation of the immune system. Methods: Ten healthy volunteers (6m, 4f, age:22-25, BMI: 21±0.6) were studied by means of a stepwise hypoglycemic hyperinsulinemic (1mU/kg/min) clamp. In 7 volunteers, a euglycemic hyperinsulinemic clamp (5mM serum glucose) of the same duration was performed as control. Plasma cytokine and cortisol levels were determined by ELISA, epinephrine was measured by HPLC. Results: During hypoglycemia, leukocyte count increased nearly 3-fold with a relative rise of granulocytes. IL-10 concentrations increased significantly, whereas TNF-α, IL1-ß and IL-6 concentrations remained

hypoglycemic clamp	*5mM glucose*	*2.3mM glucose*
Leukocytes, GPT/l	*6.8 ±0.4*	*14.4±0.8***
IL-10, pg/ml	*<7.8*	*14.6±3 2**
Epinephrine, pM	*0.2 ±0.01*	*6.1±0.2***
Cortisol, nM	*0.39±0.01*	*1.1±0.05***

[*:p<0.01, **:p<0.001] unchanged. During euglycemia, leucocyte count and cytokine levels remained constant. Discussion: Hypoglycemia causes secretion of the contrainflammatory cytokine IL-10. As IL-10 is a potent downregulator of adhesion molecules this cytokine may be involved in the release of leukocytes from vessel walls or bone marrow.Whether counterregulatory hormones directly induce IL-10 secretion under hypoglycemic conditions is subject to future studies. This study shows that modulation of blood glucose levels can substantially alter immunoregulation.

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In vivo Studies Of The Effects Of Capsaicin On Blood Glucose Levels And Insulin Binding Using Dog Models.

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A number of plants have been used in Jamaica to treat diabetes by traditional healers .One of them is Capsicum *frutences* whose active agent, capsaicin is already being used to treat Diabetic Neuropathy. This study was designed to determine if capsaicin has any effect(s) on blood sugar, insulin levels,as well as insulin binding using leucocytes. A capsaicin fraction which was isolated from Capsicum *frutences* when fed to dogs (n = 13) caused hypoglycemia that is stastically significant when compared to a control, at the 2.5 hr time interval of an Oral GlucoseTolerance Test. (5.38 mol/dl ± 0.49 versus 6.79 mol/dl ± 0.34, $p < 0.05$ for the control). These results were comfirmed by using a capsaicin standard (4.53 mol/dl ± 0.45 versus 6.6 mol/dl ± 0.46, n = 11, $p < 0.05$ for the control). Blood Sugar was measured by the Glucose oxidase method. Insulin concentration measured by the Coat a Count method at the same time showed an increase of insulin levels when compared to the control (18.83 µl/mol ± 3.6, versus 6.9µl/mol ± 1.25, n = 11, $p > 0.05$ for the control). Insulin binding was done by the Gambir *et al* method in which isolated, washed leucocytes were incubated with varying concentration of non-radioactively labeled insulin and a fixed quantity of radioactive labelled insulin. After the 3 hr incubation, cells were washed with buffer and radioactivity determined in a autogamma counter. The results showed that there was a stastically significant decrease in insulin binding of theleucocytes of the dogs injected with the capsaicin fraction when compared to the control. Binding for the capsaicin fraction was 27% ± 2.69 compared with 8% ± 1.25 (n = 10, $p>0.05$) for the control. In conclusion, it is apparent that capsaicin has a hypoglycemic effect which is mediated by a comcominant increase in blood insulin levels. The decreased insulin receptor binding may be compensatory mechanism in response to the latter and serves to prevent rapid depletion of blood glucose level which could result in a hypoglycemic coma.

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Endogenous Glucose Production

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GLUCONEOGENESIS IS NOT INCREASED IN MILDLY HYPERGLYCEMIC NON-INSULIN-DEPENDANT DIABETIC PATIENTS.

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The enhanced endogenous glucose production (EGP) of non insulin dependent diabetic subjects (NIDDM) is generally considered to result from an increased gluconeogenesis (GNG). We infused (post-absorptive state) 5 normal subjects and 5 NIDDM (glycemia 11,1 ± 1,0 mM) with [6,6-$^2H_2$] glucose (150 min) and [3-^{13}C] lactate (6 hours). Liver glutamine was non invasively sampled with phenylacetate and its labeling pattern determined (mass spectrometry) after purification of the glutamine part of urinary phenylacetylglutamine. After correction for labeled CO_2 reincorporation by pyruvate carboxylase (PC) (control test with $NaH^{13}CO_3$ infusion) this labeling pattern was used to calculate a correction factor for isotopic exchange in the oxaloacetate pool and flux through liver Krebs cycle. NIDDM had increased lactate Rt (15,3 ± 0,7 vs 10,0 ± 0,7 µmol/kg/min, $p<0,01$) and EGP (15,22 ± 0,90 vs 12,52 ± 0,33 µmol/kg/min, $p<0,05$). Uncorrected contribution of GNG to EGP were 28 ± 3 % (control) and 19 ± 4 % (NIDDM). Correction factor were comparable in controls (1,34 ± 0,02) and NIDDM (1,46 ± 0,04) and the correted relative and absolute contribution of GNG to EGP were not increased in NIDDM (27 ± 5 % and 4,1 ±0,5 µmol/kg/min) compared to controls (38 ± 3 % and 5,0 ± 0,6 µmol/kg/min). The calculated PC over pyruvate dehydrogenase activity ratio, were comparable (12,1 ± 0,6 vs 13,0 ± 1,3). Lastly hepatic fatty acid oxidation, as estimated by this model, was not increased in NIDDM. Conclusion, despite an increased lactate Rt we found no increase of GNG in the diabetic patients studied.

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RELEASE OF GLYCEROL FROM MUSCLE TISSUE IS ONLY MINOR IN OBESE AND NORMAL SUBJECTS.

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Recently, a microdialysis study reported molar concentrations of glycerol in adipose as well as muscle tissue. Since this should result in non-physiological plasma concentrations of glycerol we performed calibrated microdialysis measurements in three subject groups. In six normal weight non-diabetic subjects fasted over night arterial glycerol and abdominal adipose tissue interstitial glycerol was 39±1 and 176±25µmol·l^{-1}, respectively($p<0.001$). Interstitial glycerol in medial femoral muscle (50±6 µmol·l^{-1}) was significantly ($p<0.05$) higher than in arterial plasma but lower (0.01) than in adipose tissue. Also, we measured muscle interstitial glycerol during a euglycemic hyperinsulinemic clamp in obese (BMI:33±4, n=10) and normal weight (BMI:23±1, n=10) subjects. At steady state obese subjects had p-glucose 5.8±0.2mmol·l^{-1}, p-insulin 480±52 mU·l^{-1}, arterial glycerol 31±3µmol·l^{-1} and muscle interstitial glycerol 54±10µmol·l^{-1}, (arterial vs interstitial $p<0.01$). Normal weight subjects: p-glucose 5.7±0.1, p-insulin 297±29, arterial glycerol 16±1 and interstitial glycerol 64±20 (arterial vs interstitial $p<0.05$). The data conclude that glycerol is released only to a minor extent from muscle tissue in normal weight and obese subjects. The data do not confirm molar interstitial glycerol levels in either adipose or muscle tissue.

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EVALUATION OF THE DYNAMIC RELATIONSHIP BETWEEN FFA AND ENDOGENOUS GLUCOSE PRODUCTION IN NORMAL AND OBESE SUBJECTS. L.D. Monti, P.M. Piatti, A. Caumo, G. Valsecchi, C. Stangalini, F. Magni, S. Costa, M. Galli-Kienle, A.E. Pontiroli and G. Pozza. Istituto Scientifico H. San Raffaele, Milano, Italy.

Recent studies have suggested that free fatty acids (FFA) are the link in the suppression of endogenous glucose output (EGP) by insulin. However, the dynamic relationship between the time courses of FFA and EGP has not been fully investigated. To clarify this issue we studied FFA decline and EGP inhibition during a low-dose (0.4 mU/kg/min) hyperinsulinaemic euglycaemic clamp in normal (n=6; age=34±2 yrs; BMI=25.0±1.4 kg/m^2) and in obese subjects (n=5; age=42±4 yrs; BMI=35.7±1.1 kg/m^2). Dideuterated glucose was infused to trace glucose turnover and the hot-GINF technique was employed to obtain a reliable assessment of EGP. FFA and EGP measurements were available at 5,10,15,20,25,30,45,60,75,90,105, and 120 min. A kinetic model was used to relate FFA decline to EGP inhibition during the nonsteady state. It provided metabolic indices such as the sensitivity of EGP to FFA, S_{FFA}, and the time delay between FFA and EGP changes, τ_{FFA}. In the fasting state, FFA levels were lower in normal subjects (0.46±0.04 vs 0.64±0.06 mmol/l;$p<0.05$) while EGP (1.95±0.18 vs 1.61±0.11 mg/kg/min) was similar in the two groups. At 120 minutes, no differences in the percentages of inhibition either of FFA (66.0±6.9 vs 75.5±6.6 %) or of EGP (83.1±5.2 vs 71.5±4.1%) were found. When the nonsteady-state period was considered, differences in the FFA vs EGP relationships of the two groups emerged. In normal subjects the model fit was good and the metabolic indices were precisely estimated: S_{FFA} was 6.2±1.1 mg/kg min per mmol/l and τ_{FFA} was 11.5±5.6 min. In contrast, in four out of five obese subjects FFA decline did not account accurately for EGP inhibition. In fact the model fit was poor, especially during the first 30 min of the clamp when FFA decrease was slower than EGP inhibition. We conclude that during a hyperinsulinaemic euglycaemic clamp FFA decrease is tightly related to EGP inhibition in normal subjects. In obese subjects some other factor is likely to be involved in the suppression of EGP.

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IN SITU LIPOLYTIC RESPONSE TO PHYSIOLOGIC STRESSORS AND ISOPROTERENOL IN PIMA INDIANS AND CAUCASIANS.
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Pima Indians have a high prevalence of obesity and NIDDM. We hypothesized that lipolytic responses to stimuli would be lower in Pima Indians than in sex-, age-, and body composition-matched Caucasians. Lipolysis was measured by microdialysis *in situ* in non-diabetic Pima Indians (12 M/15 F, 30±7y, 85±18kg, 38±10% body fat; means±SD) and Caucasians (11 M/10 F, 34±7y, 105±26kg, 41±12% body fat). Glycerol concentration was measured in the dialysate of a probe inserted in the abdominal subcutaneous adipose tissue during a 30 min baseline and 1) 30 min exercise at 41±12% VO2max, 2) 30 min mental stress (color-word test), and 3) 40 min local infusion of isoproterenol added to the perfusate (10μM). Plasma epinephrine and norepinephrine were measured during baseline and in response to exercise and mental stress. Baseline dialysate concentrations of glycerol were similar in the two groups.

Relative change in dialysate glycerol concentration over baseline (%)

	Pima Indians	Caucasians	*t*-test
Exercise	38±38	41±41	NS
Mental stress	-2±11	-1±12	NS
Local isoproterenol	66±31	72±31	NS

The groups were also similar after correction for changes in local blood flow (n=44). Plasma catecholamines increased similarly in the 2 groups in response to exercise and mental stress. In conclusion, a lower lipolytic response to stimuli does not seem to explain the high susceptibility of Pima Indians to weight gain.

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ROLE OF FFA IN THE REGULATION OF BASAL GLUCOSE METABOLISM IN CONTROL AND DIABETIC RATS.
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Increased FFA concentration, experimentally induced during an hyperinsulinemic clamp, is able to inhibit glycolysis and reduce glucose uptake, finally determining insulin resistance. Hyperinsulinemia, however, markedly lowers FFA concentration. The aim of this study was to clarify the effects of high plasma FFA on glucose uptake (GU), hepatic glucose production (HGP), and gluconeogenesis (GN) in control (C) and diabetic (90% pancreatectomy) rats (D) in presence of identical basal insulin and euglycemia. In order to acutely reach euglycemia without insulin, D rats received an infusion of phloridzin (inhibitor of glucose reabsorption in the kidney: glycosuria). After a 6h fast, all rats were infused for 120 min with [3-^{3}H]-glucose, [U-^{14}C]-lactate plus a concomitant infusion of triglyceride emulsion (Intralipid plus heparin) or saline. At tracer/tracee steady state, GU was calculated as the rate of disappearance of glucose minus glycosuria. The minimal estimation of GN was calculated as the ratio between plasma ^{14}C-glucose and 2 · plasma ^{14}C-lactate multiplied HGP (glucose rate of appearance). Saline infused D rats, as expected, showed reduced GU (D: 37.1±3.3 vs. C: 66.8±3.9 μmol·kg^{-1}·min^{-1}). Elevated FFA did not modify GU in both groups (D: 32.2±2.2; C: 62.3±1.7 μmol·kg^{-1}·min^{-1}). HGP was markedly increased in D rats compared with C (D: 89.5±3.8 vs. C: 61.8±3.9 μmol·kg^{-1}·min^{-1}) and remained unaffected by FFA infusion in both groups (D: 78.9±4.4; C: 60.1±2.8 μmol·kg^{-1}·min^{-1}). GN relative contribution to HGP was almost doubled by increased FFA in C (from 18.7±6 to 34.5±8 %, or from 11.7±1.7 to 20.6±2.2 μmol·kg^{-1}·min^{-1}); it was already increased in saline infused D rats (either relatively: 54.4±4 %, or as absolute flux: 21.1±1.7 μmol·kg^{-1}·min^{-1}) but remained unaffected in FFA infused D rats (52.3±5 %, 18.7±2.2 μmol·kg^{-1}·min^{-1}). In conclusion, this results suggest that, in presence of basal insulin and euglycemia, an acute elevation of FFA concentration: 1. is not able to modify GU and overall HGP, either in diabetic or control rats and 2. increases GN in control rats, but not in D rats, where it is already elevated. In perspective, other mechanism(s) than just increased FFA and GN are responsible for augmented HGP, at least in this model of diabetes.

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FREE FATTY ACIDS METABOLISM IN HUMANS : CONTRIBUTION OF OXIDATION, INTRAHEPATIC AND EXTRAHEPATIC REESTERIFICATION.
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Plasma FFA can be either oxidized or reesterified into triglycerides (TG). This reesterification can take place in liver (the TG being then secreted as VLDL-TG) or in extrahepatic tissues. To measure these metabolic pathways five normal subjects drank deuterated water (for measurement of lipogenesis) in the evening and were infused the following morning in the post-absorptive state with [1-^{13}C] palmitate (4 hours). We determined : 1) FFA turnover rate (from the enrichment of plasma palmitate), 2) FFA oxidation rate (from the excretion rate in expired gas of $^{13}CO_2$), 3) total lipid oxidation (by indirect calorimetry, corrected for the measured lipogenesis), 4) intrahepatic reesterification (from the kinetic of ^{13}C palmitate incorporation into TG during [1-^{13}C] palmitate infusion), 5) TG turnover rate (from the kinetic of ^{13}C palmitate disappearance in TG after the end of [1-^{13}C] palmitate infusion). FFA oxidation rate (calculated using the acetate correction factor of Sidossis) was 2,76 ± 0,65 μmol/kg/min, accounting for 45% of FFA R_t (6,04 ± 1,04 μmol/kg/min) and 90 % of total lipid oxidation (3,02 ± 0,5 μmol/kg/min). Total FFA reesterification was therefore 3,27 ± 0,54 μmol/kg/min). TG turnover rate was 0,11 ± 0,05 μmol/kg/min). Lipogenesis accounted only for 4% of TG secretion rate whereas hepatic FFA reesterification accounted for about 50% representing only 0,16 ± 0,02 μmol/kg/min of plasma FFA utilisation. Extrahepatic reesterification was therefore 3,11 ± 0,53 μmol/kg/min. Conclusion : 1) FFA oxidation account for near the totality of lipid oxidation. 2) oxidation and reesterification contribute each about one half to FFA utilization. 3) lipogenesis and reesterification account for only 50-55% of TG secretion. 4) most of FFA reesterification takes place in extrahepatic tissues.

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NITRIC OXIDE INHIBITS HEPATOCYTE GLUCOSE METABOLISM.

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There is increasing evidence in literature for the existence of intrahepatic regulation of glucose production by Kupffercell products as cytokines and prostaglandins. The role of nitric oxide (NO), another Kupffercell product, is less well studied. NO has been described to influence gluconeogenesis from lactate by inhibiting rate-limiting gluconeogenic enzyme fluxes of PEPck and PC. It is not known whether NO influences other pathways of intrahepatic glucose metabolism. We aimed to study the influence of NO on gluconeogenesis from dihydroxyacetone (DHA) and on glycogen synthesis from either DHA or glucose by adding a low dose of the chemical NO-donor S-Nitroso-Acetyl Penicillamine (SNAP) to fasted, isolated rat hepatocytes. Cells were incubated for 1 hour with mentioned substrates and with or without proline or glutamine to stimulate glycogen synthesis. For results see the table:

substrate	SNAP mM addition	GLUCONEOGENESIS isotonic	glutamine	proline	GLYCOGEN SYNTHESIS glutamine	proline
DHA 10 mM	0	259 (± 57)	280 (± 33)	317 (± 39)	77,1 (±7,2)	81,7 (±1)
	0,25	264 (± 49)	273 (± 45)	307 (± 41)	15,7 (±14,6)*	11,5 (±19,9)*
glucose 20 mM	0	All expressed as umol/gdw			113 (±15,1)	120 (±15,6)
	0,25	* P < 0,05			17,9 (±21)*	39,6 (±30)*

We found that: **1.)** Gluconeogenesis from DHA was not affected by SNAP. **2.)** Glycogen synthesis from DHA and glucose was 80-90% inhibited by SNAP. **3.)** Total cell ATP levels were not affected by SNAP.
Conclusion: SNAP-derived NO causes inhibition between glucose-6-phosphate and glycogen, presumably by inhibiting glycogen synthase.

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REGULATION OF LIPOLYSIS STUDIED BY MICRODIALYSIS IN NORMAL AND SPINAL CORD INJURED SUBJECTS.

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Stress reactions with increased sympathetic activity may cause insulin resistance whereas reduced lipolytic rates due to an inherent reduced adrenergic sensitivity has been proposed to cause obesity. To elucidate the role of the sympathetic nervous system (SNS) we studied a spinal cord injured (SCI) group with decentralised SNS and a weight and age-matched control group. Subcutaneous microdialysis and blood flow measurements (^{133}Xe-clearance) were performed in umbilical and clavicular region during centrally mediated sympathoexcitation. The SCI group showed an increased fat mass (26.8 kg vs 16.1 kg, $p<0.01$), higher p-glycerol (82±7 vs 61±4, $p<0.05$), p-insulin (9.0±2 vs 5.0±0.7 mU/l, $p<0.05$) and lower p-noradrenaline (NA) (0.78±0.2 vs 1.23±0.14 nmol/l $p<0.05$) concentrations. Sympathoexcitation increased blood flow and glycerol release (rest:98±41, excit:167±43 nmol/min/100g, $p<0.05$) in umbilical region among controls, whereas no reaction was found in the decentralised region in the SCI group. P-glycerol, -FFA, -insulin, -NA and -adrenaline increased in control subjects. In spite of this, lipolysis rate was similar in both groups in both regions. In conclusion: The insulin resistance and increased adipose tissue mass found in SCI seem not to be caused by the decentralisation of the SNS.

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EVIDENCE, IN ISOLATED MICROSOMES, FOR THE INHIBITION OF GLUCOSE-6 PHOSPHATASE AFTER REFEEDING.

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It has been recently shown that the activity of liver glucose-6 phosphatase (Glc6Pase), the final enzyme of gluconeogenesis and glycogenolysis, was inhibited (Vmax) after refeeding. The inhibition was observable in homogenate and not in isolated microsomes, suggesting it was either labile or due to a metabolite lost during the isolation process. To distinguish between both hypotheses, we designed a very rapid microsome isolation procedure (less than 1 hour), from a small amount (≈1g) of fresh liver sampled in anesthetized rats. Microsomal Glc6Pase Vmax in 48h-fasted rats (0.35±0.01 µmol/min/mg prot., mean±S.E.M. n=12) was inhibited after 7h-refeeding (0.27±0.01 µmol/min/mg, n=12, $p<0.01$). The inhibition was also observable after detergent-treatment of microsomes (0.6±0.03 vs 0.45±0.2 µmol/min/mg prot., before and after refeeding, respectively, n=12, $p<0.01$). The Km of Glc6Pase (≈2.5 mM in intact membranes, 1 mM in detergent-treated membranes) was not affected by refeeding. Once they had been rapidly isolated, microsomes could be further washed, inhibition was retained. Glc6Pase inhibition was not removed upon incubation in the presence of BSA (1 mg/ml), ruling out that it could be mediated by unsaturated fatty acids or fatty acyl-CoA esters (known inhibitors of the enzyme) bound to the membrane. Our results strongly suggest that the inhibition of Glc6Pase after refeeding is not dependent on a metabolite present in homogenate and lost in microsomes. This inhibition, which might involve a covalent modification of the enzyme, is stable after rapid removal of microsomes from homogenates.

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CAN INSULIN MEDIATED SUPPRESSION of FFA AND GLYCEROL BE USED TO EVALUATE THE LIPOLYTIC ACTIVITY DURING I.V. INSULIN TOLERANCE TEST ?

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NIDDM and obesity are also characterized by resistance to the antilipolytic effects of insulin. To determine whether the insulin mediated suppression of FFA and glycerol are an indicator of the degree of insulin sensitivity, we measured FFA, Glycerol, Plasma glucose disappearance rate (K_{ITT}) during I.V. insulin tolerance test (IVITT) non-diabetic obese young women (Age:41±3 yr, BMI: 28±3.3 kg/m^2, WHR: 84±0.05). Insulin sensitivity was measured with IVITT (Regular insulin 0.05 IU/kg) The glucose levels were determined at 3 min intervals for 15 min before and after insulin administration. The FFA and glycerol levels were determined at 15 min intervals for 60 min before and after insulin administration. K_{ITT} was 4.11±1.6 %.min^{-1} .FFA were 590±254, 460±245, 260±133, 292±147, 350±190 µEq/L, Glycerol were 8.57±2.6; 5.9±1.5; 5.67±2.1; 5.76±2.3; 6.1±1.8 mg/L at 0, 15, 30, 45, 60min respectively. Suppression of FFA were 24±22 %, 54±20 %, 45±26 %, 10±4 % and suppression of glycerol were 27±19 %, 33±26 %, 31±25 %, 8±3 % at 15, 30, 45, 60 min, respectively K_{ITT} correlated only with 30 min -FFA level ($r= -0.72$, $p=0.012$) not correlated any glycerol levels. As a conclusion, levels of glycerol are insufficiently suppressed according to FFA levels during IVITT. At least 50 % decrease in FFA level at 30 min during IVTTT may reflect insulin sensitivity in android obesity.

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SMALL AMOUNTS OF FRUCTOSE AUGMENT NET HEPATIC GLUCOSE UPTAKE DURING HYPERGLYCEMIA IN INSULIN DEFICIENCY.

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Fructose activates glucokinase by releasing the enzyme from its inhibitory protein in the liver. We showed previously that intraportal infusion of small amounts of fructose markedly augmented net hepatic glucose uptake during hyperglycemic hyperinsulinemia. In this study we examined whether small amounts of fructose augment net hepatic glucose uptake during hyperglycemia with normoinsulinemia in conscious 42 h-fasted dogs. Isotopic and A-V difference methods were used. Each study consisted of an equilibration period (-140 to -40 min), a control period (-40 to 0 min), and a test period (0 to 240 min). During the latter glucose (6.5 $mg \cdot kg^{-1} \cdot min^{-1}$) was continuously given intraportally with (0.4 $mg \cdot kg^{-1} \cdot min^{-1}$) (+F) or without fructose (-F). During the study, somatostatin (0.8 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was given along with basal insulin (0.25 $mU \cdot kg^{-1} \cdot min^{-1}$) and glucagon (0.6 $ng \cdot kg^{-1} \cdot min^{-1}$) intraportally. In the +F group, fructose increased the sinusoidal blood fructose level (nmol/ml) from <16 to 139 ± 11. The average rate of net hepatic fructose uptake and fractional extraction during the test period were 2.1 ± 0.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ and 46 ± 2 %. The infusion of glucose alone elevated arterial blood glucose (μmol/ml) from 4.3 ± 0.3 to 11.2 ± 0.6 during the first 2 h after which it remained at 11.6 ± 0.8. In the presence of fructose glucose infusion elevated arterial blood glucose (μmol/ml) from 4.3 ± 0.2 to 7.4 ± 0.6 during the first 1 h after which it decreased to 6.1 ± 0.4 (3 h). With glucose infusion, net hepatic glucose balance ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) switched from output (8.9 ± 1.7 and 13.3 ± 2.8) to uptake (12.2 ± 4.4 and 29.4 ± 6.7) in -F and +F, respectively. Average net hepatic glucose uptake ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) and fractional extraction (%) (last 3 h of the test period) were higher in +F (30.6 ± 3.3 and 14.5 ± 1.4) than in -F (15.0 ± 4.4 and 5.9 ± 1.8). In conclusion, small amount of fructose markedly reduces hyperglycemia during intraportal glucose infusion by increasing net hepatic glucose uptake even when plasma insulin levels remain at the basal.

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RELATIONSHIP BETWEEN FREE-TESTOSTERONE LEVELS AND GLUCOSE EFFECTIVENESS AFTER AN ACUTE DECREASE IN FFA LEVELS IN OBESE WOMEN.

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Androgen hormones (i.e. free testosterone: FT) seem to decrease insulin sensitivity with an effect probably mediated by an increase in FFA levels. To evaluate whether an acute decrease in FFA levels may induce a reduction in FT levels and in turn increase insulin sensitivity, 21 women affected by obesity (age 41±2 yrs, BMI 37.3±0.9 kg/m^2, waist/hip range between 0.76 and 0.91) underwent two IVGTT studies separated by a one week interval. Placebo or acipimox (to inhibit lipolysis) were administered orally before the beginning of the IVGTT. Glucose and insulin concentration profiles obtained during the IVGTT, were analyzed with the minimal model of glucose disappearance which provides indices of both insulin sensitivity (SI) and glucose effect *per se* or glucose effectiveness (SG). After placebo, basal FFA were 0.8±0.5 mM and decreased to 0.2±0.03 mM after acipimox p<0.0001). Basal insulin (I) levels were 13.4±1.1 μU/ml after placebo and decreased to 11.0±1.2 μU/ml after acipimox (p<0.05). basal FT levels were 1.0±0.1 ng/ml after placebo and significantly decreased to 0.73±0.09 ng/ml after acipimox (p<0.05). During IVGTT and placebo, SI and SG were 2.26±0.36 10^{-4} $min^{-1}/\mu U/ml$ and 0.013±0.001 min^{-1}, respectively. After acipimox, SI increased to 2.92±0.40 10^{-4} $min^{-1}/\mu U/ml$ (p=0.058) and SG to 0.018±0.002 min^{-1} (p<0.01). Interestingly, the increment in SG significantly correlated with the decrement of basal FT levels (r=0.45, p<0.05). In conclusion: 1) fasting FFA concentrations seem to modulate fasting FT and insulin levels; 2) an acute decrease in FFA increase both SI and SG; 3) the increase in SG after an acute decrease in FFA levels seems related to a simultaneous reduction in FT levels.

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HUMAN SYSTEMIC, HEPATIC AND RENAL GLUCOSE, GLUTAMINE AND ALANINE METABOLISM: EFFECTS OF EPINEPHRINE.

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Glutamine (GLN) and alanine (ALA) are the most important gluconeogenic amino acids. Recent studies indicate that in humans liver and kidney are equally gluconeogenic organs. It has been proposed that epinephrine stimulates gluconeogenesis (GN) by increasing substrate availability. To test this concept we used a combination of isotope ([6-^{3}H] glucose, [U-^{14}C] glutamine, [3-^{13}C] alanine) and renal balance techniques before and during infusion of epinephrine (E) in 9 normal postabsorptive volunteers.

Tbl. 1. Systemic (syst), hepatic (hep) and renal (ren) rates ($\mu mol \cdot kg^{-1} \cdot min^{-1}$).

* p<0 05	Glucose Release			Glutamine GN			Alanine GN		
	syst	hep	ren	syst	hep	ren	syst	hep	ren
Basal	11.8	9.0	2.8	.48	.13	.35	72	.69	.03
Epinephrine	15.8*	10.3*	5.5*	.69*	.06	.64*	1.70*	1.62*	.08*

Tbl. 2. Renal net balance (NB), uptake (UPT), release (REL) ($\mu mol \cdot kg^{-1} \cdot min$) and fractional extraction (FX) (%):

* p<0.05	Glutamine				Alanine			
	NB	FX	UPT	REL	NB	FX	UPT	REL
Basal	49	9.2	.73	.24	.16	8.4	.55	.44
Epinephrine	.80*	15.1*	1.29*	.49	.01	15.0*	.81*	1.01*

Summary and conclusions: GLN GN is predominantly renal (73 ± 3 % basal, 90 ± 4 % with E) whereas ALA GN is almost exclusively hepatic (96 ± 1 % basal, 95 ± 2 % with E). E increased fractional extraction of both amino acids. The resultant increase in uptake completely accounted for the increase in renal GN from ALA and GLN. Contrary to current opinion, these results demonstrate that E increases GLN and ALA GN by increasing their transport. Isotopic use of these gluconeogenic precursors permits individual assessment of hepatic and renal GN.

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The Correlation between Insulin Resistance and the Visceral Fat vs. Skeletal Muscle Ratio (VFSMR) in Middle-Aged Women

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In addition to visceral fat accumulation, bulk of skeletal muscle mass may influence insulin sensitivity via its capacity to store glucose load. We evaluated 129 non-diabetic middle-aged women to investigate the relationships between VFSMR and metabolic variables representing insulin resistance.

Visceral and subcutaneous fat areas at the umbilical area and skeletal muscle areas at the mid-thigh were measured by computed tomogram and VFSMRs and visceral fat vs. subcutaneous fat area ratios (VSR) were calculated. 75gm oral glucose tolerance tests were performed with measuring plasma glucose, insulin and free fatty acid(FFA) levels, and areas under the curve of glucose (Glu-AUC), insulin (Ins-AUC), FFA (FFA-AUC) and glucose/insulin ratio (GIR=Glu-AUC/Ins-AUC), were calculated.

Mean age of the subjects was 50.1±5.2 years and mean VSR and VFSMR were 0.38±0.15 and 0.57±0.26, respectively. Among the metabolic variables, Glu-AUC, FFA-AUC, total cholesterol (TC) and triglyceride (TG) levels were more significantly correlated with VSR than VFSMR, but Ins-AUC, GIR and sex hormone-binding globulin (SHBG) levels were more significantly correlated with VFSMR than VSR. After multiple linear regression analysis with adjustment of age and BMI, VFSMR was the most important anthropometric parameter in Ins-AUC, GIR and SHBG level and VSR in FFA-AUC and TG level. In subjects with VFSMR more than 0.6, age and Ins-AUC were significantly increased(p<0.001) and GIR and SHBG levels were significantly decreased (p<0.001, p<0.01) than in subjects with lower VFSMR. Regardless of the magnitude of VSR, subjects with VFSMR higher than 0.6, were older and showed higher Ins-AUC, GIR and SHBG levels than in subjects with lower VFSMR.

In conclusion, VFSMR may be a better anthropometric parameter indicative of insulin resistance than VSR in middle-aged women.

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A DEFECT OF SUPPRESSION OF ENDOGENOUS GLUCOSE PRODUCTION CONTRIBUTES TO LIPID-INDUCED GLUCOSE INTOLERANCE.

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In normal subjects, a lipid infusion does not greatly impair glucose tolerance, because the inhibitory effect on glucose oxidation seems matched by a rise of non oxidative disposal. But an influence of lipids on Endogenous Glucose Production (EGP) has not been examined during an Oral Glucose Tolerance Test (OGTT). In 6 normal subjects (age 23±2 y; Body Mass Index 21.5±0.4), we performed doubly labelled OGTT (1g.kg-1 maïze glucose, naturally enriched in ^{13}C, to measure exogenous glucose appearance RaE), with a primed-continuous dideuterated glucose infusion to measure total glucose appearance RaT; EGP was calculated as RaT-RaE. Each subject underwent 2 OGTTs, first during a saline (Sa), second during an "Ivélip 20%" (Iv) infusion ($0.015ml.kg^{-1}.min^{-1}$, started 90 minutes before oral glucose charge). The lipid infusion produced a marginal (NS) rise in glucose and insulin levels, but EGP was less suppressed at time 90 min (Sa:0.94±0.38 $mg.kg^{-1}.min^{-1}$, Iv:1.77±0.53; $p<0.05$), and 330 min cumulation of EGP suppression was lower (Sa: -409±24 $mg.kg^{-1}$, Iv: -298±43; $p<0.05$). Despite identical oral charges, RaE was higher under "Ivélip" (330 min cumulation: Sa:872±53 $mg.kg^{-1}$,Iv:1025±82; $p<0.05$), suggesting an increased recycling of ^{13}C. Increased gluconeogenesis may therefore be the cause of the impaired suppression of EGP under lipid infusion.

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LIPOPROTEIN LIPASE IN MUSCLE OF hHTg RAT IS RELATED TO FATTY ACID COMPOSITION OF MUSCLE PHOSPHOLIPIDS

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Hypertriglyceridemia is closely linked to insulin resistance. Indeed, raised dietary intake of omega-3 (n-3) polyunsaturated fatty acids (PUFA) which leads to lower plasma triglyceride (TG) levels is associated with improvement in insulin action. In order to evaluate the role of lipoprotein lipase (LPL) in the hypotriglyceridemic effect of long chain n-3 FA, the expression of LPL has been measured in skeletal muscle of hereditary hypertriglyceridemic (hHTg) rats which underwent an euglycemic hyperinsulinemic clamp. Before the clamp, groups of animals were fed a basal or a high (63%) sucrose diet with or without fish oil supplement for two weeks. Results were compared to data obtained from control animals subjected to an identical protocol. The expression of LPL was increased in the soleus muscle when the n-3 FA acids were included into the basal or high sucrose diet [B: 6.6±1.1 AU; FO: 24.2±1.8 AU $p< 0.001$; HS+FO: 46.2±2.5 AU, $p< 0.001$]. In addition, similar stimulation of LPL mRNA levels by n-3 FA was found also in hHTg rats [B: 7.9±0.7 AU; FO: 26.2±2.5 AU $p< 0.001$; HS+FO: 24.4±1.5 AU $p< 0.001$]. mRNA levels correlated negatively with plasma TG levels ($r= -0.60$, $n= 16$, $p<0.01$). Moreover, we have found a very significant positive correlation ($r= 0.87$, $n= 32$, $p< 0.001$) between the muscle LPL expression and the GIR (= glucose infusion rate) as a measure of insulin sensitivity. In addition, when the LPL expression was correlated with the content of long chain PUFAs, a highly significant positive correlation between the LPL mRNA and the total percentage of Σ20-22 PUFA in skeletal muscle phospholipids ($r= 0.85$, $n= 32$, $p< 0.001$) was obtained. On the other hand, the LPL mRNA correlated negatively ($r= -0.86$, $n= 32$, $p< 0.001$) with the ratio of n-6/n-3 FAS in muscle phospholipids. Thus, our results indicate that a) increased removal of triglyceride by muscle through the activation of LPL may contribute to the TG lowering effect of fish oil, and the b) incorporation of long chain PUFAs into muscle phospholipids could be an important long-term modulator of the skeletal muscle LPL.

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UNDETECTABLE CONTRIBUTION BY KIDNEY TO GLUCOSE PRODUCTION IN POSTABSORPTIVE AND 60 H FASTED STATES.

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A-V net balance of glucose across kidney and liver and ^3H uptake after 3 h infusion of (6-^3H)glucose or (3-^3H)glucose were determined in healthy men (n=16), age 28±1 yrs, BMI 23.2±0.4 kg/m^2 after 12 and 60 h of fasting. Individual means were calculated from 9 determinations between 115 and 180 min. Net renal and splanchnic balance were calculated as A-V blood glucose differences times regional blood flow. Glucose uptakes were calculated as A-V difference of ^3H-activity in plasma glucose, divided by the ^3H-activity in arterial plasma glucose, multiplied by arterial concentration of glucose and regional flow. Results (µmol/kg/min):

	12 h		60 h	
Glucose	6-^3H	3-^3H	6-^3H	3-^3H
n	4	6	7	3
Glucose Ra	11.3±1.3	9.3±0.9	8.1±0.2	7.2±0.4
Spl ^3H-uptake	1.9±0.2		0.3±0.4	
Spl Net Balance	-9.6±1.4		-6.7±0.8	
Renal ^3H-uptake	-0.5±0.5	1.0±0.9	-0.1±0.2	0.6±0.9
Renal Net Balance	-0.2±0.3	1.0±1.8	-0.8±0.7	-1.0±0.3

Postabsorptive net balance across splanchnic bed was 9.6 and glucose uptake 1.9, equal to Ra glucose. In accord negligible net production and fractional extraction of glucose across kidney were found. In the 60 h fast 6.7 net balance across splanchnic bed, and 0.3 uptake, accounted for 92 % of total glucose production. Glucose production by kidney was not demonstrated. It is concluded that, postabsorptive contribution of renal glucose production to whole body glucose turnover is negligible; after 60 h of fasting it is about 10 % of total glucose production.

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TRIGLYCERIDE INDUCED DIABETES MELLITUS DUE TO LIPOPROTEIN LIPASE DEFICIENCY.

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The complete reversal of diabetes mellitus (DM) in two sisters with familial lipoprotein lipase (LPL) deficit and normal apolipoprotein CII levels is reported. All the family members, father aged 48, mother 44, and 3 sisters (Sib1 18, Sib2 16, Sib3 11) were studied. Postheparin plasma LPL was 76.0 mU/ml in Sib1, 117.1 in Sib2, 205.7 in Sib3, 145.0 in the father and 101.7 in the mother. GC-clamped PCR-amplified DNA from the promotor region and 1 to 10 LPL gene exons were screened for nucleotide substitution by denaturing gradient gel electrophoresis. Two polymorphies were found in father's exon 4 (GAG to GAA) both coding for Glu^{118} and in mother's exon 8 (ACC to ACA) both for Thr^{361} while a stop mutation was in mother's exon 9 (TCA to TGA) coding for termination instead of Ser^{447}. The polymorphies in exon 4 and 8 were inherited by Sib 1 and 2. HDL_3 and HDL_2 were lower than normal in the whole family, but particularly in the father and Sib 1 and 2. One to 2 years after development of severe chylomicronemia with diffuse skin lesions, insulin-resistant DM ensued in Sib 1 and 2. Current medical therapy failed normalize triglycerides (TG) and glycemia; thus a modified bilio-pancreatic diversion operation was employed to induce lipid malabsorption. Within three weeks after surgery, plasma TG and cholesterol levels decreased from 56 mM and 12.9 (with dietary restrictions) to <6.2 and 2.84 mM (on free diet) respectively. Fasting glycemia decreased from >16.6 (under daily doses of insulin ≥150 IU) to 4.4-4.5 mM (without any therapy). Body weight and fat-free mass were maintained in Sibs after surgery. Glucose uptake (M) by euglycemic hyperinsulinemic clamp (EHC) and end-clamp glucose oxidation (ECGO) were significantly lower than normal before (M=Sib1;5.61 and Sib2;12.5 µmoles/kgbw/min; ECGO = Sib1; 5.08 and Sib2; 9.24) and not significantly different from normal after surgery (M=Sib1;16.7 and Sib2;20.7 µmoles/kgbw/ min; ECGO = Sib1; 16.3 and Sib2; 19.2). In conclusion our data provide, for the first time, a direct evidence that insulin-resistant DM is determined by high levels of TG and that it can be reversed by decreasing circulating lipid concentration through a lipid malabsorption.

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Feeding suppresses gluconeogenesis significantly less in Mexican-Americans than in European-Americans: a mass isotopomer analysis study.

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In a previous study we used mass isotopomer analysis to measure gluconeogenesis in healthy Mexican-American men and found that gluconeogenesis was suppressed only modestly after feeding. The aim of this study was to compare the effect of feeding on gluconeogenesis in Mexican-Americans vs. Caucasians. Five Mexican-American men (age 33-63 years, BMI 26-30) and six European-American men (age 35-64, BMI 24-28) were infused intravenously for 6 h with U^{13}C-glucose on two occasions, in the fasting and fed states. All were normoglycemic and normoinsulinemic, with negative history of NIDDM in the immediate family. The infusion rates were 12.5 μmole/kg/h for the "fasting" study, and 20 μmole/kg/h for the "fed" study (when the subject consumed each hour a balanced enteral formula supplying 15 μmole of carbohydrate/kg/min). Mass isotopomer analysis of the pentaacetate derivative of plasma glucose was carried out following positive chemical ionization gas chromatography mass spectrometry. Glucose flux was calculated from the isotopic enrichment of the [M+6]-isotopomer. The enrichments of the [M+1]-[M+3] isotopomers were used with the equations of Katz et al to estimate gluconeogenesis. In the Mexican-Americans, the rate of gluconeogenesis via pyruvate in the fasting state was 5.81 ± 1.51 μmole/kg/min [mean ± SD] and contributed 60% of glucose flux; in the fed state the absolute rate of gluconeogenesis did not change (6.01 ± 0.63 μmole/kg/min) and its contribution to total glucose flux decreased to 37%. In the European-Americans, the rate of gluconeogenesis via pyruvate in the fasting state was 7.08 ± 1.5 μmole/kg/min and contributed 76% of glucose flux; in the fed state the absolute rate of gluconeogenesis decreased significantly (4.29 ± 0.95 μmole/kg/min) and its contribution to total glucose flux decreased to 27%. The effect of feeding on suppression of gluconeogenesis was therefore significantly greater in the European-Americans than in the Mexican-Americans. There were no significant differences between the two groups in plasma insulin levels. The results suggest that ethnic differences exist in the ability to regulate gluconeogenesis in response to feeding. The underlying factors may involve differential hormonal regulation of gluconeogenesis, or variable delivery of enteral glucose to the metabolic pool in the form of 3-carbon precursors.

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IMPORTANCE OF ALCOHOL FOR THE TRIGLYCERIDE RESPONSE AFTER INGESTION OF AN ORAL METABOLIC TOLERANCE TEST (oMTT)

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The metabolic syndrome is characterized by a cluster of diseases which often coexists: impaired glucose tolerance, NIDDM, dyslipidemia, hypertension and obesity. To prevent these diseases an early detection of predisposition is desired. After ingestion of a liquid formula which contains 4221 KJ energy, 58g mainly saturated fat, 75g carbohydrates, 30g protein and 10g alcohol in a volume of 500 ml (oMTT), in a group of 25 year old men without family history 15% of subjects (High Responders, HR) showed higher pp triglycerides (TG), higher insulin, proinsulin and glucose values which indicates an early state of the metabolic syndrome. This risk profile could be confirmed by a study in sons of parents with complete metabolic syndrome. The oMTT seems to detect the metabolic syndrome at a stage where the oGTT is not yet impaired and might be helpful to early diagnose patients high of risk. To differentiate the impact of its alcohol content we studied 15 Normal Responders (NR) and 8 HR after the ingestion of oMTT with 10g alcohol (wA) and without alcohol (oA). Blood for determination of TG was drawn before, 0.5h and 1h-9h pp.The pp TG values were lower in all subjects in the test without alcohol (AUC ΔTG oA: 372±47 mg/dl vs. ΔTG wA 438±68 mg/dl). HR showed significant higher pp TG after oMTT with alcohol (AUC ΔTG wA: 726±148 vs. ΔTG oA: 387±72 mg/dl, p=0.05). So is was only possible to discriminate NR and HR significantly in the test with alcohol (wA: AUC ΔTG NR: 353±43 vs HR: 726±148 mg/dl; p=0.006 - oA: AUC Δ TG NR: 364±63 vs HR 387±72 mg/dl, p= 0.82). Addition of alcohol to fat seems to be essential for discovering the phenomenon of TG High Response. This may indicate a crucial role of the metabolic response to alcohol (and fat) in the pathogenesis of the metabolic syndrome.

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ASSESSMENT OF HEPATIC SENSITIVITY TO GLUCAGON IN NIDDM

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NIDDM is associated with increased rates of endogenous glucose production (EGP) in both the postabsorptive and postprandial states. In order to determine whether these excessive rates of EGP are due to increased hepatic sensitivity to glucagon, 9 NIDDM and 10 nondiabetics (ND) were studied on three occasions. Endogenous hormone secretion was inhibited with somatostatin and replacement infusions of glucagon and growth hormone were given. Constant "basal" insulin infusions (defined as that necessary to maintain glucose at ~ 5 mM) were established in each individual. On one occasion, the glucagon infusion was maintained at 0.65 ng/kg/min (~100 pg/ml). On two different occasions the glucagon infusion was increased to 1.5 and 3.0 ng/kg/min for four hours elevating the glucagon concentrations to ~ 150 and 300 pg/ml, respectively. [6,6-$^2H_2$] glucose and $H^{14}CO_3$ were infused systematically to trace EGP and CO_2 incorporation into glucose (an index of gluconeogenesis). Glycogen was labeled via the direct pathway by having subjects ingest [6-^{3} H] galactose the evening prior to study. Each increase in glucagon resulted in a concomitant but transient (0-120 min) increase in EGP in both groups. The integrated increase in glucose concentration, EGP and CO_2 incorporation into glucose did not differ between groups implying comparable stimulation of EGP and gluconeogenesis. However the ratio of the release of [6-^{3}H] glucose to EGP was lower in the NIDDM than nondiabetic subjects indicating that a smaller portion of glucose released from glycogen was derived from the direct pathway. Glucagon did not alter carbohydrate or lipid oxidation in either group. We conclude that a) glucagon stimulation of glucose production, gluconeogenesis and by implication glycogenolysis, is equivalent in NIDDM and nondiabetic humans and b) a lesser portion of glycogen is derived from the direct pathway in NIDDM. These data indicate that the excessive rates of hepatic glucose release in NIDDM are unlikely to be due to enhanced sensitivity to glucagon.

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FREE FATTY ACIDS IN DIABETIC KETOACIDOSIS AND DIFFERENT CONTROL OF TYPE II DIABETES MELLITUS

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The relationship between insulin resistance, glycemia, free fatty acids (FFA) and ketogenesis substantiates a following-up the changes of total and main FFA such as C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, and C20:4 using the gas-liquid chromatography during diabetic ketoacidosis (DKA) 2 days after its coping and 2 months later on after achieving a good control of diabetes mellitus (DM). The study covered 39 patients (21 females and 18 males) with type II DM (mean age of 59±3.1 years, BMI of 26.5±0.8, and ideal body weight of 113.4±0.8%). Ten age, gender and BMI-matched controls were used. During DKA (BGL of 15.32±1.38 mmol/l; HbA/C of 11.3±0.5%, pH of 7.120±0.09; BE of -8.362±4.62; K of 4.093±0.59 mmol/l, and Na of 141.25±1.75), total FFA increased up to 1131.1±170.4 μmol/l. Two days after DKA they reduced down to 826.63±133.98 μmol/l (mean daily fasting Blood Glucose Level (BGL) of 12.0±1.2 mmol/l and postprandial BGL of 19.1±2.7 mmol/l). After 2 months at mean daily fasting BGL of 6.9±0.8 mmol/l, postprandial one of 8.3±1.4 mmol/l and HbA/C of 6.9±0.33%, the total FFA decreased down to 627.036±144.3 μmol/l remaining, however, by 16.1% higher than control values of 539.8±72.5 μmol/l. In DKA and poor control of DM, the elevation of oleic, linolic and palmitic acids and the reduction of arachidonic acid (down to 18.6±2.9 μmol/l) was most outlined. It increased 2 days after DKA up to 20.42±8.8 μmol /l and at improved control up to 25.8±13.8 μmol/l without reaching the control levels of 36.0±20.1 μmol/l. There was no essential dynamics in the percentage ratio of single FFA, of saturated and unsaturated FFA, and of monoenic and polyenic FFA. The changed ratio between the linolic and arachidonic acid was well manifested being 11.6 with DKA, 5.5 with good control, and 2.7 in healthy subjects. The significant changes of FFA in different DM control states confirm metabolism lability.

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NICOTINE IMPAIRS INSULIN-MEDIATED HEPATIC GLUCOSE HANDLING IN CONSCIOUS DOGS.

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To determine the acute effect of nicotine on insulin-mediated glucose metabolism, we investigated hepatic and peripheral glucose disposal during portal glucose infusion with euglycemic hyperinsulinemic clamp in seven healthy beagle dogs. These dogs were canulated into the femoral artery, portal, hepatic, splenic, and jejunal veins and, were implanted Doppler flow probes around the hepatic artery and portal vein ten days before the experiment. During experiments, insulin was infused intraportally (13.5 pmol/kg/min) and exogenous glucose was administered peripherally to maintain arterial euglycemia. After 120-min metabolic equilibration period and 30-min basal period (period B), glucose (38.9 μmol/kg/min) was administered into the portal vein for 180 min. During portal glucose infusion, nicotine (0.3 μg/kg/min) was administered peripherally for last 90 minutes (period PN(+)) after 90-min of period PN(-). Arterial plasma glucose and insulin levels were comparable among three periods (5.2±0.04 mM, 170±7 pM, respectively). In period PN(+), plasma nicotine concentration was 5.7±0.4 ng/ml and equal to that observed after smoking. Hepatic plasma flow was stable throughout the experiment (B, 18.2±1.8; PN(-), 19.1±2.0; PN(+), 19.8±2.4 ml/kg/min). Peripheral glucose disposal rates were comparable among three periods (B, 70.6±7.8; P, 60.6±5.0; N, 76.7±6.7 μmol/kg/min). Net hepatic glucose uptake was 4.4±1.7 in basal period, and increased to 15.6±1.1 μmol/kg/min by portal glucose delivery in period PN(-). Nicotine administration decreased net hepatic glucose uptake to 8.9±1.7 μmol/kg/min in period PN(+). In conclusion, increase in plasma nicotine levels induces insulin resistance in the liver rather than in the peripheral tissues.

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THE EFFECT OF 48 HOUR STARVATION ON MUSCLE CARBOHYDRATE AND LIPID METABOLISM IN RAT MODEL OF NIDDM.

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NIDDM is heterogenous disorder characterised by defect in insulin action and secretion, affecting carbohydrate and lipid metabolism. A rat model of NIDDM can be produced by neonatal injection of a low dose of streptozotocin(STZ) combined with later introduction of a high fat-diet. The aim of the present study was to estimate whether the 48-h starvation influence the glycogen and triacylglycerol (TG) concentrations in muscle and liver in rats with experimental NIDDM. To test this, the glycogen and TG concentration was estimated in the liver and red and white part of the gastrocnemius muscle in basal conditions and after 48-h fasting.Experiments were carried out on male Wistar rats fed from 8 to 11 week of age with isocaloric standard or high - fat diet (59% calories as fat) with previous injection of low dose of STZ (45mg/kg) or vehicle at 2 days of age (I - control group: vehicle + standard diet, II - STZ + standard diet -STZ, III - vehicle + high-fat diet-HFD, IV-STZ+high-fat diet-STZ/HFD). In all groups fasting plasma glucose, insulin, NEFA and TG were measured. Marked hyperglycemia was observed in STZ/HFD group ($p<0.05$). 48-h fasting decreased significantly glucose level in all studied groups. The highest insulin concentration was observed in HFD group, but the difference was not significant. Insulin level decreased after starvation in all studied groups, but statistical difference was observed only in HFD group ($p<0.05$). After starvation glycogen concentration fell significantly in both parts of gastrocnemius muscle in all studied groups vs starved control rats. Basal liver glycogen was markedly lower in HFD ($p<0.05$) and STZ/HFD group($p<0.05$) than in the control. Fasting caused significant decrease in liver glycogen concentrations in all studied groups vs basal conditions. A significant TG accumulation in examined tissues was observed in all studied groups in comparison to control. 48-h starvation decreased TG concentrations in white and red part of gastrocnemius muscle and liver in STZ group and STZ/HFD group. Plasma TG decreased markedly after starvation in control and STZ group ($p<0.01$, $p<0.01$). 48-h starvation did not affect plasma NEFA level in studied groups. We conclude that 48-h starvation increase TG utilization in rat model of NIDDM.

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INSULIN EFFECTS ON HEPATIC GLUCOSE PRODUCTION: EXTENT AND PATHWAYS. J. Radziuk and Z. Zhang Ottawa Civic Hospital, Ottawa, Canada

The extent to which insulin affects hepatic glucose production (HGP) and the pathways by which this occurs were assessed using dose response determinations during a 120 min recirculating perfusion of livers from twenty-five, 20h fasted rats. The perfusion medium consisted of donor rat erythrocytes in a BSA Krebs Ringer buffer. Initial concentrations of glucose were 100 mg/dl and lactate 40 mg/dl. Lactate was infused at 1 mg/min together with [U-14C]-lactate. Insulin was infused to maintain levels of 0, 70, 175 and 1050 pmol/L. Inflow and outflow concentrations of labelled and unlabelled metabolites were measured. A mathematical model relating lactate, glucose and glycogen fluxes indicated an unchanged fractional flux from lactate to glucose and an insulin dose-dependent increase in the corresponding glycolytic flux (12±4, 17±1, 21±2 and 42±6 $X10^{-3}min^{-1}$) and glycogen synthesis (1.7±0.5, 1.6±0.1, 4.4±0.6 and 6.6±2.3 $X10^{-3}min^{-1}$). This was corroborated in dispersion-corrected estimates of net glucose production: 0.89±0.07, 0.93±0.04, 0.49±0.08 and 0.33±0.08 μmol; mean lactate extraction: 38±2, 42±5, 37±3 and 38±3% and glycogen formation: 0.14±0.04, 0.12±0.02, 0.23±0.04 and 0.32±0.06 μmol glucosyl units. These data indicate, that in the fasting rat liver perfused with lactate as the principal substrate, insulin decreases net HGP by ~ 60%. It does this in the face of an unchanged gluconeogenetic flux from lactate by increasing net glycogen synthesis as well as glycolysis.

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ALTERED RATIOS OF GLYCOLYTIC TO OXIDATIVE MUSCLE ENZYME ACTIVITIES IN OBESITY

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Reduced enzymatic capacity for substrate utilization by skeletal muscle could contribute to the pathogenesis of obesity. To examine this hypothesis, the relationship between glycolytic (Gly) and oxidative (Ox) enzyme activities within skeletal muscle were determined and the proportionality of Gly to Ox was calculated. Enzyme activities were compared to patterns of fat deposition by regression analysis and adjusted for important co-variables. Vmax for hexokinase (HK), citrate synthase (CS), phosphofructokinase (PFK), glyceraldehyde phosphate dehydrogenase (GAPDH) were determined in 50 glucose-tolerant adults (24 M, 26 F), ranging from lean to obese (BMI = 19 to 41 $kg \cdot m^{-2}$). Visceral fat (VFat) was determined by CT and overall adiposity by DEXA. Insulin sensitivity (Rd) was measured using euglycemic clamps (40 $mU \cdot m^{-2} \cdot min^{-1}$) and VO_2max by cycle ergometry. PFK/CS, HK/CS, and GADPH/CS were positively correlated with VFat ($r=0.32$ to 0.38, $p<0.05$), and total adiposity ($r=0.25$ to 0.39, $p<0.05$). Glycolytic capacities were correlated with VFat, though not as strongly as the Gly/Ox ratios. Correlations between Gly/Ox ratios and VFat remained after adjusting for oxidative capacity of muscle, i.e. CS activity. Gly/Ox ratios were also correlated with VO_2max, yet significant correlations with VFat remained after adjusting for this parameter. Gly/Ox ratios were negatively correlated with insulin sensitivity; the relationship of Gly/Ox with VFat was not independent of insulin resistance. In summary, the Gly/Ox enzyme ratios are altered in visceral obesity, reinforcing the concept that perturbations in the capacity of skeletal muscle to oxidize substrates, especially in proportion to its capacity for glycolysis, may have an important role in obesity and its metabolic complications.

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LOSS OF FUNCTIONAL ZONATION IN HEPATIC ACINUS IN DIABETIC BB RATS.

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In the normal rat liver, gluconeogenesis is predominat in the periportal hepatocytes (PPH), whereas gycolysis predominant in the perivenous hepatocytes (PVH) of the hepatic acinus. To know if a diabetic state modify this functional zonation in the hepatic acinus, PPH or PVH was isolated selectively by a digitonin-collagenase method from IDDM model-BB rats, and cultured in 35 mm dishes for 1 h.

Gluconeogenesis from 5 mM lactate, 0.5 mM pyruvate and 5 mM alanine was greater in PPH than PVH (50.3±5.8 vs 33.2±4.1 nmol/mg prot/30 min without glucagon, $p<0.05$, and 66.6±6.0 vs 43.3±4.7 with 100 nM glucagon, $p<0.05$) from non-diabetic BB rats, but was not different between PPH and PVH (56.8±6.9 vs 51.6±5.7 without glucagon, NS, and 98.5±7.6 vs 93.9±6.1 with 100 nM glucagon, NS) from diabetic BB rats.

Lactate release by the addition of 20 mM glucose into the medium was greater in PVH than PPH (21.3±2.0 vs 10.5±1.7 μg/mg prot/60 min, $p<0.05$) from non-diabetic BB rats, but was not different between PPH and PVH (10.1±3.6 vs 9.1±3.0, NS) from diabetic BB rats.

Gycogen content when 20 mM glucose was added into the medium was not different between PPH and PVH from non-diabetic BB rats (33.5±2.1 vs 34.7±2.4 μg/mg prot, NS), or from diabetic BB rats (24.9±10.6 vs 18.2±5.8, NS), respectively.

In conclusion, the functional zonation in the hepatic acinus was lost in diabetic BB rats. This might be responsible for hepatic glucose overproduction in diabetes mellitus.

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THE ROLE OF LONG CHAIN POLYUNSATURATED FATTY ACID IN THE MODULATION OF INSULIN ACTION IN hHTg RAT

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We have shown previously that the *in vivo* insulin action in muscles of hereditary hypertriglyceridemic (hHTg) rats correlated negatively with the concentration of circulating triglycerides (Tg). Raised dietary intake of n-3 polyunsaturated fatty acid (PUFA) had been accompanied by increased insulin sensitivity of hHTg rats. The aim of this study was to test the role of long chain PUFA in muscle phospholipids for the modulation of *in vivo* insulin action and glucose transport under conditions of hereditary fixed and diet-induced hypertriglyceridemia. Groups of hHTG rats were fed for 2 weeks 4 types of diets: a) basal diet /BD/, b) BD with marine fish oil (BD+FO), c) high sucrose /= HS; 63 cal%/ and d) HS+FO. Then, euglycemic hyperinsulinemic (6.4 mU/kg/min) clamps were carried out in conscious rats equipped with permanent cannulas in the carotid artery and jugular vein. Skeletal muscles and the heart (collected after the clamp) were used for analyses of FA composition (gas chromatography) of skeletal muscle phospholipids (thin layer chromatography) and GLUT4 gene expression (Northern blot). Correlation analysis of fatty acid composition of skeletal muscle phospholipids with the *in vivo* insulin action of hHTg rats showed a tight positive relationship between the percentage of long-chain PUFAs (C20-22 PUFAs) and the glucose infusion rate (GIR) ($n= 16$, $r= 0.93$, $p<0.001$), and a negative correlation between the ratio of n-6/n-3 PUFA and GIR ($n= 16$ $r= -0.89$, $p<0.001$). HS feeding decreased the GLUT4 mRNA levels of hHTg rat in soleus muscle [HS: 6.9±0.2 arbitrary units (AU); BD: 13.0±1.0 AU; $p< 0.05$] and heart [HS: 1.8±0.3 AU; BD: 3.2±0.3 AU; $p< 0.05$]. Supplementation of HS diet with FO increased GLUT4 mRNA in both tissues [Soleus: 15.6±1.4 AU, $p< 0.05$; Heart: 3.4±0.2 AU, $p<0.05$]. The GLUT4 gene expression correlated positively with the long chain FA content in skeletal muscles ($n= 16$, $r= 0.55$, $p <0.05$) and in the heart ($n= 16$, $r= 0.68$, $p <0.01$). In summary: 1) the *in vivo* insulin resistance of the hHTg rats decreases with increasing proportion of the long-chain PUFAs in muscle phospholipids. 2) Data on GLUT4 mRNA tissue levels point also to a regulatory effect of C20-22 PUFAs on the gene expression for GLUT4.

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Novel Imaging Methods, Blood Flow, Exercise

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METABOLIC CONTROL OF MUSCLE GLUCOSE DISPOSAL: MAGNETIC RESONANCE SPECTROSCOPY STUDY IN HUMANS

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Although the absence of muscle intracellular free glucose (ICG) in euglycemic conditions is direct evidence of glucose transport being the rate-limiting step for glucose disposal, post-transport steps might be involved in the metabolic control of muscle glucose disposal during hyperglycemia. We have previously shown a significant accumulation of ICG (≈0.8 mmol/l) in human muscle during hyperglycemia (≈22 mM) at basal insulinemia. The present study was designed to evaluate muscle ICG accumulation during hyperglycemic hyperinsulinemia in the physiological range. Five healthy men were studied during a hyperglycemic (≈10mM) hyperinsulinemic (≈60mU/l) clamp, with an infusion of somatostatin and ^{13}C-glucose. Extracellular glucose, ICG, and glycogen were measured by ^{13}C Magnetic Resonance Spectroscopy (SMR) of the leg muscles. ICG concentration was calculated from the SMR signal, and from the plasma glucose concentration and ^{13}C enrichment. No significant ICG accumulation was observed: muscle concentration was 0.09±0.07 mmol/l during the second hour of the clamp. Over the same time period, muscle glycogen synthesis rate was 0.26±0.03 mmol/min.l reflecting a rapid glucose uptake. The finding that ICG does not accumulate in conditions mimicking the hyperglycemic hyperinsulinemic situation occuring after a large carbohydrate oral load, strongly supports the predominant role of glucose transport in controlling the overall rate of muscle glucose disposal, not only at euglycemia but also during physiological hyperglycemia.

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EFFECTS OF SHORT-TERM AEROBIC EXERCISE ON SERUM LIPID LEVELS AND LDL PARTICLE SIZE IN YOUNG MEN.

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To determine the effects of short-term aerobic exercise on serum levels of lipids, lipoproteins and apolipoproteins (apo), and LDL particle size, 31 nonobese men (mean ± SE body mass index; 22.1 ± 0.6 kg/m^2) aged 18 to 20 years had blood samples obtained just prior to and within 20 min of completion of 3 days of swimming exercise (4h/day). They consumed same diets (4300 kcal/day) during the entire study period. With exercise, they lost their weight from 66.3 ± 1.8 to 65.7 ± 1.8 kg ($P < .001$) and plasma glucose decreased from 4.83 ± 0.06 to 4.17 ± 0.17 mM ($P < .0001$) whereas nonesterified fatty acids increased from 463 ± 37 to 1078 ± 64 μM ($P < .0001$). Levels of total cholesterol decreased 9% from 4.44 ± 0.16 to 4.06 ± 0.10 mM ($P < .001$), LDL cholesterol decreased 14% from 2.74 ± 0.13 to 2.35 ± 0.10 mM ($P < .001$), apoB decreased 17% from 90 ± 4 to 75 ± 3 mg/dl ($P < .001$). Although LDL particle size decreased, postexcercise peak LDL diameter averaged 26.6 ± 0.4 nm, a figure that was shown to be less susceptibility to oxidative modification (pre-exercise, 27.1 ± 0.3 nm). Levels of triglyceride decreased 13% from 0.81 ± 0.05 to 0.71 ± 0.05 mM ($P < .1$), apoCII decreased 40% from 2.5 ± 0.2 to 1.5 ± 0.1 mg/dl ($P < .001$), apoCIII decreased 30% from 8.8 ± 0.4 to 6.2 ± 0.4 mg/dl ($P < .0001$), apoE decreased 14% from 5.0 ± 0.2 to 4.3 ± 0.2 mg/dl ($P < .01$). However, serum levels of insulin, HDL cholesterol and apoAI did not change significantly. Lp(a) levels decreased from 55 ± 10 to 46 ± 8 mg/dl ($P < .005$) in a top-tertile group whereas they did not change in the other two groups. These observations may explain in part the reduced risk of developing vascular disease in individuals who are physically active.

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MODELS TO MEASURE FLUORODEOXYGLUCOSE TRANSPORT AND PHOSPHORYLATION IN HUMAN SKELETAL MUSCLE FROM PET DATA

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Measuring glucose transport and phosphorylation in skeletal muscle is crucial for understanding of diabetes. Dynamic PET [^{18}F] fluorodeoxyglucose ([^{18}F]FDG) data contain information on transport and phosphorylation of glucose analogue FDG, but a model is required. Several FDG models and numerical identification approaches have been proposed in brain and heart. However these models have never been evaluated in human muscle tissue [^{18}F]FDG data. Here we assess the performance of two of these models. DATA. 5 normal subjects in the basal state received 8 mCi [^{18}F]FDG and dynamic scanning and plasma sampling were performed for 40 min. MODELS. Sokoloff et al. model parameters are 3 (3K model): transport in (ml/min-gram muscle) out (k2, min^{-1}), phosphorylation (k3,min^{1}). Phelphs et al. model assumes that phosphorylation (k4,min^{1}) is also present (4K model). In addition we added to both models a volume term (V,%). Parameters were estimated in each subject by weighted nonlinear least squares with optimal weights, i.e. equal to the inverse of measurement error variance (assumed proportional to counts, inversely to scanning time with a scale factor estimated a posteriori). RESULTS. The 3K model rate parameters were successfully identified: k1=0.031±0.006 (SE), mean precision 10%; k2=0.409±0.0129, 9%; k3=0.032±0.004, 6%. Also V was successfully estimated: 1.1±0.5(16%). The extraction coefficient (ml/min-gram muscle) was 0.23±0.02(3%). The 4K model rate parameters exhibited different values with expected deteriorated precision: k1=0.043±0.012(9%); k2=0.617±0.232(11%); k3=0.053±0.006(16%); k4=0.017±0.002(29%). V was reliably estimated only in 2 subjects. The extraction coefficient was 0.29±0.02(39%). The Akaike parsimony criterion was slightly in favour of 3K model. CONCLUSION. The 3K model with an additional volume term looks the most parsimonious one. The addition of the volume term and an accurate description of the measurement error are of utmost inportance for arriving at a reliable assessment of parameters and their precision. Additional work is required to i) evaluate other models in the basal state; ii) extend the models for studying elevated insulin states; iii) move from an FDG to a glucose picture by measuring the Lumped Constant under different experimental conditions.

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EFFECTS OF ENDURANCE TRAINING ON MYOCARDIAL AND SKELETAL MUSCLE FATTY ACID BETA-OXIDATION.

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We have previously shown, that endurance training reduces myocardial and increases skeletal muscle glucose uptake. To measure whether myocardial or skeletal muscle fatty acid oxidation is altered by training, we studied eight male endurance athletes (age 27±2.3 yrs, BMI 21.4±1.0 $kg/m^{2,}$ VO_2max 68±3.4ml/kg/min), and nine sedentary controls matched for characteristics other than VO_2max (49±8.0 ml/kg/min, p<0.001). Myocardial and femoral muscle fatty acid oxidation rates were measured during euglycemic hyperinsulinemia (serum insulin 60mU/L) with 14(R,S)-[^{18}F]fluoro-6-thia-heptadecanoicacid ([^{18}F]FTHA) and positron emission tomography (PET). During hyperinsulinemia whole body glucose uptake was 49 % higher in the athletes than in the controls (59±9.5 vs 40±9.4 µmol/kg/min, p<0.001). Myocardial fatty acid beta-oxidation indexes per kilogram of myocardium calculated by multiplying fractional [^{18}F]FTHA uptake and serum free fatty acid concentration were similar in the athletes and controls (10.1±5.1 vs 10.2±3.8 µmol/kg/min, ns). Skeletal muscle fatty acid beta-oxidation index in femoral region was 1.68±0.76 µmol/kg/min in the athletes and 1.15±0.41 µmol/kg/min in the controls (ns). Myocardial or skeletal fatty acid beta-oxidation rates were not correlated with VO_2max. In conclusion, myocardial and skeletal muscle fatty acid beta-oxidation rates per gram of tissue during euglycemic hyperinsulinemia are not altered in endurance athletes compared to sedentary controls.

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EXTRACELLULAR AND INTRACELLULAR GLUCOSE UNDER BASAL CONDITIONS IN MAN

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The glucose analogue, 3-O-methyl glucose (3OMG), is transported by glucose transporters but is not metabolised by intracellular enzymes. It can therefore be used to estimate the rates of glucose transport across the plasma membrane, and in a two compartment model of glucose metabolism it provides a measure of the flux from the central to the remote compartment. Using 3OMG and U-13C-glucose (U13CG) we quantified whole-body glucose kinetics in two fasting normal male subjects each on two occasions using a two compartment model. After basal blood sampling a bolus of U13CG (5 mg/kg) and 3OMG (8 mg/kg) was administered intravenously without perturbing fasting insulin concentrations. Blood samples were taken every two minutes for 20 min, then every 5 min to 90 min. Plasma concentrations of the two tracers were measured by GC-MS analysis. The parameters of the proposed model of glucose kinetics with removal from both compartments were estimated with precision. The fractional rate constants for removal from the central and peripheral compartments were similar (k_{01}:0.93±0.11; k_{02}: 0.95±0.26 $x10^{-2}$ min^{-1}; mean±SD) and both central to peripheral and peripheral to central compartments were k_{21}: 3.38±1.26; k_{12}: 5.14±2.19 $x10^{-2}$ min^{-1}. The distribution volume of the central compartment (148±16 mL/kg) indicated that this compartment can be associated with the extracellular volume. The peripheral compartment was considered to represent the intracellular volume. The model indicated that 36% of the total glucose (1.22±0.23 mmol/kg) is distributed in the intracellular fluid resulting in an intracellular glucose concentration of 1.1 mmol/L (intracellular volume of 400 mL/kg assumed). Intracellular irreversible removal represented 36% of total glucose uptake. The kinetics of glucose transport into and out of the cells is rapid (26±11 and 22±10 µmol/kg/min) compared to hepatic glucose output (11±2 µmol/kg/min). The model suggested that under basal conditions in man intracellular glucose concentration is an important contribution to the total glucose pool.

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AEROBIC CAPACITY AND INSULIN ACTION

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In this study we focused on the relationship between oxygen uptake in volitional maximal exercise treadmill test and insulin and glucose levels in oral glucose tolerance test (OGTT) in a population based sample of healthy, non-smoking, middle-aged (45 - 65 years) men (n=89). An inverse relationship was observed between $\dot{V}o_{2max}$ and 2-hour insulin level: the regression coefficient was -0.067 (SE 0.023, p=0.00). The relationship was -0.051 (SE 0.023, p=0.02) between anaerobic threshold oxygen uptake (AnT $\dot{V}o_2$) and 2-hour insulin level, -0.112 (SE 0.029, p=0.00) between aerobic threshold oxygen uptake (AerT $\dot{V}o_2$) and 2-hour insulin level. When adjusting $\dot{V}o_2$ and 2-hour insulin level to the body weight, age, exercise amount and intensity, left ventricule mass (LVM/BMI), stroke volume (SV/BMI), volitional effort (respiratory exchange ratio, RER and maximal heart rate, MHR) and smoking history no significant relationship (p=0.11-0.80) was observed between $\dot{V}o_2$ and 2-hour insulin level. When comparing $\dot{V}o_2$ and AUC for glucose we could find significant inverse relationship between $\dot{V}o_{2max}$ and AUC for glucose -0.151 (SE 0.074, p=0.04), between AnT $\dot{V}o_2$ and AUC for glucose -0.193 (SE 0.073, p= 0.01) and between AerT $\dot{V}o_2$ and AUC for glucose -0.397 (SE 0.091, p=0.00). When adjusting $\dot{V}o_2$ and AUC for glucose to the body weight, age, exercise amount and intensity, LVM/BMI, SV/BMI, volitional effort (RER and MHR) and smoking history AUC for glucose at the AerT contributed significantly to this relationship (p=0.00). Comparing the means of glucose and insulin values (oneway ANOVA) between different exercise amount and intensity groups no statistically significant relationship (p=0.14 - 0.92) in any exercise amount or intensity level was observed. In conclusion: 1. poor physical fitness described as AerT $\dot{V}o_2$ is inversely associated to AUC for glucose. 2. In these sedentary subjects no relationship between exercise amount, intensity and insulin or glucose levels was noted.

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MATHEMATICAL DEMONSTRATION THAT INSULIN RESISTANCE IN OBESE SUBJECTS IS NOT DUE TO SLOW INSULIN TRANSFER ACROSS CAPILLARIES.

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As implemented in their program MINMOD, some of the parameters and variables of the equations representing Bergman's minimal model of glucose metabolism have no simple rational relationship with the kinetic constants and constants of proportionality of the minimal model as such. In this work we implemented the original version of the minimal model, which does not suffer from this problem, and used it to investigate the source of insulin resistance among obese but otherwise healthy subjects. A frequently sampled intravenous glucose tolerance [FSIGT] test was performed in 38 healthy subjects of varying degrees of obesity [standard FSIGT test in 21 and tolbutamide FSIGT test in 17 subjects] in order to compare MINMOD and "modified" equations [M1]. The implementation of the original Bergman's minimal model leads the follow equations: $dG/dt = -[p_1^{M1}+X^{M1}]\cdot G + p_4^{M1}$ and $dX^{M1}/dt = -p_2^{M1}\cdot X^{M1}+p_3^{M1}\cdot I$, being p_1^{M1}: glucose effectiveness at zero insulin; $X_{basal} = [p_3^{M1}/p_2^{M1}]\cdot I_{basal}$ and $p_4^{M1} = [p_1^{M1}+X^{M1}_{basal}]G_{basal}$. Since there was no significant difference between the lean and obese groups as regards p_2^{M1}, the lower insulin sensitivity of the obese subjects may be attributed entirely to the difference in p_3^{M1}, i.e. to differences in k_2 and/or $[k_4 + k_6]$. Assuming that interstitial insulin [I'] is higher in obese subjects than in lean subjetcs, we have mathematically demonstrated that the proportionality constants of the model [k_4 and k_6] were lower in obese subjects than in lean subjetcs, but not the rate constant for insulin transfer across capillaries, k_2. This conclusion follows from the ratio observed between the average p_3 values of lean and obese [ob] subjects, $p_3^{M1}{}_{lean}/p_3^{M1}{}_{ob} = 1.22$ [1]; the ratio observed in this work between $[X^{M1}{}_{lean}]^{AUC}$ and $[X^{M1}{}_{ob}]^{AUC}$, the area under the curve [AUC] of $X^{M1}(t)$ during the FSIGT test, of lean and obese subjects, $[X^{M1}{}_{lean}]^{AUC}/[X^{M1}{}_{ob}]^{AUC} = 1.39$ [2]. Writting explicitly equation 2 $[(k_4 + k_6)\, I'^{AUC}]_{lean} / [(k_4 + k_6)\, I'^{AUC}]_{ob} = 1.39$ [3]. Since $I'_{lean}{}^{AUC} / I'_{ob}{}^{AUC} < 1$ this implies that $[k_4 + k_6]_{lean} / [k_4 + k_6]_{ob} > 1$ and hence $[k_4 + k_6]_{lean} > [k_4 + k_6]_{ob}$. Moreover, since explicitly equation 1 is: $[k_2\,(k_4 + k_6)]_{lean} / [k_2\,(k_4 + k_6)]_{ob} = 1.22$ [4], replacing equation 4 in equation 3: $[k_{2\,ob} / k_{2\,lean}] / [I'_{lean}{}^{AUC} / I'_{ob}{}^{AUC}] = 1.14$. Since $I'_{lean}{}^{AUC} / I'_{ob}{}^{AUC} < 1$, we concluded that $k_{2\,ob} > k_{2\,lean}$. In conclusion, our results suggest that low insulin sensitivity in these subjects is due to receptor and/or post-receptor events rather than to slow transfer of insulin across capillary endothelium into the interstitial space.

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AEROBIC CAPACITY, INSULIN ACTION AND MUSCLE MORPHOLOGY

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The purpose of the study was to investigate the relationship between oxygen consumption in volitional maximal exercise treadmill test, insulin and glucose levels in oral glucose tolerance test (OGTT) and distribution of muscle fiber type (I and II) in m. vastus lateralis in a population based sample of healthy, non-smoking, middle-aged (45-65 years) men (n=54). Marginal relationship was observed between 1-hour insulin level and muscle fiber type I: the regression coefficient was -0.079 (SE 0.043, p=0.07). A significant relationship was observed between muscle fiber type I and capillary/muscle fiber ratio: 25.4 (SE 10.3, p=0.01). Capillary/muscle fiber ratio was related to $\dot{V}o_{2max}$ 0.837 (SE 0.251, p=0.00) and AnT $\dot{V}o_2$ (anaerobic threshold) 0.407 (SE 0.182, p=0.03). An inverse relationship was observed between muscle fiber type I and RER (respiratory exchange ratio) at AerT (aerobic threshold) -119.6 (SE 57.9, p=0.04). A significant relationship was observed between average muscle fiber type I surface area (μm^2) and body weight (kg) 0.005 (SE 20.6, p=0.01). Body weight was also related to capillary/muscle fiber ratio 0.005 (SE 0.02, p=0.03). In conclusion: 1. One hour insulin level in OGTT might be affected by the histologically determined proportion of muscle fiber type I and thus indirectly by the capillary/muscle fiber ratio. 2. Energy metabolism in maximal treadmill exercise test in sedentary subjects is affected by both muscle fiber type I and capillary/muscle fiber ratio. 3. Size of the muscle fiber type I and capillary/muscle fiber ratio is affected by the body weight.

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DIFFERENCES IN KINETICS OF INTRACELLULAR pH AND 31P CELL METABOLITES IN SKELETAL MUSCLE OF NIDDM AND CONTROLS

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^{31}P NMR spectroscopy provides a noninvasive and very precise tool to monitor intracellular pH (pH_i) and cell metabolite concentrations such as PCr, Pi, and G6P.

The aim of the present study was to investigate these parameters under hyperglycemic - hyperinsulinemic clamp conditions (DeFronzo protocol with minor modifications) by ^{31}P NMR spectroscopy of the human calf muscle using a Siemens 1.5 T whole-body imager. The spectra of four patients with NIDDM (D) and three normal healthy subjects (N) were recorded at a time resolution of 3.2 min in order to get insight into the time-course of potential biochemical changes.

We found clear differences in the changes of pH_i (see Fig. 1) and PCr between N and D: both parameters were found to decrease markedly more in N as compared to D.

Only in the control group N the corresponding kinetics of G6P exhibits a marked elevation during the first 30 min and remains then almost constant. Pi increases by almost 20 % with minor differences between both groups.

Thus, this technique is capable of detecting incredibly small changes of the intracellular homeostasis of H^+, PCr, Pi, and G6P. Furthermore, the differences found provide additional insight into the impaired glucose metabolism in NIDDM patients.

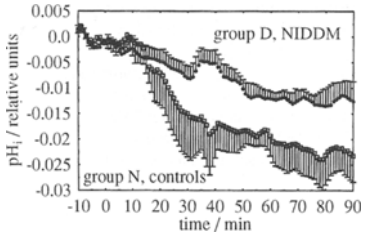

Fig. 1: Relative changes of pH_i in patients with NIDDM (means ± SEM, n=4) and controls (n=3). The time resolution of the original data was 3.2 min per spectrum. To match the clamps in each group, a linear interpolation was carried out leading to an appearantly higher time resolution of 1 min.

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EFFECTS OF AEROBIC CAPACITY AND FAT MASS ON THE INSULIN RESPONSE AFTER AN ORAL GLUCOSE LOAD

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The effects of aerobic capacity and fat mass on the insulin response after an oral glucose load were investigated in 21 college students. The maximal oxygen uptake was measured on a cycle ergometer by the leveling off criterion. Fat mass was measured with an impedance technique. Body mass index (BMI) was calculated as weight divided by squared height. All subjects underwent an oral glucose tolerance test after the overnight fasting period of 12 h. Their subjects were divided into three groups based on their insulin response after an oral glucose load: the first group showed a hyper- and prolonged-insulin response; HI (n=6), the second group showed a lower-insulin response; LI (n=6), and the third group consisted of other subjects; MI (n=9). The maximal oxygen uptake in the HI group (29.6 ± 3.3 ml/kg/min) was significantly lower than that in the LI group (42.5 ± 3.1 ml/kg/min, $P<0.05$). Although no significant difference was observed in the body mass index between the HI (24.6 ± 1.9) and the LI groups (22.2 ± 0.4, $p>0.05$), the body fat and the waist hip ratio were significantly higher in the HI group (26.8 ± 3.5 %, 0.87 ± 0.03) than in the LI group (16.2 ± 1.1 %, 0.76 ± 0.01, $P<0.05$). All subjects in the LI group performed regular exercise, while none of the subjects in the HI group performed any regular exercise. These results may thus suggest that an inactive life style, a decreased aerobic capacity and an increased body fat accumulation all appear to result in an increased insulin response after a glucose load.

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IN VIVO MICRODIALYSIS WITH 2-(^{18}F)FLUORO-2-DEOXY-D-GLUCOSE FOR STUDYING GLUCOSE METABOLISM: A NEW METHOD

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2-(^{18}F)Fluoro-2-deoxy-D-glucose (FDG) has been validated as a radioactive tracer for measuring glucose metabolism (intracellular uptake and phosphorylation) by positron emission tomography. Microdialysis, again, is a technique for measuring extracellular fluid (ECF) concentrations of substances in tissues in vivo. METHOD: We have combined microdialysis and FDG to follow changes in the extracellular FDG concentration in fasting (N=5) and nonfasting (N=5.) healthy adult Sprague-Dawley rats given a bolus injection of insulin. Anesthetized rats were inserted with microdialysis probes in the jugular vein, liver and femoral muscle. 5-min fractions of the dialysate were collected for 110 minutes; 2 IU of insulin were injected iv. 70 min after the injection of 2.0 - 3.0 mCi (specific activity 2 Ci/ng) of FDG. The proportion of FDG and FDG 6-PO_4 in tissue homogenates at the end of the experiments were determined by HPLC. RESULTS: The elimination $T_{1/2}$ in muscle of fasting rats was lower than fed rats (25±2 min vs. 34±9 min) indicating that glucose is mobilized more slowly from the ECF into the myocytes of fed than fasted rats. Insulin reduced the $T_{1/2}$ in all tissues by about 60 % implying that insulin mobilizes glucose from the plasma into the tissues and from the ECF of muscles into the myocytes. Insulin raised the proportion of FDG 6-PO_4 in all tissues - most markedly in the liver - which agrees with the concept that insulin promotes glycogen synthesis. COMMENT: These and other results obtained by our method (data not shown) agree what is known about the kinetics of glucose and the effects of insulin on glucose kinetics and metabolism. The microdialysis + FDG method is a new and valid method for studying the kinetics and metabolism of glucose in the ECF and agrees well with the results obtained with PET in humans. Pharmacological manipulations can be measured reliably with this method, as shown by the effects of insulin on FDG metabolism. Our method offers also a new approach for studying the effects of drugs on glucose metabolism.

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THE EFFECT OF SINGLE BOUT OF ENDURANCE EXERCISE ON MUSCLE CARBOHYDRATE AND LIPID METABOLISM IN RAT MODEL OF NIDDM

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A rat model of NIDDM can be produced by neonatal injection of a low dose of streptozotocin (STZ) combined with later introduction of a high fat-diet. The aim of the present study was to estimate whether the single bout of endurance exercise influence the glycogen, triacylglicerol (TG) and phospholipid concentrations in muscle and liver in rats with experimental NIDDM. To test this , the glycogen, TG and phospholipid concentration was estimated in the liver and red and white part of the gastrocnemius muscle in basal conditions and after endurance exercise on a treadmill. Experiments were carried out on male Wistar rats fed from 8 to 11 week of age with isocaloric standard or high - fat diet (59% calories as fat) with previous injection of low dose of STZ (45mg/kg) or vehicle at 2 days of age (I - control group: vehicle + standard diet, II - STZ + standard diet -STZ, III - vehicle + high- fat diet-HFD, IV-STZ + high-fat diet-STZ/HFD). In all groups plasma concentrations of fasting glucose, insulin, NEFA and TG were measured. Marked hyperglycemia was observed in STZ/HFD group ($p<0.05$). Exercise decreased significantly glucose level in all studied groups. Insulin levels decreased after exercise in all studied groups, but no statistical difference was observed. Basal liver glycogen was markedly lower in HFD ($p<0.05$) and STZ/HFD group($p<0.05$) than in the control. Glycogen concentration after exercise fell significantly in examined tissues in all groups in comparison to basal conditions, but did not differ markedly with control group. A significant TG accumulation in examined tissues was observed in all studied groups in comparison to control. Single bout of exercise decreased TG concentration in white and red part of gastrocnemius muscle in STZ/HFD group ($p<0.05$, $p<0.002$) but it remained still significantly higher in studied groups vs respective control values. Exercise did not change significantly plasma TG and NEFA concentrations.A significant decrease of phospholipids concentrations was observed after exercise in all examined tissues in STZ group vs basal conditions.We conclude that single bout of exercise has a beneficial effect on TG metabolism in rat model of NIDDM.

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THE EFFECT OF BLOOD FLOW ON MUSCLE GLUCOSE UPTAKE DURING ACUTE INSULIN RESISTANCE IN RAT

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The influence of blood flow on muscle glucose uptake during acute epinephrine (E) induced insulin resistance and ß-adrenergic blockade with propranolol (P) was investigated during an euglycemic hyperinsulinemic clamp in female rats. To assess the interstitial glucose concentration and the local skeletal blood flow, microdialysis was performed in medial femoral muscle in both legs with perfusion by 1% albumin containing ^{14}C-ethanol. The blood flow was assessed indirectly by means of the ethanol outflow : inflow ratio (%). E caused a significant decrease in glucose infusion rate which was completely restored during P-infusion (12.9±1.6 vs. 4.5±0.7, and 13.4±1.7 mg/kg/min, $p < 0.0001$). Interstitial glucose concentrations were significantly higher during ß-adrenergic stimulation (4.7±0.2 vs. 5.5±0.2 mmol/l, $p < 0.05$) which decreased during infusion with P (4.6±0.3, $p < 0.05$). Ethanol outflow : inflow ratio remained unchanged during ß-adrenergic stimulation (8.3±0.7 vs. 9.0±0.8 %, n.s) but decreased significantly during infusion with P (11.1±0.77, $p < 0.05$).
Data show that the rapid reduction of muscle glucose uptake is accompanied by an increase in interstitial glucose concentration without any alterations of the local blood flow. The results indicate that the mechanisms behind acute insulin resistance resides in the muscle cells and that the microcirculation becomes less rate-limiting for the glucose disposal in this state.

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EXERCISE INCREASES PANCREATIC ß-CELL MASS IN THE OLETF RAT, A MODEL OF NIDDM

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Both caloric restriction (D) and exercise training (Ex) ameliorate the diabetic state via similar mechanisms, one of which is an increase in insulin sensitivity. In order to determine if exercise training causes an increase in the ß-cell mass in OLETF rats that show decreases in the proliferative capacity of ß-cells and their mass (Diabetes 45:941, 1996) and whether these interventions act differently, ß-cell masses and insulin content in the pancreata of male OLETF rats were determined after a 9 week period of 70% caloric restriction or spontaneous exercise training using a training wheel. ß-cell mass was quantified by manual point counting of insulin immunostained sections.

Both interventions caused a similar increase in insulin sensitivity in the control OLETF rats [GIR $\mu mol \cdot kg^{-1} \cdot min^{-1}$: 53.9±5.7 (control) vs. 83.8±6.8 (D) and 98.8±10.7 (Ex)], which resulted in lower plasma IRI concentrations for the D group (229±33 pM) and for the Ex group (274±45 pM) as compared to the controls (371±48 pM). The ß-cell mass, adjusted for body weight, was significantly higher for the excercise trained group than for the other two groups [33.6±6.6 (Ex) vs. 24.9±4.9 (D) and 25.4±2.7 mg/kg BW (control)]. In terms of insulin content, a similar trend was found [50.1±8.9 (Ex) vs. 40.8±6.3 (D) and 27.8±8.7 µg/kg BW (control)].

These data suggest that exercise training has a positive effect in terms of ameliorating the diabetic state and that it results in an increase in both ß-cell mass and insulin sensitivity.

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STEADY STATE INTERSTITIAL MUSCLE INSULIN CONCENTRATION IS LOWER THAN IN PLASMA.

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Measurements in lymph and adipose tissue have indicated that interstitial insulin concentration is ~ 40% lower than in plasma. Hitherto, measurements of insulin in muscle interstitial fluid have not been performed. We have developed a new calibrated microdialysis technique allowing correct assessments of interstitial peptide concentrations and this was employed to estimate the insulin concentration in medial femoris muscle in 10 individuals (age:37±3, BMI:25.1±4.3) during an euglycemic hyperinsulinemic clamp. At steady state, b-glucose was 5.7±0.2 $mmol \cdot l^{-1}$, p-insulin was 188±20 $mU \cdot l^{-1}$ and interstitial muscle insulin was 99±22 $mU \cdot l^{-1}$, ($p<0.01$). At a higher insulin infusion rate steady state p-insulin concentration was 444±52 $mU \cdot l^{-1}$, and interstitial insulin 330±33 $mU \cdot l^{-1}$ ($p<0.01$). The data show for the first time that high physiological and supraphysiological plasma insulin levels give 30-50% lower interstitial concentrations of insulin in the muscle and, further, suggest the importance of capillary delivery as a regulating step for the insulin effect.

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COST BENEFIT ANALYSIS OF A PHYSICAL ACTIVITY PROGRAMME FOR PATIENTS WITH NIDDM

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Regular exercise is an accepted component of the management of NIDDM, but despite the benefits of exercise, few patients follow an exercise prescription. This study aimed to measure the cost and benefits of a holistic programme designed to encourage NIDDM patients to take regular exercise. Over 6 weeks, 234 consecutive outpatients previously diagnosed with NIDDM (107 female, 127 male; age 57 ± 10; age range 31-78,) were invited to attend a local leisure centre for exercise testing, lifestyle counselling and participation in a supervised physical activity programme. Only 27 (11%) attended testing and counselling with 14 (6%) participating in the physical activity programme for 3 months. Costs incurred were: leisure professionals time £187; exercise testing and screening £420; activity classes £272; administration £263; total cost £1142 (£81 per person). After 3 months participation, according to a questionnaire appraisal, 13 subjects benefited from enhanced physical, mental and social well being. In conclusion, the initial costs of this method were high for such a low participation rate, yet, it is clear, that individuals attending greatly valued the holistic benefits and their involvement. Alternative less expensive strategies are urgently required to encourage and motivate more NIDDM patients to exercise regularly.

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SKELETAL MUSCLE, A SITE FOR THE ANTIDIABETIC EFFECT OF S15261. AN *IN SITU* MICRODIALYSIS STUDY.

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In obese, insulin-resistant ageing rats and in a model of NIDDM (sand rat), chronic treatment with L-3 [2- [2- [4- [α - fluorenylacetylaminoethyl] benzoyloxy] ethylamino] 1-methoxyethyl]trifluoromethylbenzene (S15261) resulted in an increase of peripheral insulin sensitivity as revealed by the intravenous glucose tolerance test and by euglycaemic hyperinsulinaemic clamp. It could be considered that up to as much as 70 % of the glucose supplied i.v. is metabolized in the muscles. Since the clamp showed no effect of the drug on hepatic glucose production skeletal muscles are a putative target for S15261. **Aim of the study**: Microdialysis was used to gain insight into the substrate exchanges in the interstitial space of skeletal muscle of anesthetized (Forene®) male Sprague Dawley rats treated with vehicle or S15261 10mg/kg or 25mg/kg orally. **Methods**: The dialysis probes were inserted parallel to the muscle fibers between the soleus and the gastrocnemius and perfused with 5mM ethanol in Ringer buffer. The dialysis perfusion flow was 1µl/min. Following a stabilization period (40 min) the animals were treated with the vehicle or the drug and samples were collected every 20 min during the subsequent three hour-period. Ethanol, lactate and glucose in the dialysate were measured by enzymic methods. **Results**: The inverse ethanol outflow/inflow ratio (initial 5.06±0.4) remains constant throughout the study in any group, indicating the absence of variation in the local blood flow. Basal dialysate glucose concentration (mM) were 5.55±0.48, 4.77±0.38 and 4.78±0.19 in control, S15261 10mg/kg and S15261 25mg/kg treated rats respectively. Only in the latter two groups was a significant decrease in glucose concentration in the dialysate observed (-16% $p \leq 0.01$ and -31% $p \leq 0.001$ respectively). Similarly the lactate concentration (0.96±0.08 mM) in the dialysate was decreased only in the treated groups (a 38% decrease $p \leq 0.001$ and a 48% decrease $p \leq 0.001$ respectively) versus the initial values. **Conclusion**: The ethanol technique provides a valid measure of change in local tissue blood flow. No change was observed in control or treated groups. Both glucose and lactate concentration were decreased in S15261 treated rats only. The present results taken together with previous data favour a peripheral site of action for this drug, although the exact mechanism of action remains unknown.

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RESISTANCE TRAINING IMPROVES THE METABOLIC PROFILE IN INDIVIDUALS WITH NIDDM.

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Background and objectives: Aerobic endurance exercise has traditionally been advocated in the treatment of non-insulin-dependent diabetes mellitus (NIDDM) while the potential role of resistance training has often been overlooked. The aim of the present study was to determine the effect of circuit-type resistance training on blood pressure, lipids and long-term glycemic control (HbA1c) in NIDDM subjects.

Methods: Thirty eight NIDDM subjects were enrolled in the study; 18 NIDDM subjects participated in a 5 month individualized progressive resistance training program (moderate intensity, high-volume) twice a week, while 20 NIDDM subjects served as controls.

Results: The exercise group showed improvements in total cholesterol (6.0±.3 to 5.3±.3 mM; $p<0.01$), LDL-cholesterol (3.90±.22 to 3.35±.21 mM;$p<0.01$) and triglycerides (1.91±.25 to 1.53±.22 mM; $p<0.01$). Also the difference between the change in HbA1c (0.5%) between the groups reached statistical significance ($p<0.01$).

Conclusions: Circuit-type resistance training seems to be feasible in moderately obese, sedentary NIDDM subjects and the inclusion of circuit-type resistance training in exercise training programs for NIDDM subjects seems appropriate.

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BLUNTED VASODILATORY EFFECT OF INSULIN IN HYPERTENSIVE OBESE SUBJECTS

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We studied the vasodilatory effect of insulin in obese patients with insulin resistance and essential hypertension. The reproducibility of insulin induced changes in blood flow was measured in 8 obese hypertensive subjects (age 32±9 years; BMI 31.8±5.8 kg/m², systolic BP 149±16 mm Hg, diastolic BP 89±13 mm Hg (mean±SD)) and 10 healthy subjects (age 22±2 years (p=0.119); BMI 22.6±2.0 kg/m² (p=0.002); systolic blood pressure 125±11 mm Hg, diastolic 74±10 mm Hg (both p<0.01)) by means of venous occlusion plethysmography (Compactus 712, Gutmann, Eurasburg, Germany) on two different study days. Blood flow was measured in 10 min intervals during hyperinsulinaemic euglycaemic glucose clamps (target blood glucose 5.0 mmol/l, study duration 6 h) with three different insulin infusion steps (120 min each): 0.5, 2.5 and 5.0 mU/kg/min. Mean blood flow values of both legs were calculated. Insulin resistance of the obese hypertensive patients was indicated by a significantly lower insulin sensitivity index (1.6±0.4 ml/min/1.73 m² / µU/ml) in comparison to healthy volunteers (2.9±1.0 ml/min/1.73 m² / µU/ml; p=0.002). No significant differences were observed between both days within the groups (p=0.300 and 0.676). Infusion of insulin increased blood flow significantly (p<0.05) in healthy subjects from 3.2±0.6 ml/min/100 ml tissue during infusion step 1 (serum insulin 132±30 pmol/l) to 4.4±0.6 (802±125 pmol/l) in step 2 and 4.7±1.0 (1782±362 pmol/l) in step 3. In contrast, blood flow did not change in obese hypertensive subjects from 3.3±0.6 ml/min/100 ml tissue during infusion step 1 (serum insulin 275±167 pmol/l) to 3.7±0.5 (1267±157 pmol/l) in step 2 and 3.9±0.6 (3638±1568 pmol/l) in step 3, indicating lack of major dilatory effect of insulin on vasculature of obese hypertensive subjects. The blood flow values and their changes registered on day 2 were comparable to those registered on day 1. In conclusion, a consistant resistance to the vasodilatory effect of insulin was observed in obese hypertensive subjects. This may contribute to the development of essential hypertension in these patients.

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LOW HABITUAL TOTAL ENERGY EXPENDITURE IS ASSOCIATED WITH THE METABOLIC CARDIOVASCULAR SYNDROME N.J.Wareham[1], S.H.J.Hennings[1], C.D.Byrne[2], N.E.Day[1]. Departments of Community Medicine[1] and Clinical Biochemistry[2], University of Cambridge.

To investigate the role of habitual energy expenditure in the aetiology of the metabolic cardiovascular syndrome (MCS), 164 randomly selected volunteers aged 30-40 years were recruited from a population register and underwent a standard 75g oral glucose tolerance test. 4-day total energy expenditure were assessed using the heart rate monitoring method with individual calibration of the relationship between energy expenditure and heart rate. This method has previously been validated against doubly-labelled water and is an accurate, cheap, non-invasive and objective method for estimating energy expenditure. Energy expenditure was expressed as physical activity level (PAL), a weight independent measure where PAL = total energy expenditure (MJ/day) / basal metabolic rate (MJ/day). A sub-group of 22 subjects underwent 4 day heart rate monitoring on 3 further occasions at 4 monthly intervals. From these repeat tests the reliability coefficient for PAL was determined. This coefficient is required to adjust the odds ratio for measurement error in the exposure. The MCS was defined as the presence of one or more of 4 features of the syndrome (hypertriglyceridaemia, low HDL cholesterol, impaired glucose tolerance or diastolic hypertension). The odds ratio between increasing quartiles of PAL and the MCS was 0.68 (95% c.i. 0.47-0.99, p=0.043). Adjustment for measurement error corrected this odds ratio to 0.40. We conclude that low habitual energy expenditure is associated with this syndrome and that the strength of the association is heightened by adjusting for measurement error.

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MYOCARDIAL GLUCOSE UPTAKE IS ENHANCED IN ESSENTIAL HYPERTENSION INDUCED LEFT VENTRICULAR HYPERTROPHY.

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We have previously shown that insulin stimulated myocardial glucose uptake (GU) is increased in untreated patients with essential hypertension without left ventricular hypertrophy (LVH-). In the present study we determined whether hypertension, with or without LVH, alters insulin stimulated glucose uptake via alterations in heart oxygen consumption. For this purpose, heart GU, flow and oxygen consumption were measured in 7 nondiabetic hypertensive men with LVH (LVH+; age 42±2 yrs, LV mass 161±9 g/m²) treated with ACE-inhibitors or/and Ca-antagonists (BP 136± 15/81±5 mmHg), in 10 similarly treated hypertensive patients without LVH (LVH-,LV mass 109±14 g/m², BP 134±16/86±10 mmHg) and in 6 normotensive normal men (CONT, 129±10/79±6). Myocardial GU and oxygen comsumption were measured during euglycemic hyperinsulinemia (insulin ~70 mU/L) using PET. Myocardial GU (µmol·100g⁻¹·min⁻¹) was 38% higher in LVH+ (109±26) than in CONT (79±18, p<0.05) and averaged 96±8 µmol·100g⁻¹·min⁻¹ in LVH-. Myocardial blood flow was similar in all groups. Myocardial oxygen consumption (mL·100g⁻¹·min⁻¹) was positively correlated with myocardial glucose uptake (r=0.59, p<0.01). When glucose uptake was compared between groups using oxygen consumption as a covariate, glucose uptake rates did not anymore differ between the groups. These data demonstrate that the impact of hypertension on insulin stimulated heart glucose uptake can be attributed to differences in cardiac energy requirements as determined by direct measurement of heart oxygen consumption in vivo.

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EXCESS PURINE DEGRADATION DURING SEMI-ISCHEMIC FOREARM TEST IN DIABETIC PATIENTS.

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The aim of this study was to investigate whether the microvascular abnormality exists in the skeletal muscle of diabetic patients. In eleven diabetic patients and six healthy controls, forearm exercise was performed by squeezing a hand dynamometer for 2 minutes at one second interval under semi-ischemia by pressing upper arm with mean arterial pressure. Venous blood samples were collected from the antecubital vein to measure blood lactate, plasma ammonia (NH_3) and hypoxanthine (HX) at 0, 2, 6, 12, and 60 minutes after exercise. We defined seven patients as the excess response group (E) in whom plasma HX increment exceeds the mean+2S.D. levels of the control group (C) and the other four patients as the normal response group (N). The maximum increments in plasma HX and NH_3 in E (16.8±3.2 µmol/l and 122±60 µmol/l) were significantly greater (p<0.05) than those in C (3.6±3.0 µmol/l and 32±34 µmol/l) and N (2.9±2.9 µmol/l and 27.4±12.7 µmol/l). There were no significant differences in plasma HX and NH_3 between N and C. The maximum increment in plasma lactate was observed at 2 minutes in all three groups. The maximum increments in plasma lactate both in E (5.4±1.5 mmol/l) and N (3.6±1.0 mmol/l) were significantly higher (p<0.05) than that of C (1.7±0.5 mmol/l). Plasma lactate level in N promptly decreased to the level of C at 4 minutes. In E, however, plasma lactate level was kept still higher than those in other groups even at 10 minutes. The prevalence of diabetic retinopathy was higher in E than in N (86% vs. 25%). These data indicate that microvascular abnormality was developed also in the skeletal muscle of diabetic patients.

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Insulin resistance and hypertension:role for microcirculation?

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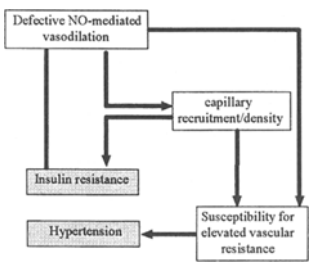
The association between hypertension and insulin resistance is well established but presently unexplained. Among several possible explanations, a connection between the two abnormalities can be envisioned at the level of the microvasculature in skeletal muscle (Figure). To test this hypothesis skin microcirculatory function was assessed in a group of 14 healthy, normotensive, glucose tolerant and non-smoking subjects, age 33±14.5 yrs (mean±SD), BMI 24.3±3.1 kg/m^2 showing a wide range in insulin sensitivity. Insulin sensitivity was assessed with the hyperinsulinaemic euglycaemic clamp technique and expressed as M/Ix100 in mg/kg/min per pmol/l. Videomicroscopy was used to measure skin capillary density and capillary recruitment after occlusion. Skin blood flow responses to acetylcholine (Ach, which acts via the endothelium) and sodium nitroprusside (SNP, a smooth muscle relaxant) were evaluated by iontophoresis in combination with laser Doppler fluxmetry. 24-h ambulatory blood pressure monitoring was used to measure blood pressure. Insulin sensitivity showed a large variation (0.87-4.41) and correlated with mean arterial blood pressure (MAP) during daytime (r=-0.50, p=0.05). Percentage capillary recruitment correlated with 24-h MAP (r=-0.58, p<0.05) and insulin sensitivity (r=+0.82, p<0.01). Furthermore, Ach-mediated vasodilation correlated with insulin sensitivity (r= +0.84, p<0.01) and percentage capillary recruitment (r= +0.69, p<0.01) but not with MAP. SNP-mediated vasodilation did not show a correlation to MAP or insulin sensitivity. In conclusion, microcirculatory function may link insulin resistance to elevated blood pressure.



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abstract has been withdrawn.

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SUPPRESSION OF INSULIN-INDUCED VASODILATION IN INSULIN RESISTENT NIDDM PATIENTS.

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Aim and Methods. To evaluate the vasoactive effect of insulin on insulin resistance, blood pressure, cardiac output and total vascular resistance of 16 NIDDM patients without macroangiopathy were investigated by non-invasive pressure wave analysis in forearm during hyperinsulinemic euglycemic clamp study. ***Results.*** During the clamp study, mean blood pressure (MBP) remained unchanged. Cardiac index (CI; cardiac output/body surface area) significantly increased by 31% (P<0.01) at 2 hour after initiating insulin infusion. In contrast, total peripheral resistance (TPR) decreased by 23% (P<0.01) at that time. Although no significant correlation between glucose infusion rate (GIR) as a marker for the muscular insulin sensitivity and the increasing rate of CI during clamp study was observed, the decreasing rate of TPR showed positive correlation with GIR (r=0.59 at 1 hour and r=0.65 at 2 hour; both P<0.05. ***Conclusion.*** These results suggest that an impairment of insulin-induced vasodilation may associate with muscular insulin resistance. Thus, increment of cardiac output and suppression of insulin-induced vasodilation may contribute to pathogenesis of hypertension in insulin resistent NIDDM patients with hyperinsulinemia.

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DIVING ON INSULIN

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Until now insulin - dependent diabetics are banned from every scuba - diving activity by the majority of the scuba diving associations and the majority of physicians. No study has been performed, to investigate, if diabetics can dive safely under controlled conditions. Based on some basic research a special open water scuba diving course for IDDM has been organized. The training included detailed metabolic control (i. e. blood glucose, hematocrite, blood pressure, fluid intake, pulse rate and lactate testing prior and after each dive, detection of exertion level and mean air consumption) in seven diabetic divers and age, sex and diving experience matched seven non - diabetic control persons. The participants for the course came from Australia, Europe and the United States. They had been selected carefully, considering their general physical and medical fitness and the quality of diabetes control and care and knowledge and management of their disease. It was found diabetic divers had to drink additional amounts of fluid (up to twice the amount required for non - diabetic divers) to effectively avoid dehydration. All diabetic divers had to reduce their short - acting insulin between 30% to 70 % before each dive and their long - acting insulin up to 50% to avoid hypoglycaemia. The reduction had to be increased according to the numbers of dives per day and the amount of diving days. Additionally they had to increase their daily carbohydrate intake up to 200 %. All adaptations had to be done with each diver on an individual basis, according to his/ her blood glucose levels measured 60, 30 minutes and directly before and after every dive - the blood glucose levels prior to the dive were aimed to be in a range between 180 - 240 mg/ dl, afterwards around 100 - 140 mg/ dl. By these limits any episode of hypoglycaemia under water was avoided and ketones were not present at any time. This research project showed that careful selection of the participants in combination with a structured training programme including special under water carbohydrate intake (glucose gel) can enable insulin - dependent diabetics to dive safely.

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INCREASED NORADRENERGIC PRESSOR RESPONSE, BUT NORMAL PERIPHERAL VASCULAR NORADRENERGIC RESPONSE IN NIDDM .

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Introduction: An increased pressor response to an i.v. infusion of noradrenaline (NA) has been reported in NIDDM, compatible with an increased vascular smooth muscle cell (VSMC) responsiveness to NA. Since systemic (i.v.) infusions are difficult to interpret as no distinction can be made between central and peripheral effects, we performed both systemic (i.v.) and local NA infusions in normotensive, non-obese, NIDDM patients to determine blood pressure, forearm arteriolar and peripheral venous NA responsiveness. **Patients and Methods:** 16 NIDDM patients, median age 61(57-63) yrs. (interquartile ranges), BMI 25.2 (24.3-26.1) kg/m^2, mean arterial pressure (MAP) 93 (89-98) mmHg, and 17 healthy volunteers (HV), 58 (53-63) yrs., BMI 24.9 (23.0-25.7) kg/m^2, MAP 93 (85-100) mmHg were studied. In *study I* (n=9 and 10, resp.) the response of forearm blood flow (FBF) to 3 doses of ***i.a.*** NA (0.025, 0.1, 0.4 μg/min) was measured by strain gauge plethysmography. Subsequently, the systemic ***i.v.*** NA dose which elevated MAP by 20 mmHg was determined (NA20). In *study II* (n=14 and 14, resp.) the venous constrictor response to a cumulative local infusion of NA was measured in a dorsal handvein.The maximal venous constrictive response (Emax) and the drug dose generating half maximal response (EC50) were calculated. **Results:** In *study I* no differences were observed in FBF responsiveness to ***i.a.*** NA. The NA20 dose was lower in the NIDDM patients relative to HV: 5.3 (4.1-7.6) vs 8.0 (6.1-10.0) μg/kg/min, $p<0.04$. In *study II* no differences were observed in EC50 and Emax between the NIDDM subjects and the HV: 1.09 (0.82-1.35) vs 0.81 (0.60-1.02) log ng/min and 94 (82-107) vs 87 (79-94)%, respectively. **Conclusions:** Non-obese normotensive NIDDM patients have an increased pressor response to NA. This increased response is not related to a defect in skeletal muscle resistance arterioles or peripheral veins. Probably a defect is present in the baroreceptor system and/or other vascular beds in NIDDM.

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INCREASE IN ENDOGENOUS LACTATE IN RESPONSE TO EXERCISE DOES NOT PROTECT THE BRAIN DURING HYPOGLYCAEMIA IN HUMANS.

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Experimental infusion of *exogenous* lactate (LACT) protects brain metabolism and function during insulin hypoglycaemia (hypo). To test the hypothesis that also spontaneous increase in *endogenous* LACT, such as that occurring during exercise, exerts similar effects, plasma counterregulatory hormones (adrenaline and noradrenaline, A and NA; glucagon, IRG; growth hormone, GH; cortisol, CORT) and autonomic and neuroglycopenic symptoms score (AS, NS) were measured in 7 normal volunteers during hypo+exercise (Study 1, S1), or hypo without exercise (Study 2, S2) or exercise without hypo (Study 3, S3) (random order). Steady-state exercise (cycloergometer, 80 min at 110 watts) was performed in S1 and S3, whereas in S2 subjects remained seated. Insulin (1 mU/kg/min) + variable glucose were infused iv from 20 to 80 min to induce hypo in S1 and S2 (plasma glucose 2.8 mmol/l) or maintain euglycaemia in S3. Results.

	Hypo+ Ex+ (S1)		**Hypo+ Ex-** (S2)		Hypo- **Ex+** (S3)	
(min)	0	80	0	80	0	80
LACT(mmol/l)	1.1±0.1	3.2±0.4	1.2±0.1	1.8±0.1	1.3±0.1	2.8±0.3
A(nmol/l)	0.3±0.1	8.1±1.1	0.2±0.04	3.7±0.8	0.3±0.1	0.6±0 2
NA(nmol/l)	2.3±0.2	7.7±0.9	1.7±0.2	2.4±0.3	1.74±0.2	6±0.9
IRG (pg/ml)	242±23	407±44	212±24	302±43	252±21	251±26
GH (μg/l)	2.7 ±1.2	80±10	1.4±0.6	37±10	1.4±0.2	31±10
CORT (nmol/l)	386±93	552±83	386±83	469±83	386±138	303±140
AS (score)	1.5±0.4	9±1.1	1±0.3	6.9±1.6	1.6±0.8	5.3±1.1
NS (score)	0.6±0.2	8±1.3	0.4±0.2	6.6±0.3	0.2±0.1	3±0.8

Conclusions. In the only physiological condition in which *endogenous* LACT increases, i.e. exercise, counterregulatory hormone and symptom responses to hypo are not blunted. The fact that the brain responds to decrease in plasma glucose, not to increase in LACT, indicates that the former, not the latter is critical for brain metabolism and function.

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EVIDENCE OF BLUNTED INSULIN KINETICS IN NIDDM PATIENTS - A MICRODIALYSIS STUDY

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Previous studies have shown that capillary delivery might be ratelimiting for the effect of insulin in both animals and man. These findings suggest that the capillary endothelium could be a regulating step of importance for mechanisms behind insulin resistance. In the present study we employed abdominal subcutaneous microdialysis (BAS ultrafiltrating membrane, cut off 30kD, 1 μl/min) during a two-step euglycemic insulin clamp. Ten male non-insulin dependent diabetic (**NIDD**) and 10 male non-diabetic control subjects (**C**) were studied after fasting overnight (Age: 57±2 (Mean±SE) vs 57±2 yrs, BMI: 29.3±0.90 vs 30.4±1.6 kg/m^2, P-Insulin: 12±2 vs 10±2 mU/l and B-HbA1c: 7.1±0.4 vs 4.7±0.2 % ($p<0.001$), respectively). During a low insulin infusion rate (**L**) (120-mU/m^2/min) mean peripheral steady state insulin concentrations during the last hour were 217±40 vs 207±15 mU/l (n.s.) and after a rapid switch to a high insulin infusion rate (**H**) (240-mU/m^2/min) mean peripheral insulin levels were 452±96 vs 543±49 (n.s.), respectively. There was a significant delay in T_{max} for interstitial insulin in **NIDD** (129±9) as compared to **C** (73±10 min) ($p<0.01$) but no significant differences between the groups in the subcutaneous insulin levels at each step were seen. Furthermore, glucose infusion rate (GIR) during the insulin clamp was lower in **NIDD** than in **C** at both **L** (7.16±0.78 vs 9.27±0.78 mg/kg/min, $p<0.05$) and **H** (8.60±0.85 vs 10.59±0.63, $p<0.05$). In conclusion, the data indicate similar abdominal subcutaneous insulin levels but a slower increase in interstitial subcutaneous insulin in NIDDM compared to non-diabetic subjects during an insulin clamp. Capillary delivery of insulin seems, thus, to be delayed in NIDDM.

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SCIATIC NERVE SEVERANCE PRODUCES HAEMODYNAMIC CHANGE AND INSULIN RESISTANCE IN MUSCLE

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Severance of the sciatic nerve *in vivo* leads to insulin resistance in previously innervated lower leg muscles. In the present study a modified constant-flow perfused rat hindlimb technique (femoral veins cannulated) was used to assess intrinsic vascular resistance, oxygen uptake, and insulin sensitivity of the 24 h-denervated lower leg (DL) compared to the sham-operated contralateral lower leg (CL) and sham-operated (both leg) control hindlimbs (S2L). Insulin (2 mU.ml^{-1})-mediated 2-deoxyglucose uptake was impaired in tibialis anterior, extensor digitorum longus, gastrocnemius white, plantaris and soleus of DL when compared to CL. There was evidence for changes in vascular resistance: perfusate flow from DL (1.87±0.2) was lower than that from CL (3.04±0.20; $P<0.05$, n=5) which was higher than that of either leg of S2L (2.2±0.1 ml.min^{-1}.leg^{-1} ;$P<0.05$, n=5). Perfusate aorta-femoral vein oxygen differences were unchanged [585±24 (DL), 610±15 (S2L) and 513±12μM (CL)], and thus the oxygen uptake ($\dot{V}O_2$) was less for DL (66.0±8.2) than CL (93.3±6.0 μmol.h^{-1}.leg^{-1};$P<0.05$, n=5). It is concluded that sciatic nerve severance in one leg leads to a loss of insulin sensitivity when compared to the contralateral leg. Perfused hindlimb studies reveal a haemodynamic change with decreased flow in the denervated lower leg, compensated by an increased flow in the contralateral lower leg. Decreased $\dot{V}O_2$ by the denervated lower leg is consistent with a decrease in muscle nutritive flow, which may account for the apparent insulin resistance.

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Weight Regulation, Leptin

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RESTING ENERGY EXPENDITURE IN SOUTH ASIANS AND WHITES WITH TYPE 2 DIABETES MELLITUS.

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Insulin resistance syndrome is more common in South Asians than whites. This study was designed to examine whether this reflects differences in energy expenditure. Resting energy expenditure (REE) was measured between 5-45 minutes by indirect calorimetry (Deltatrac) after a 10h overnight fast. There were no differences in clinical characteristics (mean ± SD) between South Asians (19M, 10F; age 54 ± 10 years; weight 78 ± 20 kg; body mass index 29 ± 7 kg/m^2; diabetes 6 ± 5 years; fat 29 ± 13%; HbA1c 8 ± 2%) and Whites (19M, 10F; age 57 ± 8 years; weight 79 ± 12 kg; body mass index 29 ± 5 kg/m^2; diabetes 5 ± 5 years; fat 29 ± 10%; HbA1c 8 ± 2%). In comparison to whites, South Asians had lower measured REE (1547 ± 258 versus 1684 ± 203 kcal/d, unpaired t-test p< 0.05) with Department of Health predicted values of 1631 ± 245 and 1641 ± 197 kcal/d respectively. In contrast REE/fat free mass was similar (30 ± 7 versus 31 ± 5 kcal/kg). We suggest that the lower REE in South Asians not only predisposes them to positive energy balance and hence weight gain and ultimately insulin resistance but will reduces the therapeutic impact of calorie restriction.

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INSULIN ACTION IN ADIPOCYTES FROM BLACK AND WHITE WOMEN: RELATIONSHIP TO VISCERAL FAT AND INSULIN SENSITIVITY INDEX, Si.

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We assessed adipocyte insulin resistance in 23 black (B) and 23 white (W) obese (BMI 35±1 vs. 36±1, mean±SEM), age-matched, premenopausal, non-diabetic women when body fat distribution was characterized by magnetic resonance imaging of visceral (VAT) and subcutaneous abdominal (SCAT) adipose tissue. B and W groups were not different with regard to VAT area (119±11 vs. 142±13 cm^2, range 33-239 in B and 25-258 in W) or VAT/SCAT area ratio (0.29±0.03 B, 0.30±0.03 W). *In vivo* insulin sensitivity of glucose metabolism was also measured as sensitivity index (Si) by the minimal model (2.3±0.5 B and 2.5±0.5 W, p=NS). *In vitro*, insulin's effects on glucose metabolism and on lipolysis were measured in collagenase-isolated abdominal subcutaneous adipocytes. Insulin sensitivity (as ED50) and responsiveness (as the difference between response at baseline and the maximal insulin concentration) were measured against lipolysis stimulated by either adenosine deaminase (ADA) or 8-bromo cyclic AMP (8brcAMP), and for glucose transport (GT) using trace amounts of ^{14}C-glucose. Insulin ED50 for ADA-stimulated lipolysis was related to VAT/SCAT ratio ($p<0.05$), similarly in B and W women. Insulin ED50 for either ADA- or 8brcAMP-stimulated lipolysis was also related to Si ($p<0.05$) similarly in B and W women. In contrast, VAT/SCAT and Si did not correlate with ED50 of insulin to stimulate GT, but it did relate to the responsiveness of GT to insulin (GTR) in W ($p<0.05$) but not in B women. In W women, both Si and GTR decreased with a high VAT/SCAT, while B women maintained a uniformly lower responsiveness of GT to insulin in adipose tissue. We conclude that a) abdominal subcutaneous adipocytes from B and W women with comparable levels of visceral adiposity are equally sensitive to the antilipolytic effect of insulin; and b) there is a dissociation between the effect of insulin on lipolysis and on GT in adipose tissue of B women. This may relate to the higher propensity to obesity and diabetes for B as compared to W women, independent of adipose tissue distribution.

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RESTING ENERGY EXPENDITURE IN NON INSULIN DEPENDENT (NIDDM) DIABETIC MEN

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Dietary manipulation remains the cornerstone of management in NIDDM. Daily calorie intake is calculated from resting energy expenditure (REE) and physical activity levels. The limited ability to measure REE results in this variable being derived by predictive equations. This study was designed to examine the validity of this approach. The clinical details (mean ± SD) of the NIDDM men were: n = 69; age 57 ± 10 years; weight 85 ± 18 kg; body mass index 28 ± 5 kg/m^2; HbA1c 8 ± 2%; duration of diabetes 5 ± 5 years). REE was measured (MREE) after 10h overnight fast by indirect calorimetry (Deltatrac) between 5-45 minutes and compared with that derived from Schofield (S), Harris-Benedict (HB), Owen (O), Department of Health (DH) and Mifflin (M). Overall no significant differences (paired t-test) were noted between MREE (kcal/d), 1752 ± 289 and 1736 ± 278 (S), 1722 ± 306 (HB), 1746 ± 188 (O) or 1789 ± 243 (DH) although that of 1656 ± 243 (M) was lower (p< 0.0001). The limit of agreement between measured and derived values (bias ± 95% CI) was very wide highlighting the problems in using predictive REE to calculate energy requirements in individuals although they appear satisfactory in NIDDM as a group. This finding is similar to non diabetics.

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IN VIVO LIPOLYSIS IN OBESE, NONDIABETIC, BLACK AND WHITE WOMEN: RELATIONSHIP TO VISCERAL FAT DISTRIBUTION

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We examined the relative contribution of visceral vs subcutaneous abdominal adipose tissue (VAT and SCAT areas, by magnetic resonance imaging) to the systemic lipolytic rates (LIPO), measured *in vivo* with deuterated glycerol and ^{13}C-palmitate infusions (as turnover rates, RaGlyc, RaPalm). LIPO was measured in the basal state in 21 black (B) and 18 white (W), obese (BMI, mean±SEM, 35±1and 34±1; % fat by DEXA, 45±1 and 46±1), premenopausal (36±1and 34±1 yrs), nondiabetic women and, in a subset of subjects (10B and 10W), during a pancreatic euglycemic clamp, at lower (42±18 pM) and then higher (114±30 pM) insulin (I) levels. B and W women were matched by VAT(116+10 and 125±13; range 33-239 and 25-258 cm^2) and VAT/SCAT. Basal RaPalm was same(139±10 vs 135±8 umole/L, p=ns) while basal RaGlyc was slightly lower in B women (244±14 vs 285±15, umole/L, $p<0.06$). The degree of suppression of LIPO by I during the clamp (%S) varied widely (56-72% and 14-69% for RaGlyc, and, 28-55% and 19-64% for RaPalm, for B and W groups, respectively). The slopes of the relationship between basal RaGlyc, basal RaPalm, %S of palmitate or glycerol and regional fat were not different between groups, even after adjusting for age, total fat mass (FM) and fat free mass (FFM), therefore results of multiple regression are for the groups combined. Basal RaPalm related significantly to WHR ($r=0.32$, $p<0.05$) and SCAT ($r=0.43$, $p<0.01$) but not to VAT($r=0.1$, p=ns), while basal RaGlyc related significantly to VAT($r=0.4$, $p<0.05$) and not to SCAT ($r=0.22$, p=ns). After adjusting for age, FM and FFM, none of the body fat distribution variables significantly predicted RaPalm or RaGlyc. %S did not relate to SCAT; only VAT was a predictor of %S after adjusting for age, FM and FFM, partial $r= -0.76$, $p<0.001$ for RaGlyc and partial $r=-0.62$, $p<0.05$ for RaPalm). In obese, nondiabetic women, the absolute amount of VAT accumulation does not appear to significantly contribute to the basal FFA turnover rates, although VAT is the best predictor of the ability of insulin to suppress lipolysis systemically.

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RELATIONSHIP BETWEEN HUMAN LEPTIN AND SUBCUTANEOUS FAT BUT NOT VISCERAL FAT

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Leptin, the protein product of the obese *(ob)* gene produced by adipocytes has been shown to be elevated in obesity and related to body mass index (BMI) and % body fat. Central visceral obesity is associated with many metabolic defects, including insulin resistance. The aim of this study was to investigate the relationship between circulating leptin levels, the type of body fat and insulin resistance in nondiabetic obese subjects before and after 12 weeks on a dietary regime. 22 subjects were included in the study (10M, 12F; BMI: 38.3 ± 0.95 ; Age 20 - 52 years). Insulin sensitivity was measured by hyperinsulinaemic euglycaemic clamp (40mU/m^2/min). Subcutaneous and visceral adipose tissue areas were determined by a single scan computerised tomography at L4. Fasting serum leptin was measured using a human leptin RIA kit (Linco). Before intervention, fasting serum leptin levels ranged from 8.13 - 81.33 ng/ml, with females having significantly higher levels than males (F: 49.0 ± 5.6; M: 17.2 ± 1.3, $p < 0.0001$). Leptin was associated with BMI ($r = 0.519$, $p = 0.0134$) and WHR ($r = -0.564$, $p = 0.0063$) but not fasting insulin ($r = 0.274$, $p = 0.2166$) or insulin sensitivity ($r = 0.198$, $p = 0.3762$). Leptin was highly correlated with subcutaneous fat ($r = 0.612$, $p = 0.0065$) but not visceral fat ($r = 0.010$, $p = 0.9643$). Insulin sensitivity was negatively correlated with visceral fat area ($r = -0.508$, $p = 0.0158$). Weight loss was accompanied by reduced subcutaneous and visceral fat areas and improved insulin sensitivity. Serum leptin levels fell significantly in the males but not in the females. The same relationships seen pretreatment between leptin levels, fat distribution and insulin sensitivity were maintained after intervention. These studies confirm the relationship between insulin resistance and visceral abdominal adiposity and suggest that circulating leptin is not related to the degree of insulin sensitivity or visceral obesity but is related to the amount of subcutaneous fat.

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DO GENES OR ENVIRONMENT EXPLAIN THE RELATIONSHIPS BETWEEN CENTRAL FAT, INSULIN AND GLUCOSE?.

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Central adiposity, a pivotal component of Syndrome X, is under strong genetic influence and closely relates to fasting insulin and glucose. Whether these inter-relationships are due to shared genetic or environmental influences is not known.

To address this, we studied a group of 220 healthy female twins (59 MZ pairs, 51 DZ pairs, mean age (±SD) 52±14 years, BMI 25.4±4.2kg/m^2). Fasting glucose was measured by the glucose oxidase method, insulin by radioimmunoassay and central fat by dual energy X-ray dispersive absorptiometry.

Central fat was positively related to log insulin ($r=0.59$, $p=0.0001$) and glucose ($r=0.49$, $p<0.001$). Log insulin related to glucose ($r=0.25$, $p=0.0002$). Univariate model-fitting indicated the heritability (proportion of phenotypic variance attributed to genetic factors) for central fat, insulin and glucose was 57%, 44% and 77% respectively. Multivariate modelling (Cholesky decomposition analysis) indicated 27% (of the 57%) of the genetic variance of central fat is due to genes shared with insulin, while no genetic influence is shared with glucose. On the other hand, the majority of heritability of glucose (73%) is due to specific genes that regulate glucose and only 4% is shared with genes that affect insulin. Shared environmental influences explained 46% of the covariance observed between insulin and central fat and 57% of the covariance between glucose and insulin. The reciprocal model of glucose influencing fasting insulin levels fit the observed data best, rather than the converse model where insulin regulates glucose.

These data suggest the relationship between insulin and central fat is not solely genetic but that substantial common environmental influences exist. These results reinforce the need for ongoing work to modify environmental influences whilst the respective genes are being identified.

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RELATIONSHIP BETWEEN PLASMA LEPTIN LEVEL AND FAT DISTRIBUTION IN JAPANESE NIDDM PATIENTS

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Leptin, recently discovered peptide secreted exclusively from adipocytes, regulates appetite and energy expenditure. In this study, we investigated the relationship between plasma leptin level and blood pressure (SBP and DBP), plasma glucose and lipid profiles (TC, TG, HDL-C and FFA) and fat distribution (V-fat and S-fat) in mildly- to moderately-obese Japanese NIDDM patients (aged 57±13, BMI 26.7±3.4, HbA1c 7.3±1.5% (SD)). Plasma leptin and insulin were measured with RIA (Linco and Eiken Co., respectively). In men, plasma leptin (5.0±2.6 ng/ml, n=73) was correlated with BMI (r=0.64), DBP (r=0.34), IRI (r=0.43), FFA (r=0.31), S-fat (r=0.77) and V-fat area (r=0.67); and in women (6.2±1.0 ng/ml, n=38) with BMI (r=0.63), IRI, (r=0.70) and S-fat area (r=0.67) ($p<0.05$ for all). There was no correlation between leptin and FPG or HbA1c. These data suggest that plasma leptin is associated with insulin, DBP, FFA and fat distribution in Japanese NIDDM patients.

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HYPERSECRETION OF GASTRIC INHIBITORY POLYPEPTIDE IN PREDIABETIC MEN WITH VISCERAL FAT ACCUMULATION.

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Visceral fat obesity in which lots of fat deposit in adipose tissue on mesenterium is frequently observed in patients with diabetes mellitus or other insulin resistant syndrome. It is unknown which factor contributes to the accumulation of visceral fat. Gastric inhibitory polypeptide (GIP) is known as an incretin. The peptide released from small intestine perfuses mesenterium to portal vein. Besides, it has direct metabolic effects on adipose tissue to incorporate fatty acids. In this study, we investigated relationship GIP secretion and clinical features of insulin resistant syndrome. All subjects were non-obese (BMI<25) men younger than 65 years of age with or without impaired glucose tolerance whose pancreatic beta cell function kept well. The fat distribution was analyzed by measuring visceral fat area / subcutaneous fat area ratio (V/S ratio) on computed tomography pictures of umbilical level. Insulin sensitivity (Si) was assessed by Bergman's minimal model method. Plasma GIP were measured by RIA system specific for human GIP. Fasting plasma insulin or incremental insulin response after 75g glucose ingestion (Σ IRI) had no correlation with V/S ratio or Si. Other metabolic markers; fasting plasma glucose, triglyceride and cholesterol had no significant correlation with those either in this case. The other hand, incremental GIP response after glucose ingestion (Σ GIP) had significant negative correlation with Si ($R=-0.637$, $P=0.046$), and had significant positive correlation with V/S ratio ($R=0.606$, $P=0.0086$). It had no correlation with BMI. These findings demonstrate the relationship between visceral fat accumulation, insulin resistance and hypersecretion of GIP, that is not mediated by insulin. We propose GIP as a promising candidate factor which accumulates visceral fat.

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IS LEPTIN RELATED TO ABDOMINAL FAT DISTRIBUTION AND INSULIN RESISTANCE IN NIDDM PATIENTS ?

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Leptin is a protein secreted by the adipose tissue. The aim of the present study was to evaluate the relationship of abdominal adipose tissue distribution measured by magnetic resonance imaging and insulin resistance to plasma leptin concentration in NIDDM subjects. Plasma leptin concentrations were measured by radioimmunoassay with a detection limit of 0.1 ng.ml^{-1}. Areas of abdominal fat were calculated from axial magnetic resonance images obtained at the level of umbilicus (L4-L5 vertebrae) in 21 men with NIDDM treated with either hypoglycemic agents (n = 17) or a diet alone (n = 4) : age 46 ± 8 (DS) months, BMI 29.3 ± 4.5 kg.m^2 (range 20.2-36.9), total body fat (skinfold thickness) 26.8 ± 5.4%, waist-to-hip ratio 0.97 ± 0.07, duration of diabetes 59 ± 47 months. The insulin tolerance test (ITT, 0.1 U/kg of intravenous regular insulin) was performed after an overnight fast, at least 48 hours after the withdrawal of metformin. The areas of subcutaneous and of deep abdominal fat were respectively 212 ± 99 cm^2 and 135 ± 55 cm^2 . Plasma leptin levels averaged 8.4 ± 6.8 ng.ml^{-1} (range 1.77 - 32.2) and were positively related to areas of subcutaneous abdominal fat (r = 0.731 , p < 0.001), BMI (r = 0.669 , p < 0.01) and total body fat (r = 0.589 , p < 0.01). In contrast, the areas of visceral adipose tissue, the waist-to-hip ratio and the blood glucose disappearance rate K_{ITT} were not related to plasma leptin concentrations. In conclusion, plasma leptin levels is not associated with deep abdominal fat and insulin resistance in the NIDDM patients of this study.

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THE ASSOCIATION OF INSULIN RESISTANCE WITH MUSCLE AND ABDOMINAL FAT IN OBESITY

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Object: To investigate the association of insulin resistance with muscle and abdominal fat in obesity. **Methods:** 18 male (BMI>26.4: n=9, <26.4: n=9) and 13 female (BMI>26.4: n=8, <26.4: n=5) obese subjects were studied. Insulin resistance was determined with the method of steady-state plasma glucose (SSPG). Visceral fat (V) and subcutaneous abdominal fat (S) were evaluated by a single scan made at the level of the navel. To measure muscle fat, triple 1 cm thickness cross-sectional scan of the mid-thigh was obtained by CT. Thigh fat inside and outside fascia (MF, SF), V and S were electronically measured by using commercially available CT software, with the region of interest window set to attenuation values of -150 to -50 Hounsfield units (HU). The mean attenuation values of thigh tissue except bone tissue inside fascia (mCT) was measured by using the same software. Increased MF correlated with low mCT and the lower mCT correspond to more fat content in muscle. **Results:** When the higher and lower SSPG groups were analyzed, the higher group shared increased V and MF in male, while in female, the more V.

Male	SSPG(mmol/dL)	V(cm)	MF(cm)	Female	SSPG(mmol/L)	V(cm)
n=9	7.7±0.7	156±14	8.4±0.5	n=7	9.8±0.8	111±12
n=9	14.3±0.4*	198±13*	12.0±1.5*	n=6	16.8±1.1*	159±13*

*p<0.05 vs lower SSPG

SSPG was significantly correlated positively with V (r=0.57, p<0.05) and MF (r=0.63, p<0.05), and negatively with mCT (r=-0.52, p<0.05) in male. In female, SSPG was significantly correlated positively with V (r=0.71, p<0.05). **Conclusion:** Insulin resistance was associated with not only visceral fat but also thigh fat inside fascia in male.

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THE RELATIONSHIP BETWEEN LEPTIN AND INSULIN SENSITIVITY IN A POPULATION-BASED STUDY IN MAURITIUS.

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It has been shown that fasting serum leptin and insulin concentrations are highly correlated, and in a small clinical study insulin sensitive men had lower leptin levels than insulin resistant men when matched for fat mass. Our aim was to examine the association between insulin resistance (assessed by HOMA model) and leptin after controlling for overall and central adiposity in a population-based cohort. Subjects were participants of a 1987 non-communicable diseases survey conducted in the multiethnic population of Mauritius. 1227 men and 1310 women of Asian Indian, Creole and Chinese ethnicity had normal glucose tolerance and fasting serum leptin measurements. Leptin levels were compared across insulin resistance quartiles within tertiles of body mass index (BMI) by analysis of covariance, and multiple linear regression models were used to assess the relationship between leptin and insulin resistance after adjusting for BMI and waist/hip ratio (WHR). Mean serum leptin concentration increased across insulin resistance quartiles in each BMI group and each sex, after controlling for BMI, WHR and age (p<0.001 for each comparison). Furthermore, insulin resistance was a significant determinant of serum leptin concentration, independent of BMI and WHR, in both men and women. These results suggest that insulin resistance and/or concentration may contribute to the relatively wide variation in leptin seen at similar levels of body mass or alternatively, leptin may play a role in the etiology of insulin resistance. In future work it will be important to determine whether the hyperleptinemia/insulin resistance relationship has a role in the natural history of obesity, NIDDM, and the other metabolic phenomena associated with insulin resistance.

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VISCERAL FAT IS ASSOCIATED WITH INCREASED PLASMINOGEN ACTIVATOR INHIBITOR-1 LEVELS IN WOMEN.

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Increased plasma Plasminogen Activator Inhibitor-1 (PAI-1) levels are a risk factor of myocardial infarction and are considered as one of the parameters of insulin resistance syndrome. The metabolic disorders observed in insulin resistance are associated with android obesity and most particularly with abdominal visceral fat accumulation. To clarify the relationship between PAI-1, insulin-resistance and visceral fat, 40 women (age:18-65 years, BMI:21-49 kg/m^2) have been studied. We have measured visceral and subcutaneous abdominal fat by computed tomography at the level of umbilicus, plasma PAI-1 activity and insulin sensitivity by hyperinsulinemic euglycemic clamp method (1mU/Kg/mn). This results show that PAI-1 levels are positively correlated to fasting insulinemia and negatively to insulin sensitivity index (r=0,48, p<0,01 and r=-0,33, p<0,05). PAI-1 levels are more strongly correlated to visceral fat (r=0,51, p<0,001), whereas no correlation is found with subcutaneous fat (r=0,23, NS). Moreover, visceral fat is positively correlated to fasting insulinemia (r=0,62, p<0,001) and negatively to insulin sensitivity index (r=-0,64, p<0,001). Stepwise multiple regression analysis showed that after adjustement for visceral fat, correlation between PAI-1 activity and fasting insulinemia disappears. These results suggest that visceral fat contributes PAI-1 elevation in insulin resistance syndrome and strengthen other data showing that in animals, PAI-1 is produced by adipose tissue, particularly visceral adipose tissue.

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FAT DISTRIBUTION AND SERUM LEPTIN LEVELS IN NON-DIABETIC OFFSPRING OF NIDDM PATIENTS

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Serum leptin levels have been associated with fat mass. Moreover, women have higher leptin levels than men. The impact of fat distribution on leptin levels, however, is a controversial issue. We studied the association of fat distribution on leptin levels in 20 non-diabetic offspring (M/F 5/15; mean age 41.3 yrs) of NIDDM patients with insulin secretion deficient phenotype (IS-group), 18 non-diabetic offspring (M/F 7/11; 40.5 yrs) of NIDDM patients with insulin resistant phenotype (IR-group) and in 14 control subjects (M/F 5/9; 41.1 yrs) without a family history of diabetes. The abdominal fat distribution (subcutaneous fat = SCFAT and intra-abdominal fat = IAFAT) was measured with computed tomography, the total fat mass (TFM) bioelectrical impedance and the whole body glucose uptake (WBGU) with the euglycemic hyperinsulinemic clamp technique.

	Control	IS	IR	ANOVA (adj.forgender[1]+TFM)
BMI, kg/m^2	25.0	24.6	28.8	p<0.05
TFM, kg	19.0	20.5	26.4	p<0.05[1]
WBGU, mg/kg/min	11.1	10.1	7.5	p<0.01
S-leptin, ng/l	12.9	13.4	23.2	p<0.001
SCFAT, cm^2	204	215	310	p =NS
IAFAT, cm^2	72	84	123	p =NS

In control and IS-group, but not in IR-group, serum leptin levels correlated with SCFAT (r=0.81, p<0.01 for controls; r=0.46, p<0.05 for the IS-group; r=0.05, p=NS for the IR-group). The significant correlations persisted after adjustment for age, gender and TFM. No relationship between IAFAT and leptin was observed. In conclusion, SCFAT was a more important determinant of serum leptin levels than IAFAT. This may explain higher leptin levels found in women than in men.

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PROSPECTIVE EXERCISE AND WEIGHT LOSS STUDY IN TYPE II DIABETIC SUBJECTS IMPROVED INSULIN RESISTANCE, LIPIDS AND LEPTINS

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Type II Diabetes Mellitus is characterized by obesity, hyperglycemia, insulin resistance, hyperlipidemia, and increased risk for cardiovascular disease. Risk factors could be reduced by diet and exercise but chronic adherence to a weight loss program or a specialized diet is known to have low success rates. The following study was designed to determine if triglyceride self-monitoring combined with an intensive 16 week exercise, weight loss and diet modification program would improve outcomes. Obese Type II DM males and females over 40 years of age, not treated with insulin or lipid lowering agents were randomized to monitored or non-monitored groups. Weight, glucose, glycohemoglobin A1C, lipids, fasting and stimulated insulin levels, leptin levels, and exercise tolerance (Max O2 levels) were measured at the beginning and end of the program. Statistical analysis was performed using a paired student's t test. Results from all subjects (n=24), showed highly significant decreases in weight (95.7±20.7 kg to 91.0±18.4 kg, p=0.001) which strongly correlated with decreases in leptin levels (r=0.4). Significant improvements were also found in fasting glucose (177.4± 40.5 to 161.3±33.3, p=0.006), triglyceride (235.0±94.6 to 179.2±70.9, p=0.003), and total cholesterol levels (219.7±24.7 to 199.9±28.7, p=0.001). Changes in glycohemoglobin A1c, HDL cholesterol and LDL cholesterol levels were not significant by 16 weeks. Fasting insulin levels were significantly decreased (22.8± 13.2 to 17.0±9.5, p=0.02) but 2 hour sustacal stimulated levels were unchanged. A significant improvement in exercise tolerance was also found by Max O2 testing (26.6±7.4 to 31.1±8.5, p=0.003). The triglyceride meter group had lower average triglycerides, however the variance in the data indicates that more subjects need to be recruited in order to show a significant effect. In summary, an intensive exercise, weight loss and diet program for Type II Diabetic patients significantly improves glucose control, lipids, insulin resistance and increases exercise tolerance, all risk factors for the cardiovascular complications of diabetes.

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PLASMA LEPTIN ASSOCIATED WITH BODY MASS INDEX AND INSULIN IN HEALTHY MALE ADOLESCENTS

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Leptin, recently discovered peptide secreted exclusively from adipocytes, regulates appetite and energy expenditure. Plasma leptin level is reported to increase in obese adults. In the present study, we measured plasma leptin, height, weight, blood pressure (SBP and DBP), heart rate, blood cell counts, plasma glucose, insulin, lipid profiles (TC, TG and HDL-C), UA and creatinine in 366 healthy male high school students (aged 16.2±0.4 and BMI 20.8±2.5 (SD)). Plasma leptin and insulin were measured with RIA (Linco and Eiken Co., respectively). Plasma leptin (2.8 ±1.6 ng/ml) was strongly correlated with body weight (r=0.48), BMI (r=0.55) and insulin (r=0.46) (P<0.001 for all). There was also correlation with age (r=0.14), SBP (r=0.21), DBP (r=0.14), heart rate (r=0.19), WBC (r=0.20), RBC (r=0.17), Hct (r=0.17), TC (r=0.16), TG (r=0.20), HDL-C (r=-0.16) and UA (r=0.20) (P<0.01 for all). Stepwise regression analysis revealed significant correlation with BMI (F=108.7), insulin (F=57.2) and WBC (F=5.9) (p<0.05 for all). We conclude that plasma leptin is associated with BMI and insulin also in healthy male adolescents. Relationship with blood cell counts was considered to need further study.

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EFFECTS OF LONG-TERM TOTAL FASTING AND INSULIN ON *OB* GENE EXPRESSION IN OBESE PATIENTS

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The aim of the present study was to explore the regulation of *obese (ob)* gene expression in severe obese females during long-term fasting (6 days). Furthermore, the effect of insulin on serum leptin levels was examined before and after fasting. Nine females were included. Age was 34 ± 3 years and BMI 46.4 ± 2.3 kg/m^2. Six days of fasting induced a significant weight loss (126.8 ± 5.3 vs. 120.5 ± 5.1 kg, p < 0.0001). Insulin-stimulated glucose uptake (hyperinsulinemic, euglycemic clamp, insulin infusion rate 1.5 mU/kg/min) was markedly reduced following fasting (M-value 5.96 ± 0.74 vs. 2.79 ± 0.23 mg/kg/min, p < 0.0001). *Ob* mRNA/ß-actin concentration (quantitated by quantitative PCR) in fat biopsies from abdominal subcutaneous adipose tissue was unchanged after 6 days of fasting (1.50 ± 0.40 vs. 1.47 ± 0.36 arb. units, NS), whereas serum leptin levels decreased significantly from 53.8 ± 4.7 ng/ml to 30.7 ± 2.0 ng/ml, p < 0.0001 during the same period. Serum leptin levels were unchanged by hyperinsulinemia for 3 hrs during the clamp prior to the fast, while hyperinsulinemia for 3 hrs after six days of fasting increased serum leptin by 25 %, p < 0.01. In conclusion: Six days of fasting reduced serum leptin by about 40 %. In contrast *ob* mRNA in abdominal subcutaneous adipose tissue was unchanged. The decrease in serum leptin levels after long-term fasting may be due to posttranscriptional events like decreased translation, increased clearance or decreased half-life. Finally, the effect of insulin on serum leptin levels seems to be dependent on the nutritional state.

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CIRCULATING LEPTIN CORRELATES TO ISLET FUNCTION IN NON-DIABETIC SUBJECTS

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It is known that circulating leptin correlates to body fat content and that administration of leptin reduces food intake. Leptin might thus be a humoral signal from fat affecting feeding behaviour, and, consequently, factors influencing the nutritional status might operate through changes in circulating leptin. We have previously shown that circulating leptin correlates to fasting insulin, to maximal insulin secretory response to a glucose + arginine challenge and to the glucose sensitivity of B cell secretion, indicating a relation between leptin and insulin secretion. In this study, we examined whether circulating leptin also correlates to other islet hormones. We therefore injected arginine i.v. (5 g) at fasting glucose and after raising circulating glucose to 14 and 28 mmol/l in 106 postmenopausal women, aged 58 years. We confirmed our previous results that circulating leptin correlates to BMI ($r=0.70$, $p<0.001$), to fasting insulin ($r=0.36$, $p<0.001$), to circulating insulin at 14 mmol/l glucose ($r=0.28$, $p=0.004$) and 28 mmol/l glucose ($r=0.33$, $p<0.001$), as well as to the insulin response to arginine at all three glucose levels ($r>0.29$, $p<0.003$ for all) in a multivariate analysis controlling for the influence of BMI. Furthermore, we demonstrated that leptin, independently of BMI, also correlates to fasting glucagon ($r=0.36$, $p<0.001$), to plasma glucagon at 14 mmol/l glucose ($r=0.20$, $p=0.047$) and to plasma glucagon at 28 mmol/l glucose ($r=0.25$, $p=0.040$) as well as to the glucagon response to arginine at all three glucose levels ($r>0.22$, $p<0.025$ for all). In contrast, circulating leptin did not correlate to plasma levels of pancreatic polypeptide (PP) ($r=0.13$, $p=0.187$). We conclude that circulating leptin correlates to both insulin and glucagon secretion but not to plasma PP in a large group of postmenopausal women. This suggests that islet function is related to circulating leptin in humans.

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LEPTIN LEVELS DO NOT PREDICT WEIGHT GAIN: A PROSPECTIVE STUDY

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Human obesity is associated with high leptin concentrations, but at any level of body fat leptin varies widely. Relatively low leptin levels may indicate leptin deficiency and risk of weight gain. Alternatively, high leptin could indicate leptin resistance and also increased risk of weight gain. Whether one or both of these mechanisms operate in humans was examined in a longitudinal study of 1988 normoglycaemic Mauritians aged 25 to 45 years who participated in population-based surveys in 1987 and 1992. Changes in BMI, waist/hip ratio (WHR) and waist circumference were compared between leptin 'resistant' 'normal' and 'sensitive' groups. Leptin 'resistance' and 'sensitivity' were defined on the basis of the Studentised residuals (SRESID) from the regression of $\log_{10}$leptin on BMI. If SRESID > 0.9, leptin was considered high or 'resistant'. If SRESID was < -0.9, subjects were considered leptin 'sensitive' or 'deficient'. There were no consistent trends in age or BMI across leptin 'resistant', 'normal' and 'sensitive' men and women. Fasting insulin decreased significantly ($p<0.001$) with increasing leptin sensitivity in men and women. After adjusting for age and BMI by analysis of covariance, there were no significant differences in the changes in BMI (percentage or absolute), waist circumference or WHR between leptin 'resistant' and 'normal' subjects except that in men WHR increased more in the leptin 'resistant' group ($p=0.001$). However, there was a tendency for smaller increases in other measures of obesity among the leptin 'resistant' subjects compared with the 'normal' subjects. Leptin 'sensitive' subjects tended to experience greater increases in the obesity parameters than 'normal', but the differences were not significant. Further adjustment for insulin did not change the results. Redefining leptin 'resistance' and 'sensitivity' as SRESID>1.2 or <-1.2 did not enhance the slight trends observed above. These findings do not support a role for leptin concentration, either high or low, in predicting weight gain. Leptin's main role in humans may be in protecting against the effects of starvation rather than over consumption, consistent with the conditions encountered during most of human evolution.

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INSULIN AND LEPTIN ARE RELATED INDEPENDENT FROM BODY MASS INDEX.

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We investigated the associations between the components of the insulin resistance syndrome and leptin levels in a sample of participants of the Rotterdam Study: 549 men and 558 women (mean age 66.8 years (SD 5.5). As part of the baseline examination immunoreactive insulin was measured after a non-fasting oral glucose load. At the follow-up examination after 2.1 years a fasting oral glucose tolerance test was performed. Insulin and leptin were measured by highly specific radioimmunoassays.

Serum leptin levels in men and women were 6.14 ng/ml (SD 4.5) and 21.7 ng/ml (15.5), respectively ($p < 0.001$). Leptin levels were associated with all components of the insulin resistance syndrome both assessed at baseline and at follow-up: body mass index, waist/hip ratio, fasting and post-load glucose, fasting and post-load insulin, triglycerides, HDL-cholesterol, and blood pressure, as well as with the presence of diabetes mellitus and hypertension (all associations: $p < 0.001$, adjusted for age and gender). When the analyses were further adjusted for body mass index, leptin remained associated with insulin only: in men an increase of 0.06 ng/ml per mU/l fasting specific insulin (95% confidence interval 0.03; 0.08, $p<0.001$) was found, whereas in women this was 0.50 ng/ml per mU/l (0.39; 0.62, $p<0.001$). The independent associations between insulin and body mass index with leptin were also present in the longitudinal analysis.

The results of this population-based study indicate that insulin levels (but not the other components of the insulin resistance syndrome) are associated with increased leptin levels independently of body mass index. This suggest that hyperinsulinemia plays a key in increased leptin levels.

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ARE LEPTIN AND OLEOYL-ESTRONE EQUIVALENT PONDEROSTAT SIGNALS IN LEAN AND OBESE SUBJECTS?

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In a recent study, a white adipose tissue component, oleoyl-estrone, induced a dose-dependent loss of weight and decreased food intake in Wistar female rats. We studied plasma concentrations of oleoyl-estrone in 17 lean subjects (8 females), and 25 obese (14 females), matched for age and fasting glucose. Obese female subjects had significantly higher percent body fat, serum leptin ($p<0,01$), and plasma oleoyl-estrone ($p=0.02$) than obese men. Both leptin and oleoyl-estrone strongly correlated with BMI, and with measures of central fat distribution. Serum leptin strongly correlated with percent body fat (PBF, bioelectric impedance) and insulin sensitivity (minimal model analysis; $r=0.72$, $p<0.0001$ and $r=-0.63$, $p=0.003$, in males; $r=0.85$ and -0.86, $p<0.0001$ in females). The same relationships were observed concerning oleoyl-estrone with percent body fat ($r=0.52$ and $r=0.69$, in males and females, respectively) and with insulin sensitivity ($r=-0.72$ and -0.76, in males and females, respectively). The associations between leptin and oleoyl-estrone with insulin sensitivity persisted after controlling for percent body fat ($r=-0.63$, $p=0.003$ in males; and $r=-0.45$, $p=0.04$ in females, for oleoyl estrone; and $r=-0.49$, $p=0.03$ in males, and $r=-0.58$, $p=0.007$ in females, for serum leptin). Fasting serum leptin correlated with plasma oleoyl-estrone ($r=0.64$, $p<0.0001$), which persisted after controlling for insulin sensitivity ($r=0.52$, $p<0.0001$) and fat mass ($r=0.38$, $p=0.01$). In summary, plasma oleoyl-estrone levels are strongly correlated with PBF and insulin sensitivity in humans, in parallel to the same associations described for serum leptin. The relationship between oleoyl-estrone and leptin levels hints at their functional relationship within the framework of body weight control.

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SERUM LEPTIN IN ELDERLY PEOPLE

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The recently discovered leptin is a novel hormone, which regulates energy intake and expenditure. Aging is associated with various alterations in food intake and energy metabolism. To investigate whether aging affects serum leptin levels, we measured serum leptin in elderly people with radioimmunoassay. The study population (n=159) included 19–20 men and 20–21 women from each of the four birth cohorts aged 65, 75, 80 and 85 years. The groups were similar with respect to body mass index (BMI), serum total and HDL cholesterol, triglyceride, insulin and blood glucose concentrations. Serum leptin concentration was similar in different age groups, but in each group it was 2–3 fold higher in females (average for all 14.8±16.2 μg/l, mean±SD) than in males (5.9±3.3 μg/l) ($p<0.05$ or less in all groups). In women, who lived longer, serum leptin concentration was 62% higher than in those, who died within 5 yrs after blood sampling (17.6±16.9 vs 10.9±11.2 μg/l, respectively, $p<0.02$). Leptin correlated positively with BMI ($r=0.40$, $p<0.001$) and insulin concentrations ($r=0.31$, $p<0.001$), as previously shown in younger population. In conclusion: 1) Leptin gender difference is present also in the elderly. 2) Aging does not affect serum leptin levels or its correlation with adiposity. 3) Higher leptin concentrations in women living longer may indicate a better nutritional status rather than be a primary factor for survival.

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LEPTIN IN NORMAL WEIGHT, OBESE AND OBESE DIABETIC SUBJECTS : EFFECT OF HYPERINSULINEMIC CLAMP AND BODY WEIGHT LOSS

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Leptin plasma levels were measured in normal weight (n = 9) and in obese (n = 50) subjects in basal conditions; after two hour euglycemic, hyperinsulinemic clamp (n = 30) or after weight loss (n = 12). In basal conditions, leptin was significantly lower in lean than in obese subjects ($p<0.001$). When obese were subdivided in three groups according to their glucose tolerance (i.e. normal, inpaired glucose tolerance and diabetics), no differences were observed in leptin levels. Obese females showed significantly higher leptin values than males ($p<0.001$). Leptin was significantly ($p<0.0001$) linked to fat body mass (FBM) when expressed both in Kg and as percent of total body weight. No significant relationships were found between plasma leptin and basal Energy Expanditure (EE) nor lipid oxidation, when these values were expressed in function of the lean body mass (LBM). Two hour euglycemic hyperinsulinemic clamp did not modify leptin levels in either lean or obese group, independently of their glucose tolerance. After four weeks hypocaloric diet, a group of obese patients (n = 12) showed a body weight loss of 7.9±1.7 Kg, corresponding to a 12.8±2.3 % decrease of their initial fat mass; at the same time leptin levels were reduced from 77.7±15.0 ng/ml to 55.6±10 ng/ml, i.e. 30.0±7.3% decrease of the initial values. No significant correlation was observed between the amount of fat loss and the reduction in plasma leptin. In conclusion, circulating leptin is, in basal conditions, influenced by sex and fat mass but not by glucose intolerance or diabetes; leptin is not affected by two hour hyperinsulinemia, but is strongly modified by fat loss.

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PLASMA LEPTIN LEVELS DO NOT CORRELATE TO PLASMA PROINSULIN LEVELS IN NORMAL ADULTS.

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It has been demonstrated that plasma leptin levels strongly correlate to body fat content. Increased body fat content , especially intraabdominal fat is accompanied by insulin resistance. We questioned whether plamsa leptin levels is correlate to insulin sensitivity in normal adult individuals. We measured fasting plasma leptin, insulin, proinsulin, C-peptide, glucose and waist/hip ratio in 213 normal subjects(men = 100, women = 113). Their fasting plasma glucose levels were below 6.1 mmol/l and they had no previous medical history and family history of diabetes mellitus. Body fat amount was determined with impedance method. BMIs were 22.4 ± 2.46 in men, 22.4 ± 2.60 in women respectively. Log fasting plasma leptin levels strongly correlate to body fat amount(r = 0.63, P = 0.000). Log fasting plasma proinsulin levels weakly correlate to body fat amount(r = 0.34, P = 0.000) and waist/hip ratio(r = 0.25, P = 0.000). However plasma leptin levels did not correlate to plamsa insulin, proinsulin, c-peptide and waist/hip ratio by partial correlation study controlling body fat amount. Fasting plasma leptin levels were significantly higher in women than men(6.45 ± 4.02 ng/ml, 2.45 ± 1.58 ng/ml, P = 0.000) although body fat amount was not differ between two groups(12.3 ± 4.0 kg in men, 15.7 ± 4.6 kg in women, P = 0.308). In conclusion, plasma leptin reflects body fat amount and may be affected by sex but does not correlate insulin sensitivity independently of body fat amount.

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POST-GASTROPLASTY RECOVERY OF IDEAL BODY WEIGHT NORMALIZES SERUM LEPTIN LEVELS OF OBESE WOMEN

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In order to better understand the significance of obesity-associated elevated serum leptin in humans, fasting leptin concentrations were measured in 8 obese women before (BMI = 37.7 ± 0.5 kg/m²) and 14 ± 2 months after successful gastroplasty restoring ideal body weight (BMI = 23.7 ± 0.6 kg/m²). Before gastroplasty, obese women showed markedly higher serum leptin levels (36.8 ± 4.8 *versus* 8.9 ± 1.8 ng/ml, $P < 0.001$) when compared to lean controls (BMI = 23.6 ± 0.7 kg/m²). After gastroplasty, serum leptin concentrations dramatically decreased to 6.4 ± 1.6 ng/ml ($P < 0.0001$) and became similar to those of controls. The minimal model-derived insulin sensitivity index S_I (10^{-5} min^{-1}/pmol.l^{-1}) was lower in obese subjects (4.74 ± 0.74, $P < 0.01$) when compared to that of controls (11.79 ± 1.74), but returned to normal levels after gastroplasty (9.15 ± 0.96). Serum leptin levels were positively related to BMI ($r = 0.882$; $P < 0.0001$; $n = 24$) and to basal plasma insulin levels ($r = 0.516$; $P < 0.01$), and inversely related to the insulin sensitivity index S_I ($r = - 0.379$; $P = 0.068$). However, these two latter correlations disappeared after adjustement for BMI. In conclusion, elevated serum leptin levels return to low normal values in post-obese women, and such normalization might contribute to favour weight regain after dieting in case of central resistance to leptin.

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SERUM LEPTIN IS INDEPENDENTLY ASSOCIATED WITH INSULIN RESISTANCE IN LEAN AND OBESE SUBJECTS

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Serum leptin is elevated in most overweight individuals in parallel with increased plasma insulin levels. We evaluated oral glucose tolerance, insulin sensitivity (frequently sampled intravenous glucose tolerance test with minimal model analysis), acute insulin response to glucose (AIRg), 0-10 minutes after the IV glucose bolus, and serum leptin levels in 19 lean [9 males (BMI 22.3, range 20.1-25), and 27 age-matched (mean 34.5 ± 2.1 years) obese (12 males; BMI 32.4 ± 0.6, range 30.1-37.2] subjects. Percent body fat (PBF) was measured using bioelectric impedance. As previously described, log transformed serum leptin was significantly associated with BMI ($r=0.66$, $p<0.0001$), PBF ($r=0.83$, $p<0.0001$) and fasting serum insulin ($r=0.61$, $p<0.01$). A strong linear association between Log_e serum leptin and insulin sensitivity ($r=-0.65$, $p=0.002$ in males; $r=-0.86$, $p<0.0001$ in females) was found. AIRg correlated with leptin levels only in those subjects with normal glucose tolerance. A Multiple Linear Regression Analysis showed that both SI and PBF, independently contributed to 52% (males) and 75% (females) of the variance of serum leptin (for SI, $p=0.02$ in males; $p=0.005$ in females). In summary, differences in leptin levels cause, or are the consequence of, differences in insulin sensitivity.

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HYPOLEPTINEMIA IN POST-OBESE WOMEN: A POSSIBLE CAUSE FOR RELAPSE OF BODY WEIGHT.

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It has recently been proposed that relatively low plasma concentrations of leptin, a hormone produced by the adipose tissue that decreases food intake and increases energy expenditure in rodents, may predispose humans to obesity. To test this hypothesis, we measured body composition (bioimpedance), energy intake (7-day food diary), energy expenditure (indirect calorimetry) and fasting plasma leptin concentration (RIA) in 6 morbidly obese women (O, 40±1y, 115±17kg, 54±3% body fat, mean±SD), 8 post-obese women 3y after surgical treatment for obesity, i.e. biliopancreatic diversion (PO, 38±9y, 73±9kg, 40±10% body fat), and 8 never-obese women (NO, 35±12y, 57±5kg, 24±7% body fat). After adjusting for metabolic rate, i.e. metabolic body size, energy intake was higher in PO than in both O (excess=2604±284kcal/d; $p<0.01$) and NO (excess=2973±263 kcal/d; $p<0.01$). Plasma leptin concentration was 9.0±4.1 ng/ml in PO, 12.2±6.7 ng/ml in NO and 46.2±14.5 ng/ml in O and correlated positively with % body fat ($n=22$, $r=0.56$; $p<0.01$) and fat mass ($n=22$, $r=0.63$; $p<0.01$). After adjusting for fat mass, mean plasma leptin concentration was 10.0±2.8 ng/ml lower in PO than in NO, and 16.5±7.8 ng/ml lower in PO than in O. Adjusted plasma leptin concentration was negatively correlated with adjusted energy intake ($n=22$, $r=-0.45$; $p=0.03$). In conclusion, our data indicate that post-obese women, who underwent biliopancreatic diversion, have higher energy intake and lower plasma leptin concentration than morbidly obese and normal weight women. These observations suggest that hypoleptinemia may promote body weight gain or relapse (after weight loss) in humans by increasing energy intake.

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PLASMA LEPTIN LEVELS IN OBESE SUBJECTS WITH GLUCOSE INTOLERANCE.

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The aim of this study was to evaluate the clinical significance of leptin in obese subjects with glucose intolerance. We sutdied 54 obese subjects (37 women, 17 men, body mass index(BMI) 33.3±6.5 kg/m^2, age 40.9±14.1 years, mean±SD) and 18 non-obese control subjects(14 women, 4 men, BMI 19.9 ±2.3, 30.7±14.2 years). Obese subjects were divided into three groups according to WHO criteria for 75g oral glucose tolerance test; with normal glucose tolerance(NGT, n=18), impaired glucose tolerance (IGT, n=16) and diabetes(N=20). Plasma leptin levels were measured using a human leptin RIA kit (Linco Company). Percent body fat was measured by impedance methods. Insulin resistance(IR) and β-cell function (β-CF) were calculated using HOMA model of Matthews et al. Plasma leptin concentration in obese subjects was higher than in control subjects and was higher in women than in men(Women:13.4 ±5.7 ng/ml vs 3.9±1.2 ng/ml,P<0.0001, Men:9.1±7.1ng/ml vs 2.7±0.9ng/ml, P<0.03). Leptin correlated significantly (P<0.0001) with BMI (r=0.57), fat precent (r=0.74) and fat mass (r=0.58), but not with fasting plasma glucose level and HbA1c. Leptin levels in the three obese groups were not significantly different. In the NGT group leptin level correlated significantly (P<0.01) with fasting insulin (r=0.73), IR(r=0.69) and β-CF(r=0.72), but did not in the IGT and DM group. In conclusion, plasma leptin was strongly correlated with body fat accumlation, but not with glucose intolerance.

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THE LONGITUDINAL CHANGES OF THE RESTING ENERGY EXPENDITURE IN THE ELDERLY DIABETIC FEMALES.

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We examined wheather the resting energy expenditure (REE) in the elderly diabetics changed with age, using the longitudinal study. The REE of 7 diabetic females (age:72+/-9 (mean +/-S.D.) YO; BMI:24.7+/-2.7 kg/m^2), treated by diet or SU agents, were measured before and after 2 to 3 years (mean period: 1034 days). The REE was measured by the indirect calorimetry. The total lean body mass (TLBM) was examined by dual energy X-ray absorptiometry. The total body fat mass per kg body weight decreased from 35+/-5.0 % to 32.6+/-7.1 during the follow-up period. TLBM did not change (31.8+/-4.8 kg vs. 32.3+/-5.1 kg).The REE per kg TLBM did not change also (31+/-5 kcal/kgday vs. 31+/-6 kcal/kgday). However, the respiratory quotient significantly decreased from 0.89+/-0.06 to 0.83+/-0.04 ($p<0.01$). The carbohydrate oxidation rate per REE significantly decreased from 51+/-11 % to 38+/-9 ($p<0.01$). The fat oxidation rate per REE significantly increased from 32+/-15% to 50+/-15 ($p<0.01$). The protein oxidation rate per REE did not change. Therefore the elderly diabetics might decrease their fat mass due to acceleration of their fat oxidation during aging.

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INCREASED PLASMA LEPTIN CONCENTRATIONS IN PATIENTS WITH CHRONIC HYPERINSULINEMIA DUE TO INSULINOMA.

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Leptin is a new hormone secreted by adipocytes which decreases food intake. The role of insulin in the regulation of leptin secretion is poorly understood and is still a matter of debate. Insulin increases leptin mRNA synthesis in rodents whereas in humans the available data are discordant. To investigate the role of chronic hyperinsulinemia in the regulation of circulating leptin concentrations we studied 13 patients with surgically confirmed insulinoma (8F/5M; aged 42.9 yr; range 19-80; BMI 26.7±1.2 kg/m^2; fasting plasma insulin 146.2±27.5 pM; mean±SEM) and 6 healthy subjects (4F/2M; aged 37.8, range 22-54, BMI 25.8±0.8 kg/m^2, fasting plasma insulin 30.7±6.3 pM, mean±SEM). Immunoreactive human leptin plasma levels were measured by radioimmunoassay. Fasting plasma leptin concentrations were significantly higher in insulinoma patients than in controls (16.7±2.7 vs 3.1±0 4 ng/ml respectively; $p<0.01$). In patients with insulinoma fasting plasma leptin concentrations positively correlated with fasting plasma insulin levels ($r=0$ 685; $p<0.01$). In contrast, leptin levels correlated neither with fasting plasma glucose nor, unexpectedly, with BMI. In summary, in patients with insulinoma: 1) plasma leptin levels are elevated; 2) a direct relationship exist between leptin and insulin concentrations. These data, therefore, support a role for insulin in the chronic regulation of leptin secretion.

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EFFECT OF WEIGHT LOSS ON RESTING ENERGY EXPENDITURE IN HYPERTENSIVE OBESE WOMEN

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Hyperinsulinemia and the associated increased sympathetic nervous activity have been proposed to the development of hypertension and obesity. The role of hyperinsulinemia in mediated resting energy expenditure (REE) and the effect of weight loss on REE between hypertensive (HO) and normotensive obese (NO) women are not understood. We measured fasting plasma glucose, insulin (FPI), lipids concentrations, REE and body composition before and after a weight loss program in 9 newly diagnosed HO and 10 NO women. As compared with age-matched lean controls, obese subjects had higher fasting plasma glucose, FPI and REE values. Although REE and FPI concentrations correlated well in simple correlation ($r=0.708$, $p<0.001$), this relationship disappeared after adjusting for values of fat free mass. Weight loss for 10% of initial weight led to significant decreases of blood pressure and FPI concentrations in both obese groups. Fasting plasma cholesterol, LDL cholesterol and triacylglycerol concentrations decreased in HO individuals. Significant fall of REE in HO ($p<0.05$) and NO ($p<0.02$) were observed following weight loss. However, the ratio of REE to FFM decreased significantly only in HO subjects (114.6±9.2 KJ/day. Kg^{-1} to 108.2±4.6 KJ/day. Kg^{-1}, $p<0.05$). In conclusion, 1). HO and NO women had higher FPI concentrations and REE than those of lean controls, 2). No significant relation between FPI and REE could be found, 3).Weight loss produced a significant decrease of REE/FFM only in HO women.

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SERUM LEPTIN CONCENTRATION IS HIGHER IN MALE BUT NOT IN FEMALE SUBJECTS WITH CUSHING SYNDROME

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Corticosteroids and insulin increase leptin expression in vivo and in vitro. To investigate whether increased serum cortisol influence leptin concentrations in humans, we analysed fasting serum leptin (RIA) and insulin (RIA) in 34 females (27 pituitary and 7 adrenal Cushing, 41.6 ± 2.7 yrs, BMI 29.6± 1 2 kg/m^2) and 16 males (16 adrenal, 39.2 ± 3.1 yrs, BMI 26.3 ± 2 3 kg/ m^2) with Cushing syndrome and fasting leptin in controls, matched for BMI and gender (34 females: 38.2 ± 2.4 yrs, 29.6 ± 2.0 kg/ m^2,16 men: 36.8 ± 3.1 yrs, 26.3 ± 1.9 kg/m^2). Cortisol in Cushing subjects was taken in 10 minute intervals over 24 hours and the mean of 144 samples was calculated for each subject. Leptin was higher both in healthy ($p<0,001$) and Cushing ($p<0,01$) females than in males. Leptin concentration was normal in female Cushing subjects (geometric mean (SEM range) 200 (163-245) pmol/l vs controls 209 (170-257) pmol/l, $p=0,51$) but higher in male Cushing subjects (106 (74-150) pmol/l vs 67 (44-101) pmol/l, $p<0,05$). Total testosterone in male Cushing subjects (2,11 ± 0,34 ng/ml) was below the normal range (>3,0 ng/ml). No difference in leptin concentration between female subjects with pituitary and adrenal Cushing was detected. Leptin correlated positively with BMI in healthy females ($r=0,79$, $p<0,01$) and males ($r=0,82$, $p<0,01$). This correlation was weaker in female ($r=0,41$, $p<0,05$) and male ($r=0,51$, $p<0,05$) Cushing subjects. Insulin correlated positively with leptin in female ($r=0,54$, $p<0,05$) and male ($r=0,72$, $p<0,01$) Cushing subjects. No correlation was observed between leptin and cortisol concentrations in subjects with Cushing syndrome. We conclude that chronic hypercortisolnemia does not affect serum leptin concentration and that insulin and adiposity are the major regulators of serum leptin levels in Cushing syndrome. High androgen levels may inhibit leptin secretion in men; this effect could possibly explain the difference in leptin concentration between male Cushing and healthy subjects.

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TOTAL ENERGY EXPENDITURE AND PHYSICAL ACTIVITY LEVEL CORRELATE WITH FASTING PLASMA LEPTIN IN CHILDREN.

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Leptin, the product of the mouse *ob* gene, is a hormone secreted by adipocytes that is known to decrease food intake and increase energy expenditure in rodents. In humans, variants in the *OB* gene have not been detected and very little is known about the action of leptin on energy expenditure and food intake. The purpose of this study was to assess the relationship between fasting plasma leptin concentrations and energy expenditure in 123 5-year old Pima Indian children (67 M/76 F). Total energy expenditure (TEE) and resting metabolic rate (RMR) were measured using doubly-labeled water and indirect calorimetry, respectively. The physical activity level (PAL) was calculated as the ratio of TEE/RMR.

	Mean ± SD	Range
Weight (kg)	23.3 ± 5.8	14.6-42.9
Body Fat (^{18}O dilution, %)	29.7 ± 7.5	17.0-49.8
TEE (kJ/d)	5982 ± 961	4243-9326
PAL (TEE/RMR)	1.34 ± 0.14	1.03-1.75
Leptin (ng/ml)	8.7 ± 10.5	0.5-56.1

Plasma leptin concentrations were positively correlated to percent body fat ($r=0.84$; $p<0.0001$) and were similar in males and females after adjusting for percent body fat. Most importantly, independently of percent body fat, leptin concentrations correlated with TEE (in absolute values [$r=0.37$; $p<0.0001$] or adjusted for body size [$r=0.42$; $p<0.0001$]) and with PAL ($r= 0.26$; $p<0.01$), but not with RMR. These results in young children suggest that, as in animal models, leptin plays a role in energy expenditure and more specifically, with energy expenditure for physical activity.

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Serum leptin levels are not affected by neuropeptide Y applications despite supression of cortisol levels and promotion of sleep in man

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In animal studies ICV infusions of neuropeptide Y (NPY) exert important effects on energy consumption and basal metabolic rate. Leptin counteracts these effects, thus a close interconnection of both regulators can be anticipated. No data are available on a potential interaction of NPY on the leptin system in man. To further explore a physiological role of NPY in humans we tested the effect of iv bolus injections of NPY (4 x 50 or 4 x 100 µg) in 11 healthy young men (age 20 to 31 yrs; BMI 22.9 ± 3.6 kg/m^2, mean ± SD). Plasma ACTH and serum levels of cortisol, GH, prolactin and leptin were measured every 20 mins between 22h00 and 07h00 and sleep was polygraphically recorded. Meals were allowed until 19h00. The secretion of ACTH ($p < 0.02$) and cortisol ($p<0.05$) was significantly reduced with the lower dose and with the higher dose of NPY sleep architecture was altered with a shortened sleep onset latency and an increased sleep period time ($p < 0.05$). All other hormones especially serum leptin levels remained largely unchanged during the 9 hrs of follow-up. These data suggest that despite significant effects of NPY on sleep and the ACTH-cortisol axis leptin may not be acutely altered by NPY in man.

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TWENTY-FOUR-HOUR RESPIRATORY QUOTIENT IS NEGATIVELY RELATED TO PLASMA LEPTIN CONCENTRATION IN PIMA MALES.

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A high respiratory quotient (RQ), i.e. a low lipid-to-carbohydrate oxidation ratio, is a predictor of body weight gain in humans. Leptin, a hormone produced by the adipose tissue, is known to be involved in the regulation of body weight, but its impact on energy and substrate metabolism in humans is unknown. We measured fasting plasma leptin concentrations by ELISA and 24-h RQ, 24-h energy expenditure, and spontaneous physical activity in a respiratory chamber in 68 non-diabetic, male Pima Indians (27±6y, 100±22kg, 34±7% body fat). Leptin was positively correlated with %body fat ($r=0.72$; $p<0.01$). There was no correlation between 24-h energy expenditure adjusted for body composition and age and plasma leptin concentration adjusted for %body fat. Similarly, spontaneous physical activity was unrelated to plasma leptin concentration. In contrast, 24-h RQ, in absolute value or adjusted for energy balance, %body fat and age, correlated negatively with adjusted plasma leptin concentration ($r=-0.24$ and $r=-0.28$; $p<0.05$, respectively). The negative correlation between 24-h RQ and plasma leptin concentration was not observed in 29 non-diabetic, female Pima Indians. In conclusion, our results indicate that a high 24-h RQ, i.e. low lipid oxidation rate, is associated with relatively low plasma leptin concentrations in male Pima Indians. This may provide an explanation for the predictive effect of a high RQ on body weight gain.

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LEPTIN LEVELS IN CHILDREN WITH DIABETES MELLITUS. W. Kiess, M. Anil, W. Blum, P. Englaro, A. Attanasio, and W. Rascher. Children's Hospital, University of Giessen, Germany, and Lilly Germany, Bad Homburg.

Leptin is produced by adipocytes and is thought to act as an afferent satiety signal regulating appetite and weight. Insulin is a potent regulator of leptin expression. To investigate whether or not leptinconcentrations in children and adolescents with diabetes (IDDM) were related to metabolic status, body weight and insulin treatment, we have measured leptin concentrations in serum from 13 newly diagnosed IDDM patients before the beginning of insulin treatment (8 girls, 5 boys, aged 4.7-17.5 years) and in 134 patients with IDDM during treatment (64 girls, 70 boys, aged 2.6-20.1 years) using a specific radioimmunoassay (RIA). Serum from children with newly diagnosed diabetes had significantly lower levels of leptin (mean 1.28±1.60 ng/ml, range 0.14-6.13 ng/ml) than healthy children (0.1 and 33.3 ug/L, median 2.2 ug/L (N=713) and than insulin treated children and adolescents (mean 5.18±5.48 ng/ml, range 0.26-29.77ng/ml) ($p<0.0001$). Leptin serum levels in patients with IDDM significantly correlated with BMI ($r=0.42$, $p<0.0001$). Multiple regression analysis showed that age and BMI were significantly correlated with leptin levels, while duration of diabetes, mean HbA1c levels, insulin dose and plasma glucose, triglyceride and cholesterol levels were not. Surprisingly and most importantly, leptin levels in young adult (Tanner stage 5) patients were significantly higher than values found in the healthy nondiabetic reference population even when adjusted for age, sex, Tanner stage and BMI. In conclusion, (1) leptin levels in children and adolescents with IDDM are directly related to body adipose tissue and age. (2) Leptin serum concentrations are low at diabetes manifestation. (3) Leptin serum concentrations in young adult patients are higher than in the reference population even when corrected for age, sex, Tanner stage and most importantly for BMI. We hypothesize that the weight gain that is observed during intensified insulin treatment regimens in adolescents and young adults with IDDM is related to high leptin levels.

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The Effect of Acarbose on Body Weight Regulation.

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Acarbose blocks absorption of carbohydrates(CHO) by inhibiting enzymes needed to digest starch. It decreases insulin responses after CHO loads. One report showed the importance of CHO ingestion for the expression of its antihyperglycemic effect. To test its efficacy in inducing weight loss, 32 obese diet-treated NIDDM women(age:59+3,HbA1c:8.2 +.3%,&BMI:40+2kg/m2) were studied.Subjects were placed on a standard 1400 ADA diet to follow with %CHO↘50%. Subjects were randomized to receive acarbose(AC)100 mg or a placebo(PL)thrice daily in a double-blind fashion for 24 wks. Subjects reported monthly their meals↘6 days of the month for calculations of %CHO,%fat,%protein,&daily calorie intake. There were no significant differ -ences between groups in age,BMI,& HbA1c.Analysis of variance,& Student's t test were used for data analysis. Our data showed 1)AC-treated group continued to lose weight over 24 wks;their mean weight loss(98.7+4.3→91.9+4.5kg)was significantly greater than PL group (96.0+5.6→95.0+5.3kg)(P∠ .03) at 24 wk.2)At the end of treatment,AC group had significantly lower HbA1c than PL group (7.4 +.3 v 8.1+.3%),&3)No significant changes in %CHO & daily calories were detected before & after 24 wks of treatment in both AC & PL groups.

These results indicate the ability of acarbose to produce weight loss in obese NIDDM in whom the % CHO intake is high, and confirm its efficacy as an effective antiobesity agent.

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COMPARISON OF PLASMA LEPTIN CONCENTRATIONS IN PATIENTS WITH TYPE I AND TYPE II DIABETES MELLITUS

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Leptin, the gene product of the ob gene, is secreted by adipocytes and its plasma concentrations are correlated with the body mass index (BMI). Mice homozygous for mutations in the ob gene do not produce leptin and develop obesity, hyperglycemia, and hyperinsulinemia. The aim of this study was to compare the plasma leptin concentrations in patients with type I diabetes mellitus (n=42: BMI: 24.8±0.7 kg/m², HbA_{1c}: 7.5±0.4%), with type II diabetes mellitus (n=119: BMI: 29.2±1.0 kg/m², HbA_{1c}: 8.0±0.6%) and in nondiabetic humans (control, n=74: BMI: 28.8±1.5 kg/m², HbA_{1c}: 5.3±0.2%). Obesity was defined by a BMI ≥ 27.8 kg/m² in women and ≥ 27.3 kg/m² in men, respectively. Leptin was determined radioimmunometrically (Linco, St. Charles, MO). Mean leptin levels were 8.7±2.6 ng/ml (obese: 13.6±3.4 ng/ml, lean: 8.0± 1.3 ng/ml) in patients with type I diabetes mellitus, 15.1±2.6 ng/ml (obese: 19.0 ±1.3 ng/ml, lean: 10.1±1.0 ng/ml) type II diabetes mellitus, and 21.5±4.5 (obese: 35.2±4.3 ng/ml, lean: 11.6±2.3 ng/ml) in nondiabetic subjects. In all groups the expected strong correlation between BMI and plasma leptin concentration was found ($p<0.0001$, Wilcoxon test). Even when corrected for BMI, women presented with higher plasma leptin levels than men (type I diabetes mellitus: 12.4 vs 4.2 ng/ml, type II diabetes mellitus: 17.9 vs 11.4 ng/ml, control: 26.1 vs 9.0 ng/ml; $p<0.0003$). Plasma leptin concentrations were also correlated with total serum cholesterol in type II diabetes mellitus ($p=0.015$), and with serum triglyceride in type I diabetes mellitus ($p=0.0032$). Conclusion: In addition to the correlation with BMI and sex, mean plasma leptin concentrations were shown to be lower in obese type I and type II diabetic patients compared with obese nondiabetic humans suggesting a defect in leptin production/secretion by adipose tissue in diabetes mellitus.

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THE EFFECT OF GLUCAGON-LIKE PEPTIDE 1 (GLP-1) ON INSULIN SENSITIVITY IN DIABETIC DOGS.

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GLP-1 is a potent incretin. It is also known to have other glucose-lowering effects which might include an increase in insulin sensitivity. To address this issue, we performed hyperinsulinemic glucose clamps with or without GLP-1, in totally depancreatized dogs. These dogs were made moderately hyperglycemic (~10mM) by a basal intraportal insulin infusion (2.04 ± 0.34 $pmol.kg^{-1}.min^{-1}$) which mimicked the residual endogenous insulin secretion in NIDDM. Since GLP also inhibits glucagon secretion, extrapancreatic glucagon was inhibited with somatostatin (0.8 $ug.kg^{-1}.min^{-1}$) and glucagon levels were clamped with intraportal glucagon (0.65 $ng.kg^{-1}.min^{-1}$). At time=0, an additional infusion of insulin (5.4 $pmol.kg^{-1}.min^{-1}$) was infused to determine glucose production and utilization. During the glucose clamp, plasma glucose specific activity was kept constant by the "hot ginf" method. In 12 paired experiments, GLP-1 infusion resulted in higher glucose requirement during the clamp than saline infusion (GLP-1: 60.9 ± 11.0 $umol.kg^{-1}.min^{-1}$ vs saline: 43.6 ± 8.4 $umol.kg^{-1}.min^{-1}$, $p<0.001$). This was due to a significantly greater glucose utilization. (GLP-1: 72.6 ± 11.0 $umol.kg^{-1}.min^{-1}$ vs saline: 56.8 ± 9.7 $umol.kg^{-1}.min^{-1}$, $p<0.001$) whereas the suppression of glucose production was not increased with GLP-1. These data suggest that: 1) GLP-1 increases insulin sensitivity in moderately hyperglycemic depancreatized dogs, independent of the incretin effect. 2) The increase in insulin sensitivity is due to GLP-1's potentiation of insulin's effect on glucose utilization.

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PLASMA HUMAN LEPTIN IN OBESE AND DIABETIC SUBJECTS AND IT'S DIURNAL VARIATION

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In order to clarify the relation between the plasma leptin level (IRL) and obesity or diabetes mellitus and it's diurnal variation, 51 untreated diabetics, 21 subjects with impaired glucose tolerance and 8 normal were studied. Most of them were examined 50g OGTT or breakfast test. Plasma IRL was determined using an immunoassay kit together with plasma glucose and IRI. Plasma IRL levels were correlated significantly with body mass index(BMI,kg/m^2) ($p<0.01$), and the value in female was higher than that in male (reqression line 0.526x-6.76 and 0.458x-7.38, respectively). For the confirmation of sex difference of plasma IRL value, BMI was divided into three grade: less than 25, 25 to 30 and more than 30. The sex difference of plasma leptin was most significant in BMI 25 to 30 ($p<0.002$). The plasma IRL concentration was determined also in 50g OGTT of untreated diabetics. Diabetics were divided according to the fasting plasma glucose: more than 200, 140-200 and less than 140mg/dl. No significant difference of plasma IRL response was found in these groups, however, plasma IRL was decreased slightly during OGTT ($p<0.01$), and this decrease was also found in breakfast test. In the diurnal variation of plasma IRL, the plasma IRL increased during midnight, peaking at am 2 or am 4 and then decreased gradually to the dawn. In conclusion, plasma IRL increased together with the increase of body weight, especially high in female, and had no relation with the severity of diabetes. The plasma IRL decreased during daytime and increased during night, peaking at am 2 or am 4.

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GLUCAGON-LIKE PEPTIDE-1 RECEPTOR AGONIST INDUCE AN INHIBITORY SIGNAL FOR FOOD INTAKE IN OBESE ZUCKER RATS.

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Because we and others have reported that GLP-1(7-36) amide reduces both food and drink intake through a central mechanism, this study was designed to determine the possible role of GLP-1 receptor in the feeding behavior of obese diabetic Zucker rats. Accordingly, identification of GLP-1 receptor in brain and the effects of receptor agonist on feeding behavior were studided in Wistar, obese (fa/fa) and lean (fa/+) littermates Zucker rats of 12-16 weeks of age. In situ hybridization showed, specific labeling of the mRNA for GLP-1 receptors in several brain regions from the three group of animals, mainly in hypothalamus. Same tissue distribution of the GLP-1 receptor was obtained after Western blot whith a specific antiserum developed by us. These results indicate that GLP-1 receptors are actually synthesized in Wistar and Zucker rats. For the i.c.v. administration of peptides, stainless steel guide cannulas that were aimed at the lateral ventricle were implanted in the rats. Food and water intake were registered for 4h after the i.c.v. administration of placebo or 5, 25 or 100 ng of GLP-1(7-36) amide or exendin-4. i.c.v. administration of both peptides results in dose-dependent reduction of both food and water intake in Wistar and in Zucker rats. These findings indicate that the synthesis of GLP-1 receptor in the hypothalamus of obese Zucker rats, permit to their agonists modulate both food and water intake through a central mechanism. Therefore, the obesity of Zucker rats may not related to alterations in the effects of GLP-1(7-36) amide in the brain.

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PLASMA LEPTIN IS INCREASED PROPORTIONALLY TO ADIPOSITY BY PHYSIOLOGIC INSULINEMIA IN INSULIN-DEPENDENT DIABETES:

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High dose insulin infusion increases circulating leptin concentrations within 4-6 hr in nondiabetic, IDDM and NIDDM subjects. The effects of a low dose regular insulin infusion which lowered glucose, but did not produce hypoglycemia, on plasma leptin was examined in 7 subjects with insulin-dependent diabetes (IDDM; 3 Female/4 Male; BMI = 24.9 ± 1.4 kg/m2; Age = 18-25 years). Long-acting insulin was withdrawn for 36 h prior to the insulin infusion which increased plasma free insulin to 187 ± 23 pM for 4 h and decreased plasma glucose from 14.8 ± 1.7 mM to 8.0 ± 1.6 mM. Plasma leptin increased from 7.0 ± 2.0 to 9.8 ± 3.3 ng/ml (%Δ = +32 ± 9, p< 0.01). Subsequently, the insulin infusion rate was decreased; plasma free insulin averaged 78 ± 17 pM and plasma glucose was stabilized at 5.3 ± 0.3 mM from 6-12 h. Plasma leptin increased progressively peaking at 12.7 ± 5.1 ng/ml at 10 h (%Δ = +50 ± 20, p< 0.025). Plasma leptin did not increase during the same time period in 9 other IDDM subjects (7 Female/2 Male; BMI = 23.2 ± 1.0 kg/m2; Age = 12-22 years) infused with somatostatin (Octreotide; 300 ng/kg/hr) plus growth hormone replacement (GH; 9.6 mIU/kg/hr x 1 hr) at 2, 5, and 8 h, however, leptin was significantly increased by 2.7 ± 0.7 ng/ml (%Δ = +39 ± 9, p< 0.005) after 14 h. The addition of rhIGF-1 (40 μg/kg) to this protocol reduced the 14 h leptin response to insulin infusion by 79 ± 36% (p< 0.05). Overall, in 16 subjects the increase of plasma leptin during insulin infusion was positively correlated with BMI (r = 0.65, p< 0.01) or the baseline leptin concentration (r = 0.77, p< 0.0005). We conclude: 1) Low dose insulin infusion producing physiologic levels of insulin and euglycemia increases plasma leptin concentrations in subjects with IDDM. 2) The increase of leptin is proportional to adiposity. 3) Somatostatin plus GH, along with IGF-1, inhibit the leptin response to insulin administration in IDDM. (Supported by the Juvenile Diabetes Foundation, Int.)

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MELANIN CONCENTRATING HORMONE (MCH) CONCENTRATION IS INCREASED IN THE HYPOTHALAMUS OF OBESE ZUCKER RATS

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The neuropeptide MCH has recently been implicated in the hypothalamic regulation of body weight. MCH mRNA was found to be overexpressed in *ob/ob* mice and this was further increased by fasting in both obese and normal mice. Central administration of MCH has been shown to stimulate food consumption in rats. The aim of this study was to determine if hypothalamic MCH levels are altered in the obese Zucker (*fa/fa*) rat compared to lean (*fa/+*) control and to assess if an increase occurs on starvation. Adult male obese and lean Zucker rats (n=16-20/group) were caged with free access to water and food. After 7 days, 8-10 animals from each group underwent a 48hr fast. All animals were sacrificed after this period and MCH levels in the hypothalamus, brain stem and cortex were measured by RIA. Hypothalamic MCH levels were significantly increased in the fed obese compared with fed lean rats [(mean±SEM) 70.4±5.0 vs. 43.1±4.5pmol/g wet weight; p<0.001]. Fasting caused MCH levels to increase in the lean rats [60.5±5.3 vs. 43.1±4.5pmol/g; p<0.05] although no further increase was seen in obese animals. There was no difference in MCH levels in the brain stem and cortex of all animals studied [all 6-9pmol/g]. We conclude that there is an increase in MCH concentration in the hypothalamus of obese Zucker rats compared to lean controls which may contribute to their abnormal regulation of energy balance. Furthermore there appears to be dysregulation of MCH release since levels are not altered on fasting.

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LEPTIN SECRETION AFTER ORAL GLUCOSE LOAD AND AFTER MIXED MEAL TEST IN YOUNG, HEALTHY SUBJECTS

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AIM: Leptin is a hormone secreted by adipocytes, which may play an important role in human obesity. Aim of this study was to investigate leptin secretion in the postabsorptive state.
METHODS: We measured leptin secretion in 23 young and healthy male subjects. After an overnight fast an oral glucose tolerance test (OGTT; 75 g glucose) and a mixed meal test (MMT) (fat 43%,carbohydrate 51%, protein 6%) over 4 hours were performed in all subjects on 2 different days. Plasma leptin levels were measured at baseline and after 2 and 4 hours using a specific RIA.
RESULTS: Mean age (±SD) in all subjects was 20±2 years. Mean body mass index (BMI) was 23±2.5 kg/m^2. As expected, fasting plasma leptin levels were highly significant correlated with BMI (r=0.774; p<0.0001). After OGTT plasma leptin levels did not change [baseline: 3.9±2.9 ng/ml (range 1.5-11.4); 2 hrs: 4.0±2.9 ng/ml (range 1.5-11.1); 4 hrs: 3.9±2.8 ng/ml (range 1.1-10.6)]. After MMT plasma leptin levels increased in all but 1 subjects [baseline: 3.8±2.9 ng/ml (range 1.3-11.6); 2 hrs: 4.0±3.4 ng/ml (range 1.4-11.8); 4 hrs: 4.6±3.9 ng/ml (range 1.4-14.7); p<0,05 (4 hrs vs. baseline)]. In MMT average increase of plasma leptin levels after 4 hours was 17% when compared to baseline (p<0,001).
CONCLUSION: We could show an acute increase of plasma leptin levels after an oral fat load (mixed meal test) whereas no acute changes in plasma leptin levels were seen after an oral glucose load (OGTT). Lipids or free fatty acids may contribute to this effect.

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WEIGHT LOSS AND INSULIN SENSITIVITY: COMPARISON OF DIFFERENT DIETARY PROGRAMMES

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Obesity and insulin resistance are associated with dyslipidemia, hypertension and increased risk of cardiovascular disease and so a secondary aim of weight loss programmes is to improve insulin sensitivity. In this study, the effects of 2 dietary programmes were compared. 11 obese non-diabetic subjects (5M/6F) were placed on a very low calorie diet (VLCD) (400kcal/day) for 8w followed by a 4w normalisation period. Another 11 subjects (5M/6F) were placed on a low fat (30g/day) diet (LFD) with no calorie restriction for 12w. Using indirect calorimetry and the hyperinsulinemic, euglycemic clamp (40mU/m^2/min), total (TGD), oxidative (GOX) and non-oxidative (NOX) glucose disposal (mg glucose/ min/g body wt/pM insulin) were determined before and after intervention. Abdominal subcutaneous (sc) and visceral (visc) fat distribution were determined by single cut CT scan.

	VLCD pre	VLCD post	LFD pre	LFD post
Body Wt	106.8 ± 2.7	94.1 ± 3.0*	107.4 ± 3.6	101.0 ± 3.3*
sc (cm^2)	539.3 ± 45.4	427.6 ± 55.4*	550.3 ± 44.8	473.5 ± 45.0*
visc (cm^2)	178.7 ± 24.4	118.3 ± 16.1*	168.2 ± 14.5	141.9 ± 16.3*
TGD	5.60 ± 0.80	8.62 ± 1.35*	4.70 ± 0.68	6.88 ± 0.80*
GOX	2.64 ± 0.20	3.51 ± 0.32*	2.56 ± 0.22	3.06 ± 0.28*
NOX	2.98 ± 0.65	5.10 ± 1.13*	2.14 ± 0.53	3.83 ± 0.62*

*p<0.05, pre vs post. Wt loss was significantly greater on VLCD than LFD, but the differences between VLCD and LFD in the changes to other parameters were not significant. Wt loss was strongly correlated with the decrease in sc (r=0.716, p=0.0002) and visc (r=0.688, p=0.0004) areas, the increase in TGD (r=0.527, p=0.0117) and GOX (r=0.617, p=0.0022), and to a lesser extent the increase in NOX (r=0.397, p=0.0673). These results show that improvements in insulin sensitivity are related to the degree of wt loss regardless of the dietary regime used.

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HYPERINSULINEMIA STIMULATES LEPTIN RELEASE INDEPENDENT OF HYPERGLYCEMIA IN NORMAL SUBJECTS

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We have previously reported that serum leptin levels increased during 72 h of clamped hyperglycemia (~ 12.6 mM) in normal subjects. It was the objective of this study to determine whether this increase was caused by hyperglycemia or by hyperinsulinemia. To this end, we measured serum leptin (by radioimmunoassay) at 2 h intervals for 72 h during euglycemic clamping (glucose ~ 5 mM, insulin ~ 67 pM, n=4), during hyperinsulinemic clamping (glucose ~5mM, insulin ~880 pM, n=4) and during hyperglycemic clamping (glucose ~ 8.8 mM, insulin ~ 286 pM, n=5) in healthy non-obese young males. During euglycemia (low insulin), serum leptin decreased by 18% over 72 h (from 3.8 to 3.1 ng/ml, $p < 0.05$); during euglycemia (high serum insulin), serum leptin rose by 70% over 72 h (from 3.4 to 5.8 ng/ml, $p < 0.001$); during hyperglycemia (moderate hyperinsulinemia), serum leptin conc. remained unchanged (5.3 to 4.9 ng/ml, NS). These data show that insulin stimulated leptin secretion dose dependently and independent of hyperglycemia. We conclude that insulin stimulated leptin secretion and that this may be a mechanism which could be responsible, at least in part, for the close relationship between serum leptin levels and obesity (which is typically associated with hyperinsulinemia).

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CONTINUOUS VERSUS INTERMITTENT VERY LOW CALORIE DIETS IN THE TREATMENT OF OBESE WOMEN.C Filozof,C Gonzalez,S Burastero,P Sanchez,C Murua,P Postel and M Perman,University of Buenos Aires.Argentina

Very low calorie diets (VLCD)are useful in the treatment of severe obesity, as they rapidly provide substantial weight losses, improving metabolic abnormalities .However, the difficulty to maintain them for long periods have limited their use.The *objective* of this study was to compare continuous(C) vs intermittent(I) VLCD in the treatment of severe obese females.Outcomes were: magnitude and composition of weight lost, adverse effects, and metabolic parameters.Group I(n=11, 41.4 ±6.8y, BMI: 40.4 ±6.1Kg/m^2), received for 5 weeks, alternating each week, a formula diet of 408 Kcal , or a low calorie diet LCD of 960 Kcal ;group C (n=11, 40.6±8.6y, BMI: 39.9 ±5.9Kg/m^2) received a formula diet of 550 Kcal for 5 weeks .At day 0 and 35, body composition (bioelectric Impedance, sum of skinfolds,diameters and perimeters , dual X-ray absorptiometry) and fasting plasma concentrations of glucose,triglycerides, cholesterol and fibrinogen were determined. Resting energy expenditure (REE)and respiratory quotient were determined by indirect calorimetry. Adverse effects were assessed by self report on a standardized questionnaire *Results:* Weight lost was similar in both groups(9.2 ±2.5 vs 8 ±2.7 Kg).It was accompanied by a significant reduction in sistolic ($p<.01$) and diastolic blood pressure($p<.001$) in both groups . Significant decreased in s-triglycerides($p<.01$) , total ($p<.001$), LDL($p<.01$) and HDL cholesterol levels($p<.001$) was found both in group C and I .In contrast, we found significant increased($p<.05$) in fibrinogen levels with mean ± SEM increasing from 3.3± 0.4 to 3.7± 0.5g/l in C and 3.1±0.2 to 3.3± 0.4g/l .The composition of weight lost was similar in both groups and there was not difference in the reduction of REE: LBM ratio between groups .Adverse effects and drop out were less frequent in I. *Conclusions:* Intermittent treatment was as effective as continuous VLCD in achieving weight loss and reduction of blood pressure,glucose and lipid levels with lower incidence of side effects. Increment in plasma fibrinogen concentration was found in both groups.

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J. W. Kolaczynski and R. V. Considine. LEPTIN AND DEXAMETHASONE IN VIVO; EFFECT OF HYPERINSULINEMIA. Thomas Jefferson University, Philadelphia, Pennsylvania, USA.

Background: Insulin and dexamethasone play a pivotal role in the control of leptin production by human fat *in vitro*. Dexamethasone and insulin acting solo but not together stimulate the Ob gene expression in and leptin production by isolated human adipocytes in the primary culture. The *in vivo* studies on the effect of dexamethasone and insulin on circulating leptin levels in humans are conflicting. Aim: We investigated effect of dexamethasone on circulating leptin levels with and without short-term exposure to supraphysiological exogenous hyperinsulinemia. Method: Four healthy male volunteers participated (age 26.5±1.5 yrs; BMI 27.1±1.4 kg/sq.meter; body fat 17.3±1.1%). Dexamethasone 10 mg in five equal doses was given twice, 8 days apart, beginning 7.00 a.m. on day 1 and ending at 7.00 a.m. on day 2 combined with or without (control) isoglycemic hyperinsulinemic (300 mU/sq. meter BSA/min) clamp commencing at 9.00 a.m. and ending at 1.00 p.m. on day 2. Results: Oral dexamethasone had no acute (up to 5 hours) effect on circulating leptin levels; in contrast, leptin levels doubled and continued to rise 24 hours later ($p<0.05$). In the three participants the elevation of leptin persisted until 8.00 a.m. of the following day (24 hours after the last dose). Short-term isoglycemic hyperinsulinemia had no apparent additional effect on the leptin rise. Summary: 1. 24-hour administration of pharmacological doses of dexamethasone has a marked stimulatory effect on circulting leptin levels; the effect appears to be sustained for the next 24 hours. Short term supraphysiological hyperinsulinemia neither potentiates nor abrogates this effect. Conclusion: The study does not support the existence of interaction of insulin and dexamethasone on leptin production by human fat *in vivo*.

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Objective : VERY LOW CALORIE DIET (VLCD) IN WEIGHT REDUCTION OF OBESE DIABETICS

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Method : Prospective intervention study.Wilcoxon Matched-paired for analysis.

13 consecutive patients with BMI over 30, associated with non-insulin dependent diabetes mellitus, and hypertension were recruited after conventional diet weight reduction therapy for at least 3 months. Parameters monitored were body mass index (BMI), waist hip ratio (WHR), fasting sugar(FBS), insulin sensitivity index (1/insulin), serum cholesterol(Chol), triglyceride(Tri), systolic blood pressure(sbp), diastolic blood pressure(dbp) and subcutaneous fat thickness over; triceps, biceps, subscapular (Sscap), and superior iliac (Siliac) and total body fat(Fat). Changes in these parameters were recorded after two week of VLCD program. Result :one patient stopped prematurely because of intolerance to the liquid diet and one patient was rejected because of poor compliance history. Ten individuals completed the program and followed up for two at least one year. Summary of results were tabulated as followings : four female and 7 male

	BMI	WHR	tricep	bicep	Sscap	Siliac	fat
pre-vlcd	34.36± 5.12	1.03± .06	39.13± 7.11	21.59± 5.06	42.74± 17.83	34.94± 6.83	41.74±3. 35
post-vlcd	32.79± 5.40	.99± .06	32 53± 6.78	18.19± 4.04	38.71± 17.13	32.04± 7.98	39.07± 4 38
P	.0033	.0109	.0117	.03	.1159	0911	.018

	sap	dip	FBAs	I/insulin	Chol	Trio
pre-vlcd	125± 15.81	80.5± 11.17	6.72±2.1	67.33± 55 45	5.57± 74	1 75±. 75
post-vlcd	118 5± 12.92	73.70± 10.81	6.54±2.2	148± 96.89	4.60± .65	2.08± 2.03
p	0587	.1386	7989	.0173	.028	.3525

Conclusion : We concluded that VLCD is safe and effective in grossly obese patients .The decreased in body weight is mainly due to lost of fat and it was associated with improvment in insulin sensitivity as showed clinically by reduction of insulin or oral hypoglycaemic agent requirement and by improvement in insulin sensitivity measured by 1/fasting insulin level.

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MARKED AND RAPID DECREASE OF PLASMA LEPTIN IN INSULIN DEFICIENT DIABETES: REVERSAL BY INSULIN.

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Evidence for regulation of circulating leptin by insulin is conflicting. We examined plasma leptin concentrations before and after the induction of insulin deficient diabetes with streptozotocin (STZ; 40 mg/kg/day x 2 days) in 22 male Sprague-Dawley rats weighing 430 ± 8 g and in 14 saline injected control rats. Within 24 hr after STZ, plasma insulin decreased from 480 ± 30 pM to 130 ± 10 pM (Δ = -73 ± 3%, $p < 0.0001$) and plasma glucose increased from 7.0 ± 0.2 mM to 24.8 mM. Concomitantly, plasma leptin decreased from 3.2 ± 0.2 ng/ml to 1.2 ± 0.1 ng/ml (Δ = -63 ± 3%, $p < 0.0001$) despite a small increase of body weight (+9 ± 1 g). Seven days after STZ, plasma insulin was 80 ± 10 pM, glucose was 25.4 ± 0.8 mM, and leptin was 0.6 ± 0.1 ng/ml (Δ = -77 ± 6%, $p < 0.0001$), while body weight had slightly declined by 7% to 400 g. Plasma leptin remained at this level for the 16 days of the study. Hyperphagia was significant at 4 days and food intake at 7 days was increased by 70% (from 34 ± 1 to 58 ± 2 g/day, $p < 0.0001$). Plasma insulin, glucose, leptin, and food intake were unchanged in control rats (All $p < 0.001$ vs STZ). A subset of STZ diabetic rats (n=8) were treated with subcutaneous lente insulin (12 U/kg twice daily) from days 14-16. Plasma glucose decreased to 11.7 ± 3.9 mM, leptin increased from 0.5 ± 0.1 to 2.9 ± 0.6 ng/ml (Δ = +445 ± 139%, $p < 0.01$), and food intake decreased by 21 ± 3% ($p < 0.0001$). The change of leptin was correlated with the degree of glucose lowering ($r = 0.75$, $p < 0.05$). There was less than a 3% increase of body weight. We conclude that insulin deficient diabetes induced by STZ produces a rapid, marked, and sustained decrease of circulating leptin that does not appear to be related to weight loss. Insulin treatment reverses the hypoleptinemia and the increase of plasma leptin after insulin is proportionate to the reduction of hyperglycemia. We hypothesize that low circulating leptin concentrations in STZ diabetes: 1) are a consequence of hypoinsulinemia; 2) could contribute along with hypoinsulinemia to diabetic hyperphagia. (Supported by the Juvenile Diabetes Association, International).

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HYPOCALORIC DIET PLUS GROWTH HORMONE THERAPY INDUCES INSULIN RESISTANCE WITHOUT BENEFIT IN SPARING LEAN BODY MASS. B. Dallagrassa, P.M. Piatti, L.D. Monti, F. Magni, I. Fermo, R. Paroni, M. Galli-Kienle, A.E. Pontiroli, G. Pozza. Istituto Scientifico H. San Raffaele, Milan, Italy.

Previous studies have shown that Growth Hormone (GH) administration during hypocaloric diet determines an important decrease in fat mass (FM) sparing fat free mass (FFM). However, little it is known about the effects of chronic GH therapy during hypocaloric diet on glucose metabolism in obese subjects. The aim of the study was to investigate the chronic effects of GH during hypocaloric diet on body weight reduction and composition, insulin sensitivity and proteolysis in 14 normal glucose-tolerant obese women. They were divided in two groups matched for age, BMI, FFM and FM. All subjects underwent a hypocaloric high-protein diet (45% protein, 35% carbohydrate and 20% fat) for 21 days. GH therapy (0.1 mg/Kg IBW s.c., every 48 hours; group 1: 6 subjects) or placebo (group 2: 8 subjects) were also administered. A euglycaemic hyperinsulinaemic (25 mU/Kg/h) clamp (ec) combined with indirect calorimetry and dideuterated glucose infusion was performed before and after the hypocaloric diet in the two groups. Before diet, during ec, M-value (M), Glucose oxidation (GO), Lipid oxidation (LO) and Hepatic Glucose Production (HGP) were similar in the two groups. After diet, body weight, FM and FFM significantly decreased by 5%, 13% ($p<0.001$ vs before diet) and 2.5% (NS vs before diet) in both groups. During ec, M and GO were lower in group 1 than in group 2 (1.6±0.2 vs 2.9±0.2, $p<0.01$ and 0.3±0.3 vs 1.8±0.2 mg/kg.FFM/min, $p<0.01$; respectively) while LO and HGP were higher in group 1 than in group 2 (1.1±0.7 vs 0.5±0.1, $p<0.01$ and 1.6±0.3 vs 0.3±0.3 mg/kg.FFM/min, $p<0.01$; respectively). 3-Methylhistidine excretion, an index of proteolysis, was decreased by 46 and 36% in groups 1 and 2, respectively (NS). In conclusion GH therapy associated to hypocaloric diet induces insulin resistance without further benefit in sparing lean body mass compared with a hypocaloric high-protein diet.

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PLASMA LEPTIN CONCENTRATIONS AND INTENSIVE INSULIN THERAPY IN INSULIN-DEPENDENT DIABETES MELLITUS.

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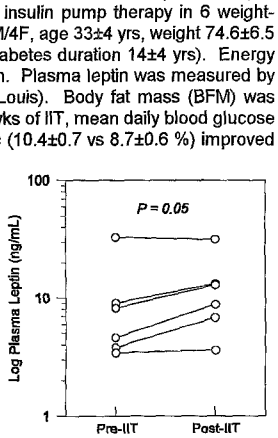
Intensive insulin therapy (IIT) in IDDM is associated with excessive weight gain and increased adiposity due to reductions in glycosuria, energy expenditure, and daily fat oxidation. In order to determine the effect of IIT on plasma leptin and the role of leptin in the weight gain and increased adiposity in intensively treated IDDM patients, we measured basal plasma leptin concentrations before and after 4 wks of insulin pump therapy in 6 weight-stable, poorly controlled IDDM patients (2M/4F, age 33±4 yrs, weight 74.6±6.5 kg, body mass index [BMI] 24±1 kg/m^2, diabetes duration 14±4 yrs). Energy intake was adjusted to prevent weight gain. Plasma leptin was measured by a specific radioimmunoassay (Linco, St. Louis). Body fat mass (BFM) was assessed by hydrostatic weighing. After 4 wks of IIT, mean daily blood glucose (11.4±0.5 mM vs 8.6±0.8 mM) and HbA1c (10.4±0.7 vs 8.7±0.6 %) improved (both $P < 0.01$) despite no change in daily insulin dose (34±5 vs 34±3 units/day). Body weight, BMI, % body fat, and BFM did not change. Fasting free insulin levels tended to increase with IIT (30 ± 8 pM vs 57±16 pM, $P = 0.1$). Basal plasma leptin levels increased with IIT (10±5 vs 13±4 ng/mL; % change from baseline 46±16%, $P = 0.05$) despite no change in body weight or adiposity. Overall, plasma leptin concentrations were positively correlated with % body fat ($r = 0.59$, $P < 0.05$) and HbA1c ($r = 0.67$, $P < 0.05$). In addition, baseline leptin levels (pre-IIT) were weakly correlated with baseline fasting free insulin ($r=0.72$, $P =0.1$). In summary, plasma leptin increased by over 40% from baseline in this group of intensively treated IDDM patients despite no change in body weight or adiposity. These results suggest that insulinemia and/or glycemia per se may regulate circulating leptin levels in IDDM independently of adiposity.



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EFFECTS OF MELATONIN SUPPLEMENTATION ON PLASMA LEPTIN LEVELS.

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Leptin, as many other hormones, shows a diurnal variation with higher night-time levels. However, the regulation of this cyclic variation is largely unknown. Melatonin, a L-tryptophane derivative that is involved in the regulation of activity and mental disposition, has a similar cyclic regulation as leptin. Since both physical activity and mental disposition are known to influence eating behaviour, the aim of the present study was to investigate whether administration of melatonin affects plasma leptin levels during prolonged fasting. The study was done twice in 6 healthy, normal weight subjects (3 males, 3 females, mean age 26±2 years, BMI 222.8±0.9 kg/m^2) after a 12 hour overnight fast. Subjects remained fasting for additional 6 hours and blood samples were taken every 30 to 60 minutes. At the beginning of the second test 30 mg melatonin were administered orally. In the absence of melatonin, as already shown, plasma leptin concentration declined significantly from 8.6±2.4 ng/ml to 6.9±1.9 ng/ml ($p<0.01$, ANOVA for repeated measures). However, administration of melatonin did not affect the decline of plasma leptin concentration over the 6 hour observation time (from 9.1±2.6 to 7.0±2.1 ng/ml, $p<0.01$ vs. baseline, ns vs. without melatonin). No significant changes of serum glucose and insulin concentrations were observed under both conditions. In conclusion, our results suggest that acute administration of high dose melatonin does not influence plasma leptin concentrations in lean healthy subjects.

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INTRAPERITONEAL INSULIN REDUCES PLASMA LEPTIN CONCENTRATION IN DIABETIC PATIENTS ON CAPD.

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Leptin, the product of *ob* gene, has been suggested to regulate food intake. High levels associate with obesity, insulin resistance and hyperinsulinaemia. We studied the effect of subcutaneous and intraperitoneal insulin therapy, with clearly different peripheral insulin concentrations, on plasma leptin. A group of 11 type I diabetic patients (6 women) with terminal kidney failure and continuous peritoneal dialysis (CAPD) therapy participated. The mean age was 45.4 ± 2.8 yrs (mean ±SE) and the duration of diabetes 28.3 ± 2.1 yrs. The patients were on CAPD and subcutaneous insulin 3-6 months prior to the studies. The study protocol included a 3-month period alternatively with subcutaneous or intraperitoneal insulin during CAPD. Insulin sensitivity was measured with hyperinsulinaemic (80 mU/m^2/min) euglycaemic clamp and other studies included body weight, HbA_{1c} and serum insulin concentration. During the 3 months with intraperitoneal insulin HbA_{1c} improved (from 9.49 ± 0.40% to 8.13 ± 0.37%, $p<0.01$) and glucose disposal rate enhanced (from 7.13 ± 0.80 mg/kg/min to 8.24 ± 0.82 mg/kg/min, $p< 0.01$) significantly. Fasting insulin concentration declined (from 17.5 ± 2.2 mU/l to 14.5 ± 2.3 mU/l, $p<0.05$). Average plasma leptin level was 20.1 ±6.2 ng/ml during subcutaneous insulin and 13.2 ± 6.4 ng/ml after 3 months with intraperitoneal insulin ($p<0.01$). There was a modest decrease in body weight during intraperitoneal insulin therapy (68.0 ± 3.9 kg to 65.9 ± 4.2 kg, $p<0.05$). Leptin concentration did not correlate to body weight, fasting insulin level, glucose disposal rate or HbA_{1c}. The change in leptin level were related to the change in insulin level and body weight. In conclusion intraperitoneal insulin reduces plasma leptin level possibly via inducing lower peripheral insulin concentration.

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BRAIN UPTAKE OF LEPTIN IN RABBIT IS SATURABLE IN VIVO

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Leptin is a peptide hormone produced by adipocytes, which provides a negative feedback signal to control body fat depot. Leptin is thought to mediate its action on food intake and weight loss by interacting with its hypothalamic receptor. We examined the biodistribution and brain uptake of radioiodinated mouse leptin (^{123}I-leptin) by dynamic gamma imaging in anesthezised New Zealand white rabbits. Leptin uptake was seen in the brain, lungs, liver and in the kidneys. In the brain increased radioactivity as a function of time was seen in the hypothalamic area. Hypothalamic to brain ratio (HT/BR) in radioactivity increased up to approximately 30-55 minutes after which a steady state in HT/BR of approximately 2.5 was achieved. When unlabelled leptin was injected 40 min after the ^{123}I- leptin, HT/BR leptin uptake ratio began to decline and remained at a lower level of approximately 1.2 thereafter. In conclusion: 1) A major proportion of leptin uptake *in vivo* occurs in the hypothalamic region of the brain with minor uptake in the lungs, kidney and the liver. 2) The uptake of leptin in the hypothalamic region is specific and saturable as indicated by the displacement after administration of unlabelled leptin.

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SERUM LEPTIN AFTER SHORT AND LONG TERM INSULIN ADMINISTRATION TO SUBJECTS WITH NIDDM.

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Aim: To correlate serum leptin levels to body fat and assess the effect of short and long term insulin administration on leptin levels in patients with NIDDM. **Material and methods:** Serum levels of the ob gene product leptin were analysed in 31 men and 22 women with NIDDM treated with diet and sulphonylurea, and related to their body mass indexes and per cent body fat measured by dual X-ray photoabsorptiometry. A subset of the subjects were randomly assigned to treatment with insulin or to continue sulphonylurea for one year. Serum leptin levels were measured in the fasting state and during a euglycaemic, hyperinsulinaemic glucose clamp before and after the treatment period. **Results:** Serum leptin levels at baseline correlated highly significantly to body mass index ($r=0.53$, $p<0.001$) and per cent body fat ($r=0.86$, $p<0.001$). Insulin infusion during euglycaemic, hyperinsulinamic clamp at baseline, produced a slight increase in serum leptin concentration towards the end of the clamp (from 6.1 ±0.8 ng/ml to 6.8 ±1.0 ng/ml ($p=0.0001$) after a mean clamp-duration of 270 min. During one year on insulin therapy, HbA_{1c} were lowered from 8.8 ± 0.4 to 8.0 ± 0.4 ($p<0.05$), body mass index increased by 6% (from 26.7 ± 0.8 to 28.3 ± 0.8 kg/m^2 ,$p<0.001$), and fasting levels of serum leptin nearly doubled (from 7.0 ±1.3 to 13.1 ±2.0 ng/ml, $p<0.0001$). **Conclusion:** In NIDDM patients, serum leptin levels correlate strongly to per cent body fat and BMI. Insulin has only a weak acute effect to increase serum levels of leptin, while long term insulin therapy increases serum leptin more than can be accounted for by the increase in BMI.

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THE REGULATION OF LEPTIN RECEPTOR LEVELS IN DIABETES.

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The leptin receptor (Ob-R) has several alternatively spliced isoforms which are expressed in a wide variety of tissues. Of these isoforms, Ob-Ra has been shown to be involved in leptin transport, while the Ob-Rb isoform appears to be involved in signal transduction in the hypothalamus. Recently Ob-Ra was found in human liver, and leptin treatment of hepatic cells in vitro was shown to decrease insulin action. These results, together with our own epidemiological evidence, suggests a possible role for leptin in the development of insulin resistance. In the current study we investigated the level of Ob-Ra gene expression in the Israeli Sand Rat, an animal model of obesity and NIDDM, using semi-quantitative RT-PCR. The level of Ob-Ra mRNA was measured in the liver, adipose tissue and hypothalamus of animals with varying degrees of obesity and insulin sensitivity. Correlations between Ob-Ra gene expression and total body fat, insulin and glucose concentrations are shown below.

	Ob-Ra mRNA expression		
n=13	Hypothalamus	Adipose Tissue	Liver
Total body fat	r=0.67*	r=0.59*	r= -0.45; n.s.
Insulin	r=0.37; n.s.	r=0.62*	r= -0.72**
Glucose	r=0.30; n.s.	r=0.11; n.s.	r= -0.55*

*p<0.05; **p<0.01

Both hypothalamic and adipose tissue Ob-Ra receptor levels increased with body fat and insulin levels, suggesting increased transport and uptake of leptin into these tissues. In contrast, Ob-Ra gene expression in the liver decreased as body fat, insulin and glucose increased. The decline in the level of Ob-Ra gene expression in the liver may reduce the impact of hyperleptinaemia on hepatic insulin action. In vivo studies are required to further explore the effect of leptin on insulin action and the regulation of leptin receptors.

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IGF-I TREATMENT OF NORMAL RATS SUPPRESSES LEPTIN mRNA IN FAT TISSUE.

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In previous studies we found that hypophysectomised (hypox) rats have low serum insulin and reduced leptin mRNA levels and that rhIGF-I treatment further suppresses leptin mRNA without changing serum insulin levels. The aim of the present study was to examine whether rhIGF-I has similar effects on leptin mRNA expression in rats with normal serum insulin and leptin mRNA levels. Methods: Leptin mRNA was determined by Northern blotting of RNA from epididymal adipose tissue of normal rats infused s.c. for 6 days with rh IGF-I (1mg/d), GH (200 mU/d) or solvent (0.1 M acetic acid) using miniosmotic pumps. In addition, fat pad weight, serum insulin, FFA, IGF-I and food intake/d was determined. Results: In normal rats, leptin mRNA levels were markedly suppressed (38.8 ± 11.7 % of controls) by treatment with rhIGF-I, whereas GH had no significant effect (80 ± 13.1 % of controls). RhIGF-I also suppressed serum insulin levels (1.07 ± 0.51 vs 1.89 ± 0.7 ng/ml in controls) and the fractional fat pad weight (0.52 ±0.13 vs. 0.31 ± 0.03 g/100g of body weight), whereas GH had no effect. Both GH and rhIGF-I treated animals had reduced FFA levels (207 ± 34 and 278 ± 42 vs 561 ± 222 in controls). There was no difference in food consumption and body weight. Conclusions: In both, hypox and normal rats, rhIGF-I treatment resulted in a marked suppression of leptin mRNA. In contrast to hypox rats, IGF-I decreased leptin mRNA together with serum insulin levels in normal rats consistent with the present concept that insulin is a major positive regulator of leptin mRNA expression in rat adipose tissue.

1070

AN INCREASED ENERGY EXPENDITURE PREDICTS INCREASE IN BODY FAT IN GIRLS.

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Studies in Pima Indiana and in infants have suggested that high energy expenditure predicts increases in body fat. This study was designed to test this hypothesis in girls as they entered puberty. A group of 69 girls in 4th and 5th grade were recruited to include equal numbers of black and white girls with a range of body composition. Body composition was measured with dual energy x-ray absorptiometry. Total daily energy expenditure (TDEE) was measured by doubly-labeled water and resting metabolic rate (RMR) was measured with a metabolic cart. Change in % body fat over two years was the primary end-point. Since it was determined that there was no main effect of race, ethnic groups were combined for the remaining analyses. A high TDEE and a high RMR predicted fat gain in these girls. When analyzed by body fat grouping, this relationship was only apparent in the obese girls. Since it was a higher energy expenditure that predicted increased body fat gain, this suggests that these girls ate proportionately more calories than the other girls.

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SEASONAL WEIGHT VARIATION IN PEOPLE WITH DIABETES - FACT OR FICTION?

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To assess the contention of many people with diabetes that they gain weight in winter and lose weight in summer we have investigated 10836 weights recorded during all the routine clinic visits of 3119 patients (age 18-93 years, 51.7% male, 31.2% diet controlled, 32.8% on oral hypoglycaemic agents, 36.0% on insulin) between February 1992 and July 1995. Efforts to fit a sine-waveform to the weight data (raw and log transformed) plotted against time of the year were unsuccessful and a smoothed mean weight plotted over time closely matched the overall mean. Linear modelling was applied to data from each clinic visit relating log transformed weight to age, gender, diabetes therapy, random plasma glucose and the interactions of these terms. The best fitting model accounted for 23.3% of the observed variability with gender, diabetes therapy, age, age squared and interactions between these as the key factors; interpatient variation accounted for over 95% of the residual variance from the model. Addition of time of year as a continuous variable or as 12 discrete factors (= months) did not improve the fit of the model. We conclude that there is no general tendency to seasonal weight variation in people with diabetes.

PS 24

Insulin Resistance, Hypertension, CHD Methods

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INSULIN SENSITIVITY AND SODIUM HANDLING IN OFFSPRING OF HYPERTENSIVE PARENTS

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Insulin mediated sodium retention has been proposed to be one link between insulin resistance and hypertension. We examined the influence of insulin sensitivity and acute hyperinsulinemia on renal sodium excretion and pressor hormones in normotensive subjects who are at risk of hypertension. **Design:** 22 fasting healthy offspring (Off) of at least one hypertensive parent and 16 controls (Con) were examined. After initial rapid infusion of 1000 mL isotonic saline, fractional sodium excretion, urinary catecholamine excretion, plasma renin, aldosterone and immuno reactive insulin concentrations were measured in 60 min intervals. During the second and third hour after sodium loading, a euglycemic, hyperinsulinemic clamp with a constant insulin infusion rate (1 mU/min/kgBW) was performed. All results are given as means $\pm$ SEM. **Results:** Both groups were closely matched for all possibly confounding variables, such as age (Con 24.2$\pm$2.3; Off 23.6$\pm$2.4 y), blood pressure (Con 112.5$\pm$2.6 / 73.3$\pm$2.3 mmHg; Off 113.2$\pm$3.2 / 68.8$\pm$2.1 mmHg), BMI (Con 22.9$\pm$2.3; Off 22,5$\pm$2.5 kg/m^2), fasting glucose and lipid parameters (all parameters not significantly different at $p<0.05$). Parameters of insulin sensitivity, i e. steady state glucose infusion rate and sensitivity index, correlated well with baseline lipid parameters ($p < 0,01$), WHR and fasting insulin ($p<0.05$), but did not differ between both groups (Con 51.6 $\pm$3.9; Off 51.5$\pm$3.1µmol/min/kgBW). Urinary sodium excretion and fractional sodium excretion were independent of family history, insulin sensitivity and steady state insulin concentration. Pressor hormones did not change significantly in response to hyperinsulinemia. **Conclusions:** In young, normotensive offspring of hypertensive patients, who are at increased genetic risk, but have not yet developped clinical or laboratory features of the metabolic syndrome, we could not find decreased insulin sensitivity. In this very early state, insulin mediated sodium retention obviously does not reach functional significance.

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INSULIN RESISTANCE IN METABOLIC CLUSTERS OF FAMILIAL HYPERURICAEMIA

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Metabolic clusters in 20 families (204 individuals) with hyperuricaemia and gout have been established by multivariat analysis of age- and sex-adjusted clinical and metabolic data using factor analysis, cluster analysis and analysis of variance (MANOVA). Regarding the degree of impairment of uric acid excretion and hyperlipoproteinaemia (HLP) six clusters could be differentiated. Highest prevalence of arteriosclerotic signs (by duplex sonography of Aa.carotes and femorales) have been found in clusters 3 (17/35 $\approx$ 49%) and 5 (10/24 $\approx$ 42%). Whereas individuals in cluster 3 were characterised by pronounced combined HLP, individuals in cluster 5 had minor deteriorations in lipid metabolism but, in contrast, the highest values for body mass index (BMI) and fasting insulin. Dividing investigated population in low (LI; <0,11 mmol/l) and high (HI; $\geq$ 0,11mmol/l) fasting insulin groups, prevalence of HI varied from 10-14% in clusters 1,2,4 to 40% in clusters 5 and 6. High fasting insulin was accompanied by significant higher BMI, waist-to-hip ratio, uric acid, systolic and diastolic blood pressures, triglycerides, ApoB, glucose and lower HDL-cholesterol. Furthermore, clusters 5 and 6 were characterised by highest values for area under the curve for glucose and insulin in oral glucose tolerance test. Insulin resistance in clusters 5 and 6 was accompanied by significant lower activity of t-plasminogen activator as well as significant higher values for α_2-antiplasmin and antigen of plasminogen activator inhibitor. These data indicate, that insulin resistance could be proved for 2 of 6 metabolic clusters found in families with hyperuricaemia and is accompanied with metabolic and hemostatic deteriorations that are known to be involved in arteriosclerosis.

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EFFECTS OF ENDOTHELIN-1 INFUSION ON INSULIN SENSITIVITY AND INSULIN SECRETION IN NORMOTENSIVE LEAN SUBJECTS

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Elevated plasma endothelin (ET)-1 levels have been described in different insulin resistant states such as syndrome X, obesity, non-insulin-dependent diabetes mellitus (NIDDM), and essential hypertension. To investigate whether physiological to pathophysiological increases in circulating ET-1 can modulate insulin levels and/or insulin sensitivity in humans, we assessed these variables during low, non-pressor dose ET-1 as compared to placebo infusion in lean normotensive healthy subjects. Ten healthy, caucasian males, age 20-30 years and with a body mass index (BMI) of 20-26 kg/m^2 were included in the study. Insulin sensitivity was assessed dynamically by the Minimal Model Approach with use of the modified frequent sampling intravenous glucose tolerance test (FSIGT). In a randomized, single blind cross-over fashion the subjects then received either an intravenous infusion of ET-1 dissolved in polygeline, or a control infusion of polygeline only (=placebo). The acute insulin response (AIR) was calculated as the average of the three peak values between 2 and 5 min after injection of glucose from which the basal insulin levels were substracted. The index of insulin sensitivity (S_I) measured during ET-1 infusion was 11.1$\pm$1.9$\times10^{-4}$ /min/mU/l versus 10.8$\pm$2.1$\times10^{-4}$/min/mU/l during placebo infusion (p=NS). ET-1 infusion markedly reduced plasma insulin levels during the FSIGT compared to placebo (ANOVA $p < 0.0001$). The acute insulin response (AIR) to glucose during ET-1 infusion was 34.8$\pm$4.3 versus 49.3$\pm$6.9 µU/ml during placebo (p=0.017). In conclusion, we have demonstrated that short term moderatly increased ET-1 levels do not impair insulin sensitivity. However, ET-1 markedly diminished the acute insulin secretion. Our data suggest that ET-1 decreases pancreatic β cell function.

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RELATIONSHIP BETWEEN HYPERURICEMIA AND INSULIN RESISTANCE

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Hyperuricemia is often associated with obesity, hypertension and dyslipidemia, and is considered to be a risk factor for cardiovascular disease, thus being similar to the insulin resistance syndrome. A low, but significant correlation was found between serum uric acid concentration and the degree of insulin resistance (GIR) estimated by the euglycemic hyperinsulinemic clamp method in 67 subjects with combined normal glucose tolerance and impaired glucose tolerance (IGT, $r=-0.278$, $p<0.05$). In a separate group of 30 non-obese subjects including 7 with IGT, UA was correlated with GIR($r=-0.518$, $p<0.01$). Plasma HDL-C and triglyceride (TG) levels were also correlated with uric acid levels. One hundred and sixty non-insulin dependent diabetes mellitus (NIDDM) patients who had undergone the clamp study were classified into 5 groups according to serum uric acid level. In the top quintile (UA:7.8$\pm$0.8), body mass index (BMI), male prevalence, plasma TG, HDL-C, fasting IRI and total IRI response (0+60+120) during the meal tolerance test were significantly higher, while age and GIR were lower than those in the bottom quintile (UA:3.4$\pm$0.5), but the differences were not significant. These findings indicate that elevated serum uric acid is a feature of the insulin resistance syndrome.

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EARLY PROTEIN RESTRICTION AND SUBSEQUENT OBESITY ARE INDEPENDENT RISK FACTORS FOR HYPERTENSION

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Recent epidemiological studies have revealed a link between fetal and early post-natal growth retardation and the development of hypertension and glucose intolerance in later life. The aim of this study was to assess whether growth retardation, caused by early protein restriction, is a risk factor for the subsequent development of hypertension that is independent from any risk associated with obesity. Pregnant Sprague Dawley rats were given either a 20% protein ('control') diet or an isocaloric 8% protein ('LP') diet throughout pregnancy and lactation. The female pups were weaned onto the diets that their mothers had been given and remained on these diets until 70 days of age. Then some of the rats were given a standard laboratory chow ('pellet') diet whilst other rats were fed a cafeteria-style high calorie ('cafeteria') diet. The rats remained on these diets throughout the remainder of the study. At one year of age the rats underwent an intra-peritoneal glucose tolerance test and had their blood pressures measured using a tail cuff technique. The body weights at this age (g) were as follows (mean (SD)): control-pellet 421.7 (31.5) (n=7), control-cafeteria 799.9 (131.3) (n=6), LP-pellet 333.7 (23.6) (n=9), LP-cafeteria 594.1 (127.1) (n=9) (early diet $p<0.001$, adult diet $p<0.001$, interaction $p=0.110$; 2-way ANOVA). Neither early ($p=0.829$) nor adult ($p=0.221$) diets were associated with alterations in glucose tolerance at this age (2-way ANOVA). Systolic blood pressures (mmHg) (mean (SD)) were: control-pellet 128 (25), control-cafeteria 157 (29), LP-pellet 158 (28), LP-cafeteria 182 (40) (early diet $p=0.025$. adult diet $p=0.026$, interaction $p=0.815$; 2-way ANOVA). The data suggest that both early protein restriction and subsequent diet-induced obesity are independent risk factors for the development of hypertension, which can present without an associated worsening of glucose tolerance at one year of age.

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PHYSIOLOGICAL LEVELS OF RAT C-PEPTIDE IMPROVES GLUCOSE UTILIZATION IN DIABETIC RATS

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Recent study suggests that supraphysiological amounts of human C-peptide (20-40nmol/l) augments glucose utilization in STZ induced diabetic(STZ-D) rats. The present study was undertaken to determine the influence of physiological conc. of rat C-peptide on in vivo glucose utilization in STZ-D (60mg/kg) rats by using an euglycemic clamp technique. Two different insulin infusion rates, 3.0(low-dose) and 30.0(high-dose) mU/kg•min, were used. Rat C-peptide I or II was infused at a rate of 0.05 nmol/kg•min, which increased plasma levels from 0.15±0.03 to 0.37±0.03 nmol /l, from 0.13±0.03 to 0.40±0.06nmol/l, respectively. (fasting levels in controls were 0.53±0.06 nmol/l). Plasma insulin levels during the low- and high-dose insulin infusion were 40 and 400 μU/ml, respectively, and blood glucose was clamped at 7.5mmol/l by periodic adjustment of the *i.v.* glucose infusion rate. Glucose metabolic clearance rate (MCR) was 160% greater ($P<0.05$) in STZ-D rats recieving C-peptide I during the low-dose insulin period(17.9±4.0 ml/kg•min, n=6) than in STZ-D control rats(7.2 ±0.8ml/kg•min, n=6). Likewise, MCR in STZ-D rats given C-peptide II(13.6±3.3ml/kg•min) tended to be greater than in STZ-D control rats ($0.05<p<0.1$). MCR of STZ-D rats infused with either C-peptide I or II was similar to the MCR found in the non-diabetic control rats (15.1±2.0 ml/kg•min). C-peptide I and II did not significantly influence MCR during the high-dose insulin period. It is concluded that phyiological amounts of rat C-peptide I and II improves glucose utilization in STZ-D rats.

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INSULIN RESISTANCE IN ESSENTIAL HYPERTENSION AND ITS ASSOCIATION TO ASYMPTOMATIC ATHEROSCLEROSIS

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Insulin sensitivity was determined in subjects with essential hypertension, and effects of antihypertensive drugs on insulin sensitivities were evaluated. Furthermore, the relation between carotid atherosclerosis and insulin resistance was studied. Using SSPG method, insulin sensitivity was measured in non-obese and non-diabetic subjects with essential hypertension. SSPG was significantly ($p<0.05$) higher in subjects with borderline HT (n=86) and HT (n=90) compared with normal (n=24) subjects. SSPG was significantly ($p<0.01$) correlated with systolic (r=0.37) and diastolic (r=0.31) blood pressures in hypertensive subjects. In essential hypertension, with normal glucose tolerance, insulin resistance was noted in 75%. Intra-platelet Ca^{2+} after collagen stimulation was higher in subjects with HT. Intra-platelet Ca^{2+} after collagen stimulation decreased during insulin infusion, and basal Ca^{2+} correlated with SSPG. SSPG reduced significantly by α1 blocker, bunazosin (-26%), ACE inhibitor, cilazapril (-26%), β-blocker (K-channel opener), tilisolol (-18%), and long acting Ca antagonist, amlodipine (-20%) & benidipine (-20%). The relation between early asymptomatic carotid atherosclerosis and these risk factors was examined in 72 non-diabetic subjects with essential hypertension aged 50-59 years. Intima-media thickness of carotid artery was assessed by B-mode ultrasonography. By multiple regression analysis, steady state plasma glucose was the strongest risk, followed by lower high density lipoprotein and systolic blood pressure. In hypertension, insulin resistance which is presumed to be derived from intracellular Ca^{2+} derangement, further elevates blood pressure and aggravates atherosclerosis. The above antihypertensive drugs help to correct the vicious cycle.

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HYPERINSULINEMIA IN MICROVASCULAR ANGINA IS COMBINED WITH HIGH Na+/Li+ COUNTERTRANSPORT.

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Stimulated hyperinsulinemia during OGTT has been demonstrated in patients with microvascular angina (MA). Insulin resistance is frequently associated with overactivity of red blood cell Na+/Li+ countertransport (Na+/Li+ CTT), a specific marker of in vivo Na+/H+ antiport activity. The present study was aimed to assess this system in 12 females (mean age 57+6 years) with MA and in 10 sex and age-matched controls. Na+/Li+ CTT was evaluated as Li+ efflux from Li+ loaded erythrocytes. Insulin levels were evaluated by RIA. Results:

TESTS	CONTROLS	MA	p
Na+/Li+ CTT (umol/L/h)	320±49	642±220	0.0001
Glucose (mg/%)			
Fasting	76±15	85±7	0.2455
90 min.	95±43	129±47	0.1444
180 mi	74±20	102±39	0.0855
Insulin (uU/ml)			
Fasting	10±2	22±23	0.0443
90 min.	41±20	102±73	0.0006
180 min.	16±8	62±46	0.0159

As is shown, hyperinsulinemia is combined with an accelerated Na+/Li+ CTT. This overactivity may cause microvascular dysfunction through intracellular alcalosis which may decrease insulin sensitivity and increase vascular response to constrictor stimuli.

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BLOOD PRESSURE CIRCADIAN RHYTHM IN "NORMOTENSIVE" INSULIN DEPENDENT DIABETIC PATIENTS.

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Objective: To assess the strength of the association between the alterations of blood pressure circadian rhythm and the presence of microalbuminuria(M) in "normotensive"(according manometric JNC V criteria) IDDM patients. As a secondary goal, to assess the correlation between hypertension (determined by ambulatory blood pressure monitoring [ABPM]) and several clinical and biochemical covariates.**Methods**: We studied 84 IDDM patients, normotensive in manometric tests (JNC V criteria), 42 male, aged 36.8±13.0 yr.; in them we performed a 24 hours ABPM study. The level of metabolic control was established through HbA1c determinations.M was determined by RIA (cut-off point: 15 micrograms/minute).Statistical analysis: 1)univariate methods (t-test,Mann-Whitney,Pearson and non-parametric correlations);2) Factor Analysis (varimax rotation);3) multiple logistic regression (Quasi-Newton;Maximum likelihood).Soft: CSS/Statistica,StatSoft,Tulsa,1993.**Results**:58.4% of the sample showed alterations of circadian rhythm (non-deeper status); positive M was observed in 30.9%.In factor analysis, a consistent link was found among systo-diastolic (SD) alteration of circadian rhythm , positive M, retinopathy, diabetes duration and LDL levels. SD alteration was positively and significantly associated with the presence of M both in the univariate correlation model (R=0.25;p=0.02) and in the logistic regression (OR=2.94;95%CI=1.13-7.69;p=0.03).Isolated diastolic rhythm alterations correlated significantly with diabetes duration in the logistic model (p=0.01).Hypertension determined by ABPM according mean blood pressure (JNC V criteria),was associated (logistic regression) with age, first degree relatives positive antecedents and HbA1c level.**Conclusions**:1) SD alterations of circadian rhythm were significantly associated to positive M. 2) High blood pressure values by ABPM correlated with age, family history of hypertension and HbA1c levels. 3)ABPM may be an useful method for precocious detection of blood pressure alterations. Further studies should demonstrate the real potential of this method in this field.

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EFFECTS OF TWO PHARMACOLOGICAL THERAPIES FOR ISCHAEMIC HEART DISEASE ON INSULIN SENSITIVITY.

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Aims: To evaluate insulin sensitivity (IS) in patients with ischaemic heart disease (IHD) and to study its evolution with two conventional pharmacological therapies. **Patients and Methods**: We have studied 20 patients with IHD demonstrated by treadmill exercise test (ST depression >0.1 mV) and/or coronary angiography. Patients with history of glucose intolerance, body mass index (BMI) >30 Kg/m2, fasting plasma glucose (FPG) >140 mg/dl or under any medication with potential effects on IS were excluded. Fifteen healthy volunteers, matched for sex, age and BMI were considered as controls. To assess IS, an insulin suppression test (IST) was performed both in controls and patients. IHD patients were then randomly treated with either acetylsalicilic acid (ASA)(200 mg/d)+atenolol (50 mg/d) [Group BETA; n=10] or ASA (200 mg/d)+isosorbide mononitrate (40 mg/d)+captopril (25 mg/d) [Group ACEI; n=10]. After 6 months, they underwent a second IST. Steady-state plasma glucose (SSPG), insulin sensitivity index (ISI=1000xglucose infusion rate/SSPG) FPG and fasting plasma insulin (FPI) were considered as a measure of IS. **Results**: As a group, IHD patients (n=20) showed higher SSPG (237,90±36,04 vs 123,36±24,43 mg/dl; p<0,001) and lower ISI (25,81±4,35 vs 50,33±9,37 dl/kg•min; P<0,001) values than Control Group. After 6 months, 8 patients from the ACEI Group and 7 patients from the BETA Group could be reevaluated. Data are summarized below (*p<0,001 vs Before Treatment):

	SSPG		ISI	
	Before Treatment	After Treatment	Before Treatment	After Treatment
BETA	235,75±43,23	245,75±30,34	26,30±5,49	24,75±2,83
ACEI	240,05±28,66	162,90±74,64*	25,33±3,22	46,79±26,03*

There were no differences among groups in age, sex, BMI, FPG, FPI. **Conclusions**: Preliminary results of this study show that IHD patients have a decrease in IS. Six months treatment with isosorbide mononitrate and captopril, but not atenolol, improve IS. Finally, SSPG and ISI obtained from IST are more powerful markers of IS than FPG and FPI.

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RELATIONSHIP BETWEEN 24-h AMBULATORY BLOOD PRESSURE AND URINARY ALBUMIN EXCRETION IN PATIENTS WITH IMPAIRED GLUCOSE TOLERANCE

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In order to investigate the relationship between UAE and change of ABPM in IGT patients, ABPM was performed in 18 subjects with high UAE (UAE>10ug/min) and in 26 subjects with normal UAE (UAE <10ug/min). The occasional blood pressure, biochemical index and UAE were measured, and heart autonomic nerve function was evaluated.

The results showed that the occasional DBP and MBP were significantly higher in the IGT with elevated UAE than that of IGT with normal UAE (DBP 88±2.3mmHg vs 80±2.1mmHg, MBP: 103±2.7mmHg vs 95±2.6mmHg, P<0.05) . The peak value of SBP (173±6 vs 153±4mmHg), DBP (134±7 vs 108± 4mmHg) and abnormal SBP load (29. 6±5.85 vs 14.3±3.19%) were higher in high UAE group than that of normal UAE group in ABPM examination. 24-h DBP(87±3 vs 81± 2mmHg) and nighttime DBP(80 ± 3 vs 71 ± 2mmHg) were increased. Nocturnal blood pressure fall of SBP (5.73±2.12 vs 11.5±1.82 mmHg) and MBP (7.22±2.18 vs 13.2±1.73mmHg) were decreased in high UAE group. The reduction of nocturnal blood pressure was decreased (P< 0.05) and the profile of 24-h blood pressure was smoother compared with normal UAE group. A significant positive correlation was found between the UAE and nighttime DBP, blood pressure load of SBP and DBP, 24hDBP and 24hSBP, r=0.4860~0.3070, P< 0.05; and there was a negative correlation between the UAE and the decrease rate in nighttime blood pressure (SBP, DBP and MBP), r=-0.3977~0.4670, p<0.01. No correlation was found between UAE and occasional blood pressure, autonomic nerve score, BMI, Ch, Tg, blood glucose or insulin level.

Conclusion: Our data suggests that the change of ABPM in high UAE group was similar to diabetic nephropathy and the UAE level was correlated with most index of ABPM.

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PARTICULARITIES OF INSULIN ACTION ON MYOCARDIAL FUNCTION AND METABOLISM IN DIABETIC DOGS

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The aim of this investigation was to evaluate the action of insulin (I) on myocardial metabolism and function in diabetes (D). Experiments were performed on 18 control dogs and 12 dogs with alloxane induced D. Catheterization and extracorporal autoperfusion of coronary artery, catheterization and continuous drainage of coronary sinus (CS) were used without opening the chest under anaesthesia. The heart reactions to I were estimated by changes of heart contractility (HC), coronary artery resistance (CAR), coronary arterio-venous difference (CAVD) by pO_2, pCO_2, pH, glucose (G) and FFA. In control dogs I injection (1.0 IU/kg, i.v.) initially on 5-10 min of reaction induced slight and unstable increase of CAR and HC (+4.8±0.3% and +9.2±1.2%, rsp.) which later were followed by more pronounced their decrease (-12.0±0.9% and -13.5±2.5%, rsp.). The latter changes were accompanied by the increase of CAVD by G (from 0.4±0.06 to 0.7±0.07 mmol/l), reduction of CAVD by FFA (from 0.18±0.05 to 0.07±0.02 mmol/l) and elevation of CS blood O_2 saturation (+15.5±4.28%). The heart reaction changes in mild and moderate D were similar to those in control dogs but their magnitude was accentuated. On the contrary in dogs with severe D (G - 14.6±0.38 mmol/l) during the first hour after I injection only slight decrease of CAR (- 2.0±0.4%) and increase of HC (+5.2±0.5%) took place while CAVD by G and FFA as well as CS blood O_2 saturation remained practically unchanged. These data indicate that secondary insulin resistance in severe D includes also attenuation of I action on myocardial function and metabolism.

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ANGIOTENSIN-CONVERTING ENZYME GENOTYPING, INSULIN RESISTANCE IN HYPERTENSIVE YOUNG MEN.

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Recent studies have reported that angiotensin-converting enzyme gene (ACE) polymorphism to be connected with altered sensitivity of tissues to insulin. We studied the insertion/deletion (I/D) polymorphism of ACE gene among 131 young males with borderline hypertension (BHT) and 87 normotensive (NT) young males as a control group using polymerase chain reaction (PCR). The frequencies of insertion and deletion ACE alleles for NT and BHT groups were 0.448 and 0.552 , 0.443 and 0.557, respectively. These differences were not significant. OGTT was performed among 47 BHT patients and 40 NT persons. BHT patients with II genotype had higher average glucose concentration in two hours after glucose loading compared to ID and DD genotypes patients (statistical significant difference was between II genotype and ID genotype). There was some increase in glucose concentration of 40 NT patients with II genotype (point 30, 60 and 120 min) compared to other genotypes, but not statistical significant. There was the most increment of glycemia during OGTT in two hours in NT persons with II genotype to other genotypes (II vs ID, $p<0.01$). Levels of insulinemia obtained during OGTT (point 0, 60, 120 min) were not significant difference among persons with various genotypes in both groups. We have concluded that BHT patients with II genotype and NT persons with II genotype have less insulin sensitivity compared to persons with DD and ID genotypes.

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INSULIN SENSITIVITY STUDIES IN OFFSPRING OF PATIENTS WITH CORONARY HEART DISEASE.

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Background: Insulin resistance is proposed to be a risk factor for development of coronary heart disease. Cross sectional studies demonstrating correlation between fasting insulin levels and presence of coronary heart disease fail to look at the temporal relation required to demonstrate causation.

Aim: to determine whether significant insulin resistance predates clinical manifestation of CHD (offspring of CHD taken as surrogates for pre-CHD) \

Design: Case control; *cases:* 7 offspring of coronary heart disease patients; *controls:* 6 controls without any family history of CHD; *matching:* group, for age and gender. *Outcome measure:* M value on hyperinsulinaemic euglycaemic clamps at insulin infusion rate of 40mU/m^2 (insulin concentration: 89mU/L and 100mU/L).

Results: There was no significant difference between cases and controls as regards anthropometry [BMI: 21.5+1.45 vs. 20.3+1.69, p=0.6; waist to hip ratio: 0.877+0.51 vs. 0.873+0.50, p=0.90]; glucose area under curve after oral glucose load (11407+26.7 vs. 12045+25.0 mg.min.dL^{-1}, p=0.184); and M value [(mg/kg/min) 5.2+1.7 vs. 6.9+1.9, p=0.167. There was no significant difference between cholesterol, HDL and triglyceride levels.

Conclusions: The offsprings of patients with CHD did not have different glucose disposal rates on hyperinsulinaemic euglycaemic clamp studies than controls. The cause effect relation between insulin resistance and coronary heart disease remains to be proven.

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CIRCARDIAN BLOOD PRESSURE, CORTISOL AND PROLACTIN IN DIABETIC AUTONOMIC NEUROPATHY

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The aim of the study was to investigated the relationship between circardian rhythm of blood pressure (BP) and autonomic neuropathy in noninsulin dependent diabetes mellitus. Ambulatory blood pressure (ABP) was measured every 15 min for 24 h in 50 diabetics without hypertension and in 40 healthy controls. The autonomic control in diabetics was evaluated by R.R interval measurements during deep breathing and uprising. The diurnal cycle of BP was assessed by the difference of BP between daytime and nighttime, the night/day ratio of BP and the percent decrease in nighttime BP. We investigated diurnal rhythms of cortisol and prolactin determined by RIA method. The results of our study showed significantly smaller diurnal noctural differences in diabetics than in nondiabetic controls. A subgroup of diabetics with autonomic neuropathy showed significantly smaller diurnal noctural differences of BP, lower ratio between day/night in diastolic BP (0,98 v.s 0,86; p 0,001) and significantly lower parcent (10%) decrease in diastolic BP compared to the group without autonomic neuropathy. A significant decrease of the daily levels of cortisol and increase of prolactin were present in investigated diabetics. It seems that vagal dysfunction may be the main cause of the higher nighttime BP in autonomic neuropathy. ABP monitoring may be useful in evaluating diabetic autonomic neropathy.

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RELATIONSHIP BETWEEN INSULIN SENSITIVITY AND DEHYDROEPIANDROSTERONE-SULFATE IN PATIENTS WITH ISCHAEMIC HEART DISEASE.

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Decreased insulin sensitivity (IS) has been related with the pathogenesis of ischaemic heart disease (IHD). Dehydropiandrosterone-sulfate (DHEA-S) has been suggested as the possible link between these two entities. **Aims**: To clarify the relationship between IS and DHEA-S in patients with IHD and without known metabolic disorders. **Patients and Methods**: We have studied 32 male patients with typical coronary angina and IHD demonstrated by treadmill exercise test (ST depression >0.1 mV) and/or coronary angiography. Patients with history of glucose intolerance, body mass index (BMI) >30 Kg/m2, fasting plasma glucose >140 mg/dl or under any medication with potential effects on IS were excluded. Eleven healthy male volunteers, matched for age and BMI were considered as controls. Insulin sensitivity was assessed by an insulin suppression test, and DHEA-S levels were measured before the test (basal) and at the end of it (during hyperinsulinemia). Steady-state plasma glucose (SSPG) and insulin sensitivity index (ISI=1000xglucose infusion rate/SSPG) were considered as a measure of IS. **Results**: Insulin resistance, defined by an ISI below the mean-2SD of the control group, was present in 78% of IHD patients. As a group, they displayed lower ISI (30,62±12,11 vs 49,82±9,62 dl/kg•min; P<0,0001) and higher SSPG (217,74±59,76 vs 124,67±24,98 mg/dl; p<0,0001) than controls. DHEA-S values were lower in IHD than in controls, both basal (1224±796 vs 1935±1260 ng/ml; P<0,05) and during hyperinsulinemia (1083±761 vs 1683±1051 ng/ml; P<0,05). DHEA-S levels decreased with hyperinsulinemia, both in patients (1224±796 vs 1083±761 ng/ml; P<0,0005) and controls (1935±1260 vs 1683±1051 ng/ml; P<0,0005). The magnitude of that decrease was the same in both groups. No correlation between ISI or SSPG and DHEA-S was found. **Conclusions**: IS is decreased in patients with IHD, even when metabolic abnormalities associated with reduced IS are excluded. These patients also show decreased DHEA-S levels, which are even more reduced when acute induced hyperinsulinemia is achieved although, as the decline was similar to that of controls, there does not seem to be resistance to that particular action of insulin.

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ALTERATIONS IN CARBOHYDRATE METABOLISM IN A SAMPLE OF THE ARGENTINE HYPERTENSIVE POPULATION. A.García, G.Burlando,E.López González,L.de Loredo, C.D.González and Committee of Hypertension, Argentine Society of Diabetes, Argentina.

Objective: to evaluate the distribution of the alterations in carbohydrate metabolism (determined through oral glucose tolerance test [GTT]) in a sample of hypertensive subjects, determining also the strength of the association between the presence of an abnormal test result and several clinical and biochemical independent covariates. **Methods**: We studied 292 hypertensive patients (according JNC V criteria),105 women (age:55.2±11.7) and 187 men (age: 54.4±10.0) without a previously known alteration in carbohydrate metabolism. GTT was performed according WHO criteria.Statistical analysis: 1) univariate methods (t-test, Mann-Whitney, Pearson and non-parametric correlations) and, 2) multiple logistic regression [MLR] (Quasi-Newton; Maximum Likelihood).Soft:CSS/Statistica , StatSoft,Tulsa,1993. **Results**:29.8% of the total sample showed glucose intolerance (Fleiss quadratic 95% confidence interval: 24.7-35.4%). Both in uni and in multivariate analysis, this intolerance was significantly associated to: age (adjusted [MLR] OR=1.03 per yr;95%CI=1.01-1.05;p=0.01), BMI (adjusted OR= 1.99 per Kg/m^2; 95%CI=1.12-3.51; p=0.01) and first degree relatives antecedents of diabetes (adjusted OR=2.61; 95%CI=1.50-4.52, p=0.0006). Male sex correlated significantly only in the univariate analysis (rS=0.11,p<0.05).**Conclusions**:1) Approx.30% of studied patients showed impairment of glucose tolerance. 2) In this sample, this intolerance was positively associated with age, BMI and first degree relatives history. 3)May be of great importance to evaluate glucose tolerance in hypertensive patients, specially in obese over 50 yrs with a positive family history.

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FREQUENCY OF HYPERTENSION IN DIABETIC PATIENTS IN CONGO

Service maladies Métabolique et Endocriniennes
C.H.U. BP 2725 BRAZZAVILLE
H. MBADINGA-MUPANGU ; H. MONABEKA ; P. KIBEKE

The objective is to study the frequency of hypertension in diabetic patients of Congo. It is a retrospective study of diabetic patients admetted in our Service between July 1991 and June 1996..

The hypertension criteri are the the ones World Health Organisation 160/95. (W.H.O.)

This studey conmed 1297 patients, 874 men, 423 wonen divided into two groups : group I : diabetc patients with hypertension, groupe II : diabetc without hypertension.

Hypertension was diagnosed in 152 (11,71 %) of 1297 diabetic patients . There are 73 men (39,91 %) and 79 women (60,09 %).

The patients' age in comprised between 30 and 70 years old.

Among those suffring of hypertension, 2,5 % have an increase in diastolic blood pressure only, 6% that of systolic and 91,5% that of both (diastolic and systolic). In 25% hypertension precedes the discovery of diabetes and in 75 % its discovery coincide with that of diabetes. Twenty seven (1,17,76 %) are requirin insulin, 125 (82,23 %) not requiring insulin, 4(2,6%) of thise requiring insulin are related to malnutrition and 9 five (3,6%) fibrocalculous.

In conclusion; the frequency pf hypertension in low in the diabetic patients followed in our Service. The weakness can be explained by existence of a carodiology service which also takes care of some diabetic patients with hypertension.

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INCIDENCE OF INSULIN RESISTANCE SYNDROME IN CHILDREN AGED 7-16 FROM FAMILIES WITH HYPERTENSION AND OBESITY.

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During the examination of villagers from Eastern Poland 105 school age children coming from 30 families were examined where both parents had hypertension and/or obesity. The aim of the study was to search for family aggregation of insulin resistance syndrome elements. Body weight, height, waist to hip ratio measurements and oral glucose tolerance, blood lipids, fasting serum insulin and microalbuminuria estimations were performed. Diabetes Mellitus (DM) was recognised in 8,8 %, impaired glucose tolerance (IGT) in 15,5% of parents. None of the examined children did not have DM, IGT or hypertension. 36% of the examined had obesity. 45% proved to have low HDL cholesterol level below 1mM/L. In 39% fasting insulin level was above 10 mIU/ml and in 10% - above 20 mIU/ml. In 18% of the offspring we found coexistence of low HDL level and hyperinsulinemia. 7,3% of the had microalbuminuria > 50 µg/ml. Every fifth child from the families with hypertension and obesity in parents showed the symptoms which can be connected with the presence of insulin resistance syndrome. Children from such families should be subject to an early diabetes prevention.

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USEFULLNESS OF A MODIFIED LOW DOSE INSULIN TOLERANCE TEST FOR EVALUATION OF INSULIN SENSITIVITY IN NIDDM.

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Background: The assessment of insulin sensitivity by glucose clamp technique is the reference method, but is unusefullness in clinical practice. The conventional insulin tolerance test (ITT) using an insulin dose of 0.1 unit/kg is a simple and rapid method and correlate with glucose clamp, but may result in hypoglycaemia in patients without insulinresistance. In order to avoid this problem, we describe an ITT technique using a lower dose of insulin on a 3h continuous IV infusion (O.O4 U/kg/h). Since a direct negative feed-back of insulin on βcells was described, T1/2PCP (time necessary to decrease initial plasma C peptide by half) is considered to correlate with insulin resistance (IR). Design and methods: 1) Eleven healthy normal weight control subjects underwent an ITT. 2) Six subjects (three healthy volunteers and three NIDDM patients) were studied with an ITT and a euglycaemic hyperinsulinaemic clamp. With that technique, insulin sensitivity was derived from the average amount of glucose infused at the steady state. 3) ITT was carried out in 88 NIDDM patients. Three tertiles were defined according to the T1/2 PCP: A (<110 mn; n=28), B (110 to 150 mn; n=31) and C (>150 mn; n=29). Clinical and metabolic data were compared in A and C subgroups. Results: 1) Mean T1/2PCP in controls range from 25 to 72 mn. 2) There was a good negative correlation (r=0.88; p<0.05) between insulin resistance derived from the T1/2 PCP and that obtained from the clamp study (glucose disposal rate). 3) In the subgroups A and C of NIDDM patients, there was no difference in age, known duration of diabetes, fasting insulin concentration, urinary C peptide and cholesterol. Body mass index, blood pressure at rest, average glycaemia (based on a 8 points monitoring during 2-3 days), HbA1c, PCP before and 10 mn after 1mg glucagon IV infusion and fasting triglycerides were significantly higher in subgroup C, but there was a consistent overlap of individual values between the two subgroups. Conclusion: 1) The decrease of plasma C peptide during a short insulin tolerance test employing 0.4 units/kg/h insulin correlate with euglycaemic hyperinsulinaemic clamp results in controls and NIDDM patients. 2) T1/2 PCP results are concordant with clinical and biological parameters of insulin resistance in NIDDM patients. This test seems to be a simple and reliable method for assessment of insulin sensitivity in NIDDM.

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DERMAL GLUCOCORTICOID SENSITIVITY IS ASSOCIATED WITH INSULIN RESISTANCE AND RELATED CARDIOVASCULAR RISK FACTORS

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The cluster of cardiovascular risk factors comprising the metabolic syndrome (hypertension, glucose intolerance and dyslipidaemia) resemble the effects of Cushing's syndrome or glucocorticoid administration but cannot be explained by increased circulating levels of glucocorticoids. Recent evidence, however, suggests that in hypertension tissue sensitivity to glucocorticoids may be increased, as judged by more intense dermal vasoconstriction following topical application of glucocorticoids. To determine whether increased glucocorticoid sensitivity might be associated with other components of the metabolic syndrome, we studied 32 men (aged 50-57 years) of whom 8 had impaired glucose tolerance (IGT). Forearm dermal blanching was measured on a visual analogue scale after overnight application of beclomethasone dipropionate. (13μl/cm^2, 0.1-10μg/ml) Increased dermal glucocorticoid sensitivity correlated with higher systolic BP (r=0.34, p=0.05), higher 120min plasma glucose after 75g oral glucose (r=0.54, $p<0.001$), greater insulin resistance as measured by a short insulin tolerance test (r=0.43, p=0.016), increased fasting triglyceride (r=0.36, p=0.04) but not BMI or waist/hip ratio. In regression analyses the associations between glucocorticoid sensitivity and glucose intolerance/insulin resistance were independent of obesity and persisted after exclusion of the subjects with IGT. As dermal vasoconstrictor sensitivity reflects tissue sensitivity to glucocorticoids, thus the present data suggest an increase in tissue sensitivity to glucocorticoids mediates the association between cardiovascular risk factors and insulin resistance.

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Measurement of Insulin Resistance in a Group of Obese Versus Non Obese Costaricans, Usein Harano's Method of Steady State Plasma Glucose

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Background: Insulin resistance (IR) is a phenomenon of great interest, to the point of having the World Health Organisation recommend its study, specifically as to the need of "conducting large population studies to determine the real prevalence of the syndrome by age and sex". In Costa Rica no large estudies of IR have been conducted, since classic methods for its measurement are expensive and time consuming. In this study we applied a simple method of steady state plasma glucose (SSPG) to evaluate IR in a population of obese and non obese patients, both to prove the applicability of the method in our country and to evaluate the effects of obesity on insulin resistance.

Method: The SSPG method of Harano was used, with personal modifications to simplify equipment requirements. After a 12 hour fasting, a solution of ocreotide acetate (Sandostatin$^{(R)}$) (50ug bolus, infusion at 75 ug/h), insulin (7.5 mU/kg bolus, infusion at 0.77 mU/kg/min), glucose (6 mg/kg/min), potassium (0.5 mmol/kg/min), water and blood (25 cc) was infused for two hours, with measurement of blood glucose at 0 and 120 minutes. Glucose level at 120 minutes correlate directly with the degree of IR. The method was applied to 13 patients, 7 with body mass index (BMI)$>$25 (obese), and 6 non obese (BMI$<$25).

Results: No significant difference in initial glucose level was found between the obese and non obese groups (94.929 mg% versus 80.5 mg%, p$>$0.1 respectively), but at 2 hours the difference was clear (244.429 mg% versus 129.5 mg%, p$<$0.0004, respectively).

Conclusions:

1) Obese Costarican subjects have significantly higher levels of insulin resistance than non obese subjects.
2) Harano's SSPG method can be applied with existing equipment in Costarican national hospitals to measure insulin sensivity.

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IMPROVEMENT IN INSULIN SENSITIVITY BUT NOT IN GLUCOSE EFFECTIVENESS FOLLOWING CHANGES IN BODY COMPOSITION.

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In normal subjects insulin sensitivity is related to the body composition with a positively correlation to the relative muscle mass and a negative correlation to the relative fat mass. The purpose of the present study was to evaluate the relation between changes in insulin sensitivity and noninsulin mediated glucose disposal (glucose effectiveness, S_G) and the changes in body composition following weight reduction. 12 obese subjects (11♀, 1♂), age 45.8 $\pm$ 10.5 years, body weight (BW) 99.7 $\pm$ 13.3 kg, BMI 35.3 $\pm$ 2.8 kg/m^2 were examined before and one year after treatment with Orlistat (pancreaslipase inhibitor) for 12 month. At inclusion and two years later an oral glucose tolerance test, a frequently sampled intravenous glucose tolerance test (FSIVGTT) and a DXA whole body scanning was performed. The included subjects obtained quite different changes in weight ranging from a loss of 18.9 kg to a gain of 6.2 kg. Corresponding ranges for changes in lean body mass (LBM) were -4.6 to 0.9 kg, in fat mass (FM) -17.9 to 8.7 kg, relative FM -12.2 to 5.5% , insulin sensitivity (SI) from -2.6 to 2.1 10^{-4}min^{-1}uU^{-1}ml^{-1} and S_G 0 - 0.027 min^{-1}. No significant changes in S_E was observed. A stepwise multiple regression analysis revealed that the strongest predictor for change in SI was delta FM (r=0.81, p$<$0.003), explaining 67% of the variation in SI.

Conclusion: There is a strong correlation between change in insulin sensitivity and change in body weight. Change in total body fat mass is the strongest predictor for change in insulin sensitivity. S_G seems to be independent of changes in body composition.

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THE BREATH TEST USING PURE [1-^{13}C] GLUCOSE : A NEW SIMPLE METHOD FOR EVALUATING THE GLUCOSE OXIDATION CAPACITY.

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Stable isotope of carbon, ^{13}C has been widely used as a tracer of metabolic analyses, and breath tests using the isotope have become valuable clinical tests, in which ^{13}C-labeled compounds are administered and the the ^{13}C/^{12}C ratio(δ^{13}C) of the expired CO_2 is measured. The aim of this study was to examine the glucose oxidation capacity in non insulin dependent diabetes mellitus (NIDDM) patients using [1-^{13}C] glucose. After an overnight fast, aqueous solution of 75g of usual glucose and 1g of pure [1-^{13}C] glucose was administered orally and the δ^{13}C was measured in the NIDDM patients and the controls at 1, 2, 3, 4, and 5 hrs using gaschromatography-mass spectrometer. The glucose oxidation capacity was impaired in the NIDDM group compared with the control group. The delta over the baseline (Δ^{13}C) of δ^{13}C in the expired CO_2 at 3hrs after the load was significantly lower in the NIDDM group (37.21±8.13, n=9) than the control group(50.73±7.76, n=4) (p=0.017). The glucose oxidation capacity was also measured under the insulin-nonstimulating condition, wherein only 1g of [1-^{13}C] glucose dissolved in saline was ingested, and the δ^{13}C in the breath CO_2 was determined in the 2 groups. The Δ^{13}C at 2hrs was 51.53±16.17 in the NIDDM patients(n=11) and 76.54±16.58 in the controls(n=4) (p=0.02), suggesting that the glucose oxidation capacity at the basal state was also impaired in the NIDDM compared with the controls. We also examined the glucose oxidation in the NIDDM patients before and after treatments using 1g of [1-^{13}C] glucose. The Δ^{13}C in the breath CO_2 at 2hrs was 46.97±13.99 before treatments(n=9) and 58.59±9.83 after treatments(n=6). In conclusion, the breath test using [1-^{13}C] glucose is a simple and useful method for evaluating the glucose oxidation capacity. The present results suggest that the glucose oxidation capacity is impaired in NIDDM under both endogenous insulin-stimulating and nonstimulating conditions. The impairment of glucose oxidation at basal state may be partly reversible by treatments of hyperglycemia.

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NO EVIDENCE OF SEASONAL VARIATION IN INSULIN SENSITIVITY IN YOUNG ADULTS

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Insulin resistance is of pathogenetic importance for the development of NIDDM and essential hypertension. Not much is known of the variation in insulin sensitivity in the individual over longer periods. We measured insulin sensitivity (Si) and glucose effectiveness (Sg) in healthy male adults (24.2±1.8 y, mean±SD) (N=10) five times over a period of 15 months. Data from a FSIVGTT (minimal model), indirect calorimetry, BMI, maximal aerobic capacity (V_{O_2}-max), 24-hour blood pressure and fat free mass (FFM) was measured. ANOVA was used to detect differences between examinations and linear regression analysis to examine the slope of individual Si and Sg for every patient. Slopes were compared by T-test. Regression coefficients for Si and Sg for every single person did not differ from zero (Si: p=0.7; Sg: p=0.3). The coefficient of variation (CV) for Si was 24.0% and the fractional SD was 3.7% (SD: 2.51), whereas the CV for Sg was 26.0% and the fractional SD was 16.0% (SD: 6.4). The seasonal pattern in presentation of NIDDM can be described as following a sinus curve. Applying a very flexible multiple linear regression model to explain any kind of seasonal variation in S_I and S_G did not reveal any trends in variation. The model, expected to follow a sinus curve, was without restrictions as to when the insulin sensitivity might be highest or lowest and allowed for different levels of insulin sensitivity (or glucose effectiveness) between individuals and different phases, e.g. individuals could have high and low insulin sensitivity at different times of the year. The model also allowed us to test the impact of covariates, e.g. the additional variables measured in the study. Si (p=0.38), Sg (p=0.71), fasting insulin (p=0.98), maximal aerobic capacity (p=0.13), BMI (p=0.64), energy expenditure (p=0.19), 24-hour blood pressure (systolic: p=0.37 and diastolic: p=0.48) and fat free mass (p=0.92) were constant over time. In conclusion we observed no significant seasonal variation in insulin sensitivity and glucose effectiveness in healthy sedentary young men. Therefore indices of these two variables obtained at different times of the year, appear to be comparable.

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COMPARISON OF EUGLYCAEMIC CLAMP AND LOW-DOSE INSULIN INFUSION IN ASSESSING INSULIN SENSITIVITY IN NIDDM PATIENTS.

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The hyperinsulinaemic euglycaemic clamp is widely used as the gold standard in the assessment of insulin sensitivity. However, although it provides a good index of insulin-mediated glucose-uptake, lipolysis is completed inhibited at much lower concentrations of insulin than are commonly obtained during the clamp. The low-dose insulin infusion, which achieves physiological insulinaemia, allows more detailed assessment of NEFA metabolism. The aim of this study was to compare clamp measures of insulin sensitivity (M values) with those obtained during low-dose insulin infusion. 13 men with NIDDM (age 56.2 ± 2.4 (mean ± SEM); BMI 28.7 ± 0.8 kg m^{-2}), treated with diet alone were studied. During the clamp insulin was infused at 2 mU kg^{-1} min^{-1} for 180 min, whilst during the low-dose protocol insulin was infused sequentially at 0.005, 0.01 and 0.05 U kg^{-1} min^{-1} for 60 min at each dose. Insulin concentrations during the clamps were 183 ± 9 mU L^{-1}, whilst during low-dose infusion they were 15.9 ± 2.0, 22.1 ± 2.7 and 62.8 ± 4.5 mU L^{-1} respectively. Blood glucose and plasma NEFA during the low-dose insulin infusion were plotted against log plasma insulin, and the glucose and NEFA concentrations at an insulin level of 30 mU L^{-1} were calculated and used as measures of insulin sensitivity (I_{30}). Mean M value was 33.4 ± 2.2 μmol kg BW^{-1} min^{-1}. The correlation between clamp M values and insulin infusion I_{30} values for glucose was 0.64 ($p < 0.05$). There was no correlation between either of these indices of glucose metabolism and the I_{30} values for NEFA. The low-dose insulin infusion demonstrates that insulin sensitivity, as assessed by measures of glucose metabolism, may not equate with other actions of insulin such as inhibition of lipolysis.

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CHRONIC CIGARETTE SMOKING AND INSULIN RESISTANCE IN PATIENTS WITH NIDDM.

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Aim of this study was to evaluate the effects of chronic cigarette smoking on insulin sensitivity in patients with NIDDM. We examined 28 smokers and 12 nonsmokers with NIDDM. The two groups were comparable for sex, age, body mass index, waist/hip ratio, alcohol consumption, degree of physical activity, glycometabolic control as well as diabetes duration and treatment. Plasma glucose, insulin and C-peptide concentrations following an oral glucose load were measured. Total, non-oxidative and oxidative rates of insulin-mediated glucose disposal were assessed by using a 4 hour euglycemic hyperinsulinemic clamp (20 mU/min·m^2 body surface area) in combination with [^{3}H]-3-D-glucose infusion and indirect calorimetry. Insulin and C-peptide responses to oral glucose load were significantly higher in smokers than in nonsmokers, while glucose levels were not substantially different. During insulin clamp total glucose disposal was markedly reduced in smokers than in nonsmokers (19±1.2 vs 33±5 μmol/min·kg fat free mass; p<0.001), in a dose-dependent manner (F=6.8, p<0.001 by ANOVA when subjects were categorized for number of cigarettes smoked per day). Oxidative (9±1 vs 14±2 μmol/min·kg fat free mass; p<0.01) and non-oxidative (10±1 vs 19±4 μmol/min·kg fat free mass; p<0.01) pathways of insulin-mediated intracellular glucose metabolism were similarly reduced in smokers vs nonsmokers. Plasma free fatty acid levels (240±33 vs 130±23 μEq/L; p<0.05) and lipid oxidation rate (1.39±0.1 vs 0.95±0.2 μmol/min·kg fat free mass; p<0.05) were less suppressed by hyperinsulinemia in smokers than nonsmokers. In conclusion, chronic cigarette smoking seems to markedly aggravate insulin resistance in patients with NIDDM. Smoking cessation might favourably affect cardiovascular risk and long-term glycometabolic control through an improvement of insulin sensitivity in NIDDM.

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Insulin Sensitivity in NIDDM: Insulin Modified Intravenous Glucose Tolerance vs Isoglycemic Hyperinsulinemic Clamp

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This study compares estimates of insulin sensitivity derived from minimal modelling (MINMOD) of a 4-h insulin-modified frequent sampled intravenous glucose tolerance test (FSIVGTT) with the isoglycemic glucose 'clamp' in subjects with NIDDM, also assessing the reproducibility of the FSIVGTT Twelve men aged 59 ±2.6 years, time from diagnosis 6.3±0.6 years, BMI 28 1±1 0 kg/m^2 (mean±SE) after a 12-hour overnight fast, underwent a FSIVGTT (300 mg/kg glucose at 0 min; 0.05 U/kg insulin at 20 min) and an isoglycemic hyperinsulinemic clamp (40 mU.$min^{-1}m^{-2}$) in random order 2-4 weeks apart. Reproducibility of the FSIVGTT was tested in four patients carried out on three separate occasions. FSIVGTT data were assessed by means of standard MINMOD and both univariate and multivariate techniques. The sensitivity index for the FSIVGTT ranged from 0.162 to 3.292 (mean 1.378) $*10^{-4}$*mL/(min*μU) for the standard approach and from 0.163 to 2.727 (mean 1.180) $*10^{-4}$*mL/(min*μU)] for the multivariate analysis. For SI_{clamp}, the minimal value was 3.06, the maximum 10.86 and the mean 6.19 $*10^{-4}$ $*L^{-2}$/(min *pmol). The correlation of the insulin sensitivity indices between the clamp and the FSIVGTT was 0.51 (p=0.056, n.s.) for the univariate and 0.67 (p=0.017) for the multivariate analyses. Repeated FSIVGTTs showed a lower variability for the multivariate than for the standard approach.

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SMOKING AND MUSCLE BLOOD FLOW AND GLUCOSE UPTAKE ASSESSED BY POSITRON EMISSION TOMOGRAPHY

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Previous studies have suggested that smoking is associated with decreased insulin sensitivity. We hypothetized that this might be caused by decreased muscle blood flow. We studied 22 healthy non-diabetic men, eight smokers and 16 non-smokers. Mean age was 37 years and BMI 24.8 kg/m^2 in both groups, VO_2 max was 35±9 mL/kg/min in smokers and 42±8 mL/kg/min in non-smokers (p=0.11). Basal and insulin-stimulated femoral muscle blood flow was measured using [^{15}O]-water and glucose uptake using [^{18}F]-fluorodeoxyglucose with positron emission tomography. Whole body glucose uptake was assessed using the hyperinsulinemic (5 mU/kg/min) euglycemic clamp technique. Fasting plasma insulin was 9.4±3.4 mU/L in smokers and 6.3±2.4 mU/L in non-smokers (p=0.016). In the basal state, muscle blood flow was 50% lower in smokers (18±4 mL/kg/min) than in non-smokers (36±20 mL/kg/min; p=0.006). Respective insulin-stimulated values were 30±10 and 53±36 mL/kg/min (p=0.038). There was no difference between smokers and non-smokers in whole body glucose uptake (49±13 vs 48±10 μmol/kg/min) or in femoral glucose uptake (116±32 vs 106±39 μmol/kg/min). We conclude that differences in skeletal muscle blood flow between smokers and non-smokers are not reflected in differences in insulin sensitivity. Our finding does not support the idea that muscle blood flow is a major determinant of insulin sensitivity.

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ASSESSING GLUCOSE EFFECTIVENESS WITH THE MINIMAL MODEL: INSIGHTS FROM AN IVGTT AT BASAL INSULIN

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The minimal model estimate of glucose effectiveness (S_G) is affected by an error that is related with the monocompartmental approximation of the model and is dependent on the insulin profile during the IVGTT. It is commonly believed that S_G can be accurately estimated by maintaining insulin at the basal level during the test. However, a recent theoretical analysis by our group, showing that glucose decay at basal insulin is two-exponential (and not monoexponential as predicted by the minimal model), casts doubts on S_G validity even under such optimized conditions. In this study we assessed the validity of S_G estimated from an IVGTT at basal insulin against a model-independent measure of glucose effectiveness. For this purpose we analyzed IVGTT studies in 12 NIDDM subjects who showed no incremental insulin response to the glucose bolus. We found that glucose decay was two-exponential (with fast and slow time constants equal to 0.2 and 0.01 min^{-1}, respectively) and that the model well-described the glucose data only from 20 min onwards, i.e. when the contribution of the fast time constant became negligible. When the model was identified ignoring the glucose data collected in the first 20 min of the test, S_G resulted similar to the slow time constant of the two-exponential decay in each subject. S_G validity was tested against a model-independent measure of glucose effectiveness (GE). GE was derived from the area under the glycaemic excursion and was mathematically shown to be equivalent to the clamp measure of glucose effectiveness. To allow a comparison with GE (ml/kg min), S_G (min^{-1}) was multiplied by the minimal model volume (V, ml/kg). S_GV and GE resulted similar (3.5±0.6 vs 3.4±0.6 ml/kg min) and highly correlated (r=0.98). In conclusion, although the minimal model suffers from the monocompartmental approximation, S_GV estimated from an IVGTT at basal insulin is equivalent to a model-independent measure of glucose effectiveness directly derived from the glucose curve.

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PARADOXICAL ASSOCIATIONS BETWEEN SMOKING AND GLUCOSE METABOLISM

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We studied the relationship between smoking and glucose metabolism in non-diabetic subjects. Glucose tolerance was assessed in a general population sample of 2,484 subjects, aged 50-74, with an oral glucose tolerance test. Subjects with diabetes (n=200, WHO-criteria) were excluded. Multiple linear regression analyses were performed using fasting- (FPG) and post-load plasma glucose (2h-PG) levels and HbA_{1c} as dependent variables. Smoking and ex-smoking were used as independent variables and adjusted for age, gender, BMI, waist to hip ratio, triglycerides, HDL-cholesterol, hypertension, alcohol consumption, fasting specific insulin and plasma glucose levels. After adjustment, there was no association between smoking and fasting plasma glucose. Smokers, as well as ex-smokers, had a lower post-load plasma glucose and smokers had a higher HbA_{1c}. Thus, while smokers had a similar fasting plasma glucose and a lower post-load glucose, they had a higher HbA_{1c}. Increased glycosylation caused by smoking seems to be the most logical explanation. Apparently, smoking dissociates glucose levels and levels of glycosylation. The underlying mechanism remains to be established.

Table — Glucose and HbA_{1c} according to smoking category.

	non-smokers n = 756	ex-smokers n = 770	smokers n = 730
FPG (mmol/l)	5.4 (0 5)	5.5 (0 6)*	5.5 (0.6) *
2h-PG (mmol/l)	5.8 (1.6)	5.6 (1.7)*	5.3 (1.6) *
β		-0.2 (-0.3 – 0.0)*	-0.5 (-0.6 – -0.3)*
HbA_{1c} (%)	5.3 (0.5)	5.3 (0.5)	5.5 (0.5) *
β		0.0 (0.0 – 0.1)	0.2 (0.2 – 0.3)*

Data are non-adjusted mean values (SD), and adjusted regression coefficients β (95% Confidence Interval)

*) $p < 0.05$ vs non-smokers.

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ESTIMATING INSULIN SENSITIVITY FROM ORAL GLUCOSE TOLERANCE TEST

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A kinetic model of glucose distribution and metabolism was used to explore whether the plasma glucose and insulin measurements following a 75 g oral glucose tolerance test (OGTT) can provide a useful measure of insulin sensitivity. The glucose kinetic model is non-linear incorporating glucose transporters as a mechanism for glucose uptake such that glucose disappearance rate becomes saturable. The glucose entry to the model assumes that 50% of the ingested glucose will appear in the systemic circulation within two hours as an output flux of a cascade of three compartments. During the OGTT the increment in plasma insulin concentration above basal acting from a remote compartment controls the number of available glucose transporters and the rate of glucose uptake while reducing hepatic glucose production rate from its basal steady state value. Mean values of plasma glucose and insulin concentrations were taken from published OGTTs in three groups of subjects. A controls group, a group with impaired glucose tolerance (IGT) and a group with NIDDM. Simulation of these results indicates an increase in glucose production rate in NIDDM (14.43 μmol/min/kg) compared to the value in controls (9.06 μmol/min/kg) and (10.12 μmol/min/kg) in IGT. During the OGTT only the controls and IGT groups increased the number of functioning glucose transporters and the rate of glucose uptake by (60% and 60%) and (30% and 30%) respectively. Basal glucose production rate was reduced by 80% in the controls and by 60% and 40% in IGT and NIDDM groups. The insulin sensitivity defined as the ratio of the change in glucose utilisation rate (maximum value minus basal) to the increase in plasma insulin concentration from basal value were 0.37, 0.16 and 0.16 in the controls, NIDDM and IGT groups respectively.

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SMOKING IS ASSOCIATED WITH HIGHER INSULIN LEVELS AND LOWER SEX HORMONE BINDING GLOBULIN IN NIDDM MALE PATIENTS
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Both insulin resistance and normal insulin action have been reported in smokers.In view of the great importance of insulin resistance in the pathogenesis of NIDDM,we investigated the concentrations of fasting plasma insulin (IRI) and sex hormone binding globulin (SHBG),both indices of insulin action,in 95 male NIDDM patients (mean age 62.06,range 30-75 years),on treatment either with diet or/and oral hypoglycemic agents.In the study group 68 patients neither smoked nor had been smokers in the past 10 years whereas 27 consumed at the time of the investigation up to 20 cigarettes/day (range 8-20) for more than 5 years.We applied multiple analysis of variance to detect differences between means and the LSD test for post-hoc comparisons.The groups did not differ significantly in the degree of glycaemic control,as assessed by fasting glucose and HbA1c levels nor in the body mass index.The latter,as well age and duration of the disease,were entered as covariates in the statistical model.It was found that IRI (Mean±SEM) was higher in the smokers group (109.56±11.2 versus 86.8±8.06 pmol/L),p=0.05,whereas SHBG was significantly lower(33.6±5.06 versus 41.8±6.84 nmol/L),p=0.03.No difference was detected in total,HDL cholesterol and triglycerides levels.It is concluded that chronic cigarette smoking may exacerbate the inherent insulin resistance characteristic of the diabetic syndrome as shown by the increased fasting insulin and low SHBG concentrations detected in our study group.

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VALIDATED MEASURE OF GLUCOSE EFFECTIVENESS AND ITS METABOLIC COMPONENTS FROM I.V. GLUCOSE INJECTION.
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Glucose effectiveness (GE) is a major determinant of glucose tolerance, and is particularly important to the determination of tolerance in the prediabetic state. To quantify GE, and its components of glucose-mediated stimulation of glucose uptake ($GE_{(uptake)}$) and suppression of hepatic glucose output ($GE_{(HGO)}$), we injected cold glucose (0.3 g/kg), into which 3-^{3}H-glucose (1.2 μCi/kg) was added (n=8 dogs). The dynamic insulin response was prevented by somatostatin, and basal hormones were replaced. After rising with injection, glucose fell towards basal, with a $t_{½}$ of 28±4 min (K_G = 0.88 min^{-1}); tracer decline was more rapid ($t_{½}$ = 20±1 min; p=0.047). Cold and hot data were analyzed with a validated 2-compartment model of glucose kinetics: compartment 1 reflects the plasma pool, from which R_d is considered independent of insulin; compartment 2 is the remote, interstitial space, the primary site of insulin-stimulated R_d. Total GE, expressed as a fraction of the plasma glucose pool, averaged 0.0298±0.0022 min^{-1} (range: 0.0202 to 0.0409). Glucose's action on R_d ($GE_{(uptake)}$) dominated overall GE, averaging 0.0206±0.0014 min^{-1} (71.6±6.1% of total); $GE_{(HGO)}$ was 0.0092±0.0024 min^{-1} (28.4±6.1% of total). Model-based GE estimates were confirmed by independent measures in the same dogs obtained from hyperglycemic clamps at basal insulin (p>0.38 by paired t-test, after all measures converted to similar units of dl/min). These data indicate that glucose-stimulated R_d, not suppression of HGO, dominates glucose effectiveness. Also, similar values are found from dynamic and steady-state protocols. Glucose effectiveness is a major component of glucose tolerance which can be accurately measured. Its overall role in the pathogenesis of NIDDM remains to be elucidated.

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INSULIN RESISTANCE IN FAMILIAL COMBINED HYPERLIPIDEMIA.
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Goals. To study the insulin sensitivity index (Si), using the Minimal model method, and its relationship with central obesity in 31 non-smokers, normotensive Familial Combined Hyperlipidemia (FCH) male subjects. *Subjects*: 31 FCH males, mean age 48.2±9.6 y., with central obesity (WHR ≥0.95; n=23) were compared with 20 control subjects of similar age, gender and body weight. Secondary hyperlipidemia was ruled out and plasma TC, TG, HDL-C, Apo B, FFA, glucose and insulin at baseline and during OGTT (75 g glucose) were measured. Intravenous glucose tolerance test (Minimal model method) modified with insulin was performed. *Results:* The Si index was 1.65±0.96 $x10^{-4}$ min^{-1} mU/l in FCH subjects and 2.86±1.22 $x10^{-4}$ min^{-1} mU/l in controls (p<0.01). In the 8 FCH subjects with WHR <0.95 Si was 2.66±0.96 $x10^{-4}$ min^{-1} mU/l and AUC glucose 858±99 pmol/l/min, significantly different (p<0.01) from FCH with WHR ≥0.95 and Si 1.30±0.68 $x10^{-4}$ min^{-1} mU/l and AUC glucose 1203±201 pmol/l/min. In this group, fasting and 120 min plasma glucose, triglycerides and 120 min plasma insulin were significantly higher (p<0.01) than in FCH with WHR <0.95. In all FCH subjects the Si index correlated negatively with the BMI, WHR, FFA and triglyceride concentrations. *Conclusions:* Insulin resistance (expressed as low Si values) is a frequent finding in patients with FCH, with or without abnormal glucose tolerance. Central obesity exacerbates the hyperglycemia, hyperinsulinemia, and hypertriglyceridemia. These abnormalities could predispose to the elevated cardiovascular risk present in FCH subjects.

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COMPARISON OF INSULIN TOLERANCE TEST WITH VARIOUS SIMPLE AND SAFE METHODS IN THE PREDICTION OF INSULIN SENSITIVITY.
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In the measurement of insulin sensitivity, intravenous insulin tolerance test (IVITT) has been shown to have a high correlation with the euglycemic hyperinsulinemic clamp method. We compared IVITT with levels of plasma Insulin/Glucose ratio (IGR), levels of plasma insulin/C-peptid ratio (ICR), fasting and 1-h postload insulin levels in 58 nondiabetic obese subjects (Mean BMI :28.9±3.4 kg/m2, Age: 42.8±8.5 yrs). The glucose levels were determined at 3 min intervals for 15 min after insulin injection. Insulin sensitivity was estimated with blood glucose disappearance rate (KITT). KITT 3.52±2.38%.min-1; fasting IGR 35.2±25.8 pM/mM;1-h postload IGR 138.5±74 pM/mM; fasting ICR 0.14±0.13 pM; 1-h postload ICR 0.25±0.15 pM, fasting and 1-h post load insulin levels were 124.2±48, 882±364 pM, respectively. KITT correlated only with fasting insulin level (r=0.52, p<0.05) and fasting IGR (r=-0.47, p<0.05). Fasting insulin level correlated with 1-h postload insulin level (r=0.58, p<0.05) Fasting IGR correlated with 1-h postload IGR (r=0.55, p<0.05) and fasting ICR (r=0.65, p=0.003) Fasting ICR correlated with 1-h postload ICR (r=0.67,p<0.001). As a conclusion, fasting IGR, in terms of having a good correlation with IVITT, is shown to be a simple and safe method in the measurement of insulin resistance in obesity in epidemiological studies.

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LONG-TERM AMYLOPECTIN FEEDING CAUSES TISSUE-SPECIFIC CHANGES IN INSULIN RESPONSIVENESS

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Dietary starch consists of a mixture of amylose (AMOSE) and amylopectin (AMPEC). AMOSE is more slowly digested and absorbed than AMPEC. We have previously shown that long-term AMPEC feeding causes whole body insulin resistance with respect to AMOSE feeding. The present study was conducted in order to define the tissue-specific effects of long-term AMOSE- or AMPEC-feeding and thereby identify the primary lesion responsible for the onset of insulin resistance in the AMPEC-fed rat. Male Wistar rats were maintained on AMOSE or AMPEC diets for a period of 26 weeks, at which time hyperinsulinemic-euglycemic clamps were performed at either basal (50 μU/ml) or mid-physiological (150 μU/ml) plasma insulin concentrations. Immediately following sacrifice, the liver, soleus muscle and adipose tissues were excised and frozen until assayed for the rate of glucose uptake, glycogenesis and lipogenesis. At physiological plasma insulin concentrations, glucose metabolic flux was significantly greater in AMOSE-fed than AMPEC-fed animals in all tissues but brown adipose tissue (BAT). In particular, glycogenesis was impaired in AMPEC animals relative to AMOSE animals. For example, the rate of glycogenesis in liver from AMOSE-fed animals (117 ± 10 nmol glucose incorporated/min/g) was double that observed in AMPEC-fed rats (54 ± 8 nmol glucose incorporated/min/g; $p < 0.01$). Despite this extensive decrease in tissue-specific insulin responsiveness, AMPEC-fed animals displayed no impairment in whole body glucose disposal due to compensatory glucose flux through lipogenesis in BAT. The rate of lipogenesis in BAT from AMPEC-fed rats (443 ± 77 nmol glucose incorporated/min/g) was more than double that from AMOSE-fed animals (203 ± 34 nmol glucose incorporated/min/g; $p < 0.05$). These tissue-specific changes in glucose metabolic flux may be indicative of a 'pre-insulin resistant' state similar to that which occurs during the onset of obesity.

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CONSEQUENCES OF UNDERMODELING ON GLUCOSE EFFECTIVENESS AND INSULIN SENSITIVITY FROM THE MINIMAL MODELS ASSESSED BY MONTE CARLO SIMULATION.

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The cold (CMM) and hot (HMM) minimal models of the IVGTT are a powerful tool to investigate glucose metabolism in vivo. They allow to estimate, from glucose, tracer glucose and insulin data, metabolic indices of the glucose-insulin system, namely glucose effectiveness (GE) and insulin sensitivity (IS) (of peripheral uptake & endogenous production those of the CMM, S_G and S_I, of peripheral uptake only those of the HMM, S_G^* and S_I^*). Recently, the MM single compartment approximation of glucose kinetics has been questioned on a theoretical basis. Here, its consequences are investigated via Monte Carlo (MC) simulation, by using a physiologically based two compartment model of the glucose-insulin system as a reference model (RM). The RM allows to generate a high number of noisy synthetic data sets of glucose, tracer glucose and insulin plasma concentrations during an IVGTT, which are then analyzed with the CMM and the HMM. The CMM and HMM indices are then compared with the "true" ones from MC simulation. Results (mean of 400 runs) show that: A) correlation of S_G=1.99 10^{-2} min^{-1} with the RM GE=1.33 10^{-2} min^{-1} is weak (r=0.54), B) S_I=2.39 10^{-4} min^{-1} per μU ml^{-1} is well correlated with the RM IS=6.46 10^{-4} min^{-1} per μU ml^{-1} (r=0.92), but severely underestimates it, C) S_G^*=1.01 10^{-2} min^{-1} is correlated (r=0.68) with the RM clearance rate FCR=1.59 10^{-2} min^{-1}, but not with the RM GE GE^*=0.89 10^{-2} min^{-1} (r=0.34), D) HMM insulin sensitivity S_I^*=3.66 10^{-4} min^{-1} per μU ml^{-1} is well correlated (r=0.91) with the RM index IS^*=5.02 10^{-4} min^{-1} per μU ml^{-1}. Correlations and comparison of means improve, for the HMM indexes only, if a volume of distribution is used. These results show that S_G is the parameter most affected by the single compartment approximation and that the indices of HMM are much more robust than those of the CMM.

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Insulin Resistance in Diabetes, Pathogenesis of NIDDM

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TIME-DEPENDENT REGULATION OF INSULIN RECEPTORS, GLUT4, HEXOKINASE II, AND GLYCOGEN SYNTHASE BY INSULIN IN RAT MUSCLES

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Our aim was to study the time-course of insulin-dependent regulation and expression of the insulin receptor (IR), insulin dependent glucose transporter (GLUT4), hexokinase II (HK II), and glycogen synthase (GS) in skeletal muscle of rats. Conscious rats were exposed to euglycemic (6 mM) hyperinsulinemia (100 mU/l) for 0, 0.5, 2, 4, 8, or 12 hours, and subsequently soleus, gastrocnemius, and rectus muscles were freeze clamped and studied in vitro. Insulin receptor number and affinity for insulin was unaffected by in vivo insulin exposure, and receptor number correlated with basal glucose uptake ($r=0.9$, $p<0.025$). IR tyrosine kinase activity (IRTK), however, was increased almost twofold ($p<0.01$) after in vivo insulin stimulation (0.5–12 h), and correlated with insulin levels during the clamp ($r=0.4$, $p<0.01$). A transient, muscle-type dependent reduction (4 and 8 h, $p<0.05$) of GLUT4 and no change in GS protein levels were found. GS activity in both soleus and gastrocnemius muscles showed a transient increase (0.5 h, $p<0.01$) in fractional velocity with no change in Vmax. This was accompanied by a transient increase of GLUT4, GS, and HK II mRNA (0.5 h, $p<0.1$) after in vivo insulin exposure. Insulin levels during the clamp correlated with GLUT4 protein in gastrocnemius muscles ($r=0.4$, $p<0.05$) and with GLUT4 mRNA in soleus muscles ($r=0.67$, $p<0.005$). These data indicate that primary insulin receptor signalling is unaffected by prolonged in vivo insulin stimulation in muscle, whereas the insulin response can be modified at the post-receptor level by transient effects of insulin on mRNA or protein levels and enzyme activities in certain effector systems.

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AGGRAVATING EFFECT OF FATTY MUSCLE AND FATTY LIVER ON HYPERINSULINEMIA IN PATIENTS WITH NIDDM

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Recently, triglyceride deposition in skeletal muscle has been reported to be associated with insulin resistance in obese patients. In the present study, we examined the effect of triglyceride deposition in muscle and liver, which is the major target organ of insulin, on plasma insulin level in patients with NIDDM. For 37 NIDDM patients (BMI 24.28±11.13kg/m^2, 21 men, 16 women), the abdominal CT was performed to evaluate mean hounsfield unit of psoas muscle (PmCT) of the umbilical region, liver (LmCT) and spleen (SmCT). We evaluated fatty muscle and fatty liver by the ratio of PmCT/SmCT and LmCT/SmCT respectively. Visceral fat area (V), subcutaneous fat area (S) and V/S ratio were measured by the abdominal CT of the umbilical region. Furthermore, we evaluated insulin response by the oral glucose tolerance test. Fasting plasma insulin level (FIRI) inversely correlated significantly with PmCT/SmCT ratio and LmCT/SmCT ratio ($r=-0.59$; $r=-0.66$, $p<0.05$, respectively). There were no significant relationship between V/S ratio and FIRI. In 16 female cases, FIRI inversely correlated significantly with PmCT/SmCT ratio and LmCT/SmCT ratio ($r=-0.75$; $r=-0.83$, $p<0.05$, respectively). In conclusion, not only fatty muscle but also fatty liver may contribute to fasting hyperinsulinemia in patients with NIDDM.

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GLUT 4 EXPRESSION IS MUSCLE GROUP SPECIFIC IN TYPE II DIABETES MELLITUS. D.S. Hardin and J. Karczewski. University of Texas, Houston,TX USA.

Significance: We have previously described muscle group specificity of GLUT 4 expression in rats and in human controls and athletes. However, to date, muscle group specificity of GLUT 4 expression in human diabetics has not been evaluated. Such specificity could have ramifications for the interpretation of data regarding the relationship of GLUT 4 and insulin sensitivity in humans. We hypothesized that expression of GLUT 4 is muscle-group specific in human diabetics. Methods: In order to test our hypothesis, 9 non-insulin dependent diabetes mellitus subjects, "NIDDM" (ages:39-53;HbA1c=5.9-11.4;BMI=21-52) underwent percutaneous biopsies of 3 separate muscle groups: vastus lateralis (V), gastrocnemius (G) and biceps (B). Each muscle sample was snap-frozen in liquid nitrogen for future quantification of GLUT 4 content. GLUT 4 was isolated by SDS-PAGE and immunoblot analysis and was quantified with I^{125}-labelled anti-rat GLUT 4 antibody and radionucleotide counting. Results: Muscle-group specific expression of GLUT 4 was determined by calculating a percentile for each muscle group (% of total gamma counts relative to the total counts in all three muscle groups) in each individual subject. Results for all patients reveal significant and consistent differences between expression of GLUT 4 in the leg muscles (G=40±9%, V=33±7%, and B=34±6%; $p<0.05$ between V and the other two muscle groups). Also noted was a positive correlation between BMI and GLUT 4 content in all muscle groups (G:r=0.64,V:r=0.71,B:r=0.75). A negative correlation was noted for HbA1c with biceps (r=-.65) and vastus (r= -.61); however this relationship was not observed in gastrocnemius (r=-.29). Summary: 1) Muscle-group specific expression of GLUT 4 was observed in these NIDDM subjects; 2) GLUT 4 expression was significantly lower in V than in G or B; 3) Positive correlation was observed between GLUT 4 and body mass index in all muscle groups; 4) negative correlation was observed between GLUT 4 content and HbA1c in V and B. Speculation: In humans, V is the most commonly sampled muscle group for determining the role of GLUT 4 in mediating insulin resistance. Perhaps additional muscle groups should be studied before dismissing down-regulation of GLUT 4 expression as a mechanism of human insulin resistance.

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EFFECT OF MATERNAL DIABETES ON BETA CELL FUNCTION AND INSULIN SENSITIVITY IN NON DIABETIC OFFSPRINGS.

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The aim of our study was to analyze the effect of paternal and maternal antecedents of NIDDM on beta cell function and insulin resistance parameters in non-diabetic offsprings of NIDDM. Ninety-nine non-diabetic first-degree offsprings: 31 with a NIDDM father (Group A) and 68 with a NIDDM mother (Group B) were studied. We measured total and HDL-cholesterol, triglycerides, fasting serum glucose, insulin and C-peptide. We performed a 5 mg/kg ideal weight continous infusion of glucose (CIGMA) to evaluate beta cell function and insulin sensitivity from achieved values of glucose, C-peptide and insulin respectively. The groups were matched by age, sex and BMI. Group B have higher waist/hip ratio (0.93 ± 0.08 vs 0.87 ± 0.08, p = 0.007), higher fasting (96.1 ± 54.9 vs 77.6 ± 20.5 pmol/l, p = 0.01) and achieved (243.0 ± 142.1 vs 195.6 ± 77.5 pmol/l, p= 0.03) insulin levels than Group A. Blood pressure, triglycerides, cholesterol, fasting and achieved serum glucose, C-peptide, beta cell function and insulin sensitivity were not different. We concluded that offsprings of NIDDM mothers exhibit higher waist/hip ratio and hyperinsulinemia, factors that are linked to an increase risk for NIDDM.

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EVIDENCE OF AN INCREASED NUMBER OF TYPE IIb MUSCLE FIBRES IN INSULIN RESISTANT RELATIVES OF NIDDM PATIENTS.

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Insulin resistance is a common feature in healthy first-degree relatives of NIDDM patients. To explore the mechanism(s) behind this condition in more detail, a percutaneous muscle biopsy (vastus lateralis) was performed in 25 first-degree relatives of NIDDM patients (R) and 21 matched controls (C) to examine muscle fibre composition and capillary density. Insulin-stimulated glucose uptake (Rd) was measured employing a hyperinsulinaemic (insulin infusion rate 0.6 mU/kg/min) euglycaemic clamp and maximal aerobic work capacity (VO_2max) was determined using a bicycle ergometer test. All had a normal OGTT. Rd (5.76±0.35 vs 8.06±0.36 mg/kg LBW/min, $p<0.001$) and VO_2max (49.3±2.8 vs 57.2±3.5 ml/kg LBW/min, $0.05<p<0.10$) were decreased in R vs C. An increased number of type IIb fibres (29.5±2.5 vs 21.0±2.8 %, $p<0.05$) was observed in R compared to C, whereas no significant differences were found in the other muscle fibre types or the capillary density between the two groups. Correlations were observed between number of type I fibres (positive), number of type IIb fibres (negative) and capillary density (positive) vs Rd (Type I fibres: $r=0.39$, $p<0.01$; type IIb fibres: $r= - 0.51$, $p<0.001$; capillaries/ type 1 fibre: $r=0.44$, $p<0.01$; capillaries/IIb fibre: $r=0.35$, $p<0.05$) as well as VO_2max (Type I fibres: $r=0.47$, $p<0.01$; type IIb fibres: $r= - 0.42$, $p<0.01$; capillaries/type 1 fibre: $r=0.39$, $p<0.05$; capillaries/IIb fibre: $r=0.45$, $p<0.01$). In a multiple linear regression analysis with Rd as the dependent variable, VO_2max ($p<0.001$), family history of NIDDM and number of type IIb fibres ($p<0.05$) significantly determined the level of Rd ($r^2=0.64$), whereas capillary density did not. In conclusion, insulin resistant first-degree relatives of NIDDM patients are characterized by an increased number of type IIb muscle fibres. Wether this finding reflects a reduced physical activity level and fitness in R or is of primary genetic origin remains to be determined.

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FACTORS WHICH DETERMINE THE WORSENING OF INTRAVENOUS GLUCOSE TOLERANCE IN THE OFFSPRING OF PATIENTS WITH NIDDM

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Objective. In order to evaluate the factors which determine the worsening of intravenous glucose tolerance in subjetcs at high risk for developing NIDDM 15 glucose-tolerant offspring of NIDDM patients and 21 control subjects were studied. **Research design and methods**. Each subject underwent a frequently sampled intravenous glucose tolerance [FSIGT] test. K_G index was calculated between 10 and 40 min of an FSIGT test. Insulin sensitivity [S_I], glucose effectiveness at zero insulin [GEZI] and first and second phases of insulin responsiveness [Φ_1 and Φ_2] were estimated using glucose and insulin kinetics minimal models. The acute insulin response to glucose [AIRg] was calculated as the area under the insulin curve above basal betwen 0 and 10 min., and the suprabasal insulin effect was determined by the product of S_I times AIRg. **Results.** Offspring showed a lower S_I [14.1 ± 7 5 vs 9.25 ± 4.20 $\times 10^{-5}$ $min^{-1}(pmol \cdot l^{-1})^{-1}$, $p<0.01$] and a similar AIRg [3284 ± 2280 vs 3105 ± 1499 $pmol \cdot l^{-1}$, NS] than controls. Sample division according to median of K_G showed that control subjects with low tolerance had a lower AIRg [4417 ± 2531 vs 2043 ± 1068 $pmol \cdot l^{-1}$, $p<0.05$] and lower suprabasal insulin effect [0.057 ± 0.03 vs 0.023 ± 0.009 min^{-1}, $p<0.05$] than controls with high tolerance. Offspring with low tolerance had a lower AIRg [2574 ± 1197 vs 3707 ± 1656 $pmol \cdot l^{-1}$, $p<0.05$] and a lower GEZI [0.101 ± 0.05 vs 0.212 ± 0.08 $\times 10^{-1}$ min^{-1}, $p<0.05$] than offspring with high tolerance. Offspring with high and low tolerance showed lower Φ_1 [375 ± 155 vs 272 ± 181 vs 698 ± 336 $(pmol \cdot l^{-1})min(mmol \cdot l^{-1})$, NS] than controls with high tolerance. **Conclusions**. Our data suggest that a decrease in GEZI and in AIRg are the main factors responsible for the worsening of intravenous glucose tolerance in offspring of NIDDM patients.

1116

ACUTE AND TISSUE SPECIFIC REGULATION OF GLUTAMINE: FRUCTOSE-6-PHOSPHATE AMIDOTRANSFERASE BY INSULIN IN VIVO

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Previous studies have shown that chronic changes (4 days) in glycemia and insulinemia can regulate glutamine:fructose-6-phosphate amidotransferase (GFA) in human and rat skeletal muscle. We examined whether GFA regulation by insulin exhibits tissue specificity and whether it responds to acute hyperinsulinemia induced by a 2-hour insulin clamp (insulin infusion rate 18 mU/kg'min) in nondiabetic (glucose 6.6±0.5 mmol/l, n=16) and STZ-diabetic (20.1±1.4 mmol/l, n=16) rats. In the nondiabetic rats, 2 hours of hyperinsulinemia increased liver GFA activity by 21 % from 244±16 to 295±14 pmol/mg'min ($p<0.02$). In the diabetic rats, both basal (182±17, $p<0.01$) and insulin-stimulated (241±10, $p<0.01$) GFA activities were significantly lower than in the nondiabetic rats. The insulin induced 32 % increase in GFA was significant also in the STZ rats within the 2 hour period ($p<0.01$). In hindlimb muscle, GFA activities were 50-54 % lower in the diabetic vs nondiabetic rats both basally (18.9±5.5 vs 34.9±4.3, $p<0.02$) and during hyperinsulinemia (21.1±2.3 vs 31.7±4.4, $p<0.05$) but insulin didn't change GFA acutely in either group. In the brain, GFA activities were comparable both basally (80±13 vs 93±6) and during hyperinsulinemia (84±7 vs 83±4, diabetic vs control rats). These data demonstrate that GFA is chronically downregulated by the combination of hyperglycemia and hypoinsulinemia in insulin sensitive tissues such as liver and in skeletal muscle but remains unaltered in the brain, which is insulin insensitive. In the liver, GFA activity is acutely increased by insulin implying that the time course for insulin regulation of GFA is shorter than has been previously thought.

1117

CAFETERIA DIET CAUSES FEATURES OF INSULIN RESISTANCE IN MATERNAL PROTEIN-RESTRICTED RAT OFFSPRING

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The aim of the present study was to investigate whether the interaction of early maternal protein restriction combined with a subsequent cafeteria diet would affect the glucose and lipid metabolism in the offspring. Rats were exposed to either a maternal 20% (C) or an isocaloric 8% (LP) protein diet during fetal and postnatal life. All offspring were weaned on to a normal laboratory chow until 6 weeks, at which time they continued on either the same diet or were fed a cafeteria diet. After 48hrs starvationt, at 12 weeks of age, significantly increased plasma insulin concentrations were seen in LP rats on a cafeteria diet compared to male C rats on the same diet [606 (479-766) vs 462 (392-542) pmol/l, geometric mean (95% CI) of n=10 per group; p=0.03]. This was despite a 16% reduction in body weight in LP rats compared to C rats ($p<0.001$). Nevertheless, in both C and LP rats fed cafetria diet, the blood glucose (6.0 ± 0.1 vs 5.9 ± 0.1 mmol/l, mean ± SEM, respectively), plasma triglyceride [1.82 (1.64-2.23) vs 1.27 (1.00-3.36) mmol/l] and non-esterified fatty acid concentrations [1.28 (1.12-1.46) vs 1.36 (0.97-1.90) mmol/l] were comparable. Furthermore, cafeteria diet did not result in a significant increase in insulin concentrations in the female LP rats compared to C rats, suggesting that some factor(s) associated with gender protected these rats [174 (111-274) vs 221 (180-273) pmol/l, respectively]. Thus, male rats exposed to maternal protein-restricted diet during early development and fed a high fat-high calorie diet in later life were becoming insulin resistant at only 12 weeks of age although this was not sufficient to affect their blood glucose levels or lipid profile.

1118

GLUCOSE TOLERANCE IS RELATED TO LIVER PROTEIN KINASE A ACTIVITY IN NORMAL AND DIABETIC MONKEYS.

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It is well known that impaired glucose tolerance is a hallmark of insulin resistant states, including older age and type 2 diabetes mellitus. We have previously shown that the basal activity of liver protein kinase A (PKA), a serine/threonine kinase, was strongly inversely related to age in a group of monkeys (age between 13 to 29 years) with a wide range of insulin sensitivity. In these same monkeys (4 were normal, 5 were hyperinsulinemic, and 7 had type 2 diabetes) we now report that the basal activity of liver PKA (1.8 to 39.1%) is positively related to glucose tolerance (glucose disappearance rate from 0.6 to 4.3 %/min) as measured during an intravenous glucose tolerance test ($r=0.54$, $p<0.05$). In order to determine whether PKA activity was related to glucose tolerance without age as a confounding variable, basal fasting PKA activity was measured in liver samples from 10 young adult (7 yr old) monkeys. The monkeys ranged in body weight from 7 to 12 kg, in body fat from 4 to 19 %, in fasting plasma glucose from 2.6 to 3.6 mmol/l, in fasting plasma insulin from 162 to 462 pmol/l, in glucose disappearance rate from 2.2 to 5.3 %/min, and in acute (0-10 min) insulin response to intravenous glucose (AIR) from 510 to 1704 pmol/l/min. Basal PKA activities ranged between 2.2 to 21.8 %. PKA activity was strongly positively related to glucose disappearance rate in these young adult monkeys ($r=0.78$, $p<0.005$). We conclude that the impaired glucose tolerance associated with aging may be due in part to a decrease in basal liver PKA activity, and that basal liver PKA activity may determine glucose disappearance rates in lean young adult rhesus monkeys.

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INSULIN SENSITIVITY IN FIRST DEGREE RELATIVES OF NIDDM PATIENTS.

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This Study was designed to predict whom of the first degree relatives of type II Diabetes is Vulnerable to become diabetic.

The Study was conducted on 15 obese (groupI) and 15 non obese (group II) obese first degree relatives of type II diabetics compared with 10 healthy control Subjects of matched age and sex with no family history of diabetes or other endocrinopathies.

Intravenous glucose tolerance test, C peptide levels after intravenous glucagon, glucose decay constant, incremental areas under glucose and insulin curves were determined. Also, first and second phase insulin secretion were estimated and insulin sensitivity (S_I), glucose effectivenes (S_G) were calculated using MINMOOD computer program. Our results revelead only a significant change in insulin senstivity between group I & II (Reduction 40.91, $P< 0.028$) while there was no significant change on comparing between group I & controls & group II & controls.

These results indicate that the obese first degree relatives of NIDDM patients are more suceptible to diabetes than non obese relatives.

1120

IN VIVO INSULIN INCREASES THE ACTIVITY OF SKELETAL MUSCLE PROTEIN KINASE A IN PREDIABETIC MONKEYS.

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Insulin activation of skeletal muscle glycogen synthase and glucose disposal is defective in both prediabetic and diabetic primates. The reduced activation of glycogen synthase by insulin could be the cause of lower glucose disposal rates, and could be the result of the failure of insulin to inhibit cAMP-dependent protein kinase activity, a.k.a. protein kinase A (PKA). To examine this proposed mechanism, PKA activity was measured in skeletal muscle (vastus lateralis) samples freeze-clamped *in situ* before (basal fasting) and during a euglycemic hyperinsulinemic clamp in 27 rhesus monkeys. Nine of the monkeys were normal (normal fasting glucose and insulin), 8 were prediabetic (normal fasting glucose and hyperinsulinemia) and 10 had spontaneous type 2 diabetes (hyperglycemia). Insulin lowered PKA activity in normal monkeys (basal vs insulin-stimulated, 14.4 ± 3.2 vs $8.1 \pm 1.8\%$, $p<0.05$), but raised PKA activity in prediabetic monkeys (5.4 ± 1.4 vs 10.5 ± 2.6, $p<0.05$). PKA activity was unaffected by insulin in the diabetic monkeys (6.7 ± 1.8 vs $7.5 \pm 1.4\%$). Basal PKA activity was higher in normal monkeys compared to prediabetic ($p<0.05$) and diabetic monkeys ($p<0.05$). Basal PKA activity was inversely related to the insulin-stimulated minus basal change in PKA activity ($r=-0.72$, $p<0.001$). We conclude that in vivo insulin during a euglycemic hyperinsulinemic clamp decreases skeletal muscle PKA activity in normal monkeys but is unable to decrease the activity of PKA in insulin resistant (prediabetic and diabetic) monkeys. Also, the insulin resistant state is characterized by low basal fasting skeletal muscle PKA activity.

1121

THE INFLUENCE OF FAMILY HISTORY ON INSULIN SECRETION AND SENSITIVITY IN NORMAL GLUCOSE TOLERANT OFFSPRING OF NIDDM PROBANDS

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Normal glucose tolerant first degree relatives of non-insulin dependent diabetic probands have been shown to have metabolic abnormalities including impaired first phase insulin secretion, insulin resistance and hyper-proinsulinaemia. The current study aimed to evaluate insulin levels at fasting and 2 hours after 75g oral glucose in relation to the family history of NIDDM

Patients and methods:

109 subjects, normal glucose tolerant by the WHO criteria were drawn from an incidence study cohort. 38 had maternal inheritance, 24 paternal, 16 bi-parental and 31 nil inheritance of NIDDM on history. Basic demographic data was collected and all subjects underwent a standard 75g oral glucose tolerant test. Homeostasis model assessment and the insulinogenic index at 120 minutes were used to estimate ß-cell function and insulin sensitivity.

Results:

The groups were matched with respect to age, gender, systolic and diastolic blood pressure. Body mass index was marginally different between groups ($p=0.062$). There was a significant difference between groups for waist-hip ratio ($p=0.021$). No significant differences were found between the groups for fasting insulin ($p=8601$), insulin at 120 minutes ($p=0.587$), insulin-glucose ratio at 0 and 120 minutes ($p=0.1589$ and $p=0.8478$), the insulinogenic index at 120 minutes ($p=0.9748$), % ß-cell function or insulin sensitivity as assessed by the HOMA model ($p=0.6989$ and $p=0.1994$).

Conclusion:

The presence of a single or bi-parental family history of NIDDM does not confer a special risk for the disorder in terms of ß-cell function or insulin resistance as computed by the HOMA model in the Durban Indian population.

1122

INCREASED ACTIVITY OF MEMBRANE GLYCOPROTEIN PC-1 IN JAPANESE NIDDM PATIENTS WITH INSULIN RESISTANCE

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Insulin resistance is one of the characteristic features of non-insulin-dependent diabetes mellitus (NIDDM). Recent studies revealed that the membrane glycoprotein PC-1 inhibited insulin receptor tyrosine kinase and played a role at least in part in the insulin resistance of NIDDM. In order to know whether PC-1 activity of fibroblasts is also elevated in Japanese NIDDM patients, we measured the PC-1 activity of cultured fibroblasts obtained from 12 NIDDM patients (BMI; 27.5 ± 7.8, mean $\pm$SD) including 7 insulin resistant patients with reduced glucose infusion rate (GIR; 1.45 ± 0.26 mg/kg/min) during euglycemic clamp study and 7 nondiabetic controls (BMI; 23.8 ± 2.6). A diabetic patient with Werner's syndrome (WS; 29yr, male; GIR 1.21 mg/kg/min) was also a subject of this study. Dermal fibroblasts taken from these subjects by forearm skin biopsy were cultured in DMEM with 10% FCS, and the PC-1 activity of the fibroblasts were measured using [^{35}S] 3'-phosphoadenosine, 5'-phosphosulphate as a substrate. Levels of PC-1 activity of 12 NIDDM patients and 7 insulin resistant patients were 98.3 ± 31.8 and 107.9 ± 26.3 nmol/mg/min, and were higher than controls (42.6 ± 13.8 nmol/mg/min, $p<0.01$). PC-1 activity of the patient with WS was also elevated (99.0 nmol/mg/min). The results suggest that the insulin resistance is correlated with PC-1 activity of skin fibroblasts in Japanese NIDDM patients and a patient with WS.

1123

EARLY INSULIN SECRETORY DEFECT RATHER THAN INSULIN RESISTANCE AS A METABOLIC DEFECT IN OFFSPRING OF NIDDM

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Pathogenesis of non-insulin-dependent diabetes mellitus(NIDDM) is heterogeneous; both insulin secretory defect and peripheral insulin resistance contribute to the development of NIDDM. The purpose of this study was to clarify the mechanism of early metabolic defect causing NIDDM in the offspring of Korean NIDDM patients. Eight control subjects(age 25.5±1.6; BMI 22.8±0.9) and six healthy offspring of NIDDM patients(age 25.4±2.7; BMI 22.9±1.7)participated in this study. 75g oral glucose tolerance test(GTT) with C-peptide and insulin response, euglycemic hyperinsulinemic clamp test, and hyperglycemic clamp test were performed. First and second phases of insulin secretion were defined as the mean of 0-10min and 10-120min. insulin secretion during hyperglycemic clamp test, respectively. Oral GTT revealed that all of the study subjects had normal glucose tolerance levels. The offspring of NIDDM and control subjects showed similar insulin secretory capacity in oral GTT(insulin area under the curve; control 75.4±14.6 vs. offspring 72.6±21.1μU/ml x hr). There was no significant difference in peripheral insulin sensitivity between control and offspring groups(control 8.13±1.42 vs. offspring 8.34±1.01mg/kg/min). Two out of the six offspring of NIDDM had decreases in first and second phases of insulin secretion in hyperglycemic clamp test compared to normal control subjects, and the mean values were slightly lower in offspring group (first 27.7±7.1, second 46.4±10.4μU/ml) compared to control group (first 37.7±5.8, second 71.9±19.9μU/ml). In conclusion, early metabolic defects of Korean offspring of NIDDM maybe related to insulin secretory defect rather than peripheral insulin resistance.

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SKELETAL MUSCLE TRIGLYCERIDES IN IMPAIRED GLUCOSE TOLERANCE AND NIDDM.

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Increased triglyceride content is believed to be an important factor for insulin resistance in skeletal muscles. In the Malmö study 125 65-year old men, originally identified in a health screening project, were subjected to a hyperinsulinaemic euglycaemic 2-h clamp combined with indirect calorimetry and a needle biopsy of the vastus lateralis muscle at the end of the clamp. Muscle triglycerides were analysed with a fluorometric assay and expressed in mmol/kg dry muscle weight. At the preceding OGTT 56 men had NIDDM, 34 impaired glucose tolerance (IGT) and 35 normal glucose tolerance (NGT) (WHO criteria). All groups were well matched for BMI (27.6, 27.8 and 27.1), for percent body fat (25.1, 24.0 and 22.5) and fat distribution (wast to hip ratio 0.99, 0.98 and 0.97) whereas maximal oxygen uptake was lower in the NIDDM and IGT groups (2.1, 2.3 and 2.4 l/min, p=0.004). Mean fasting glucose was 8.6, 4.8 and 4.6 mmol/l and serum triglycerides 2.2, 1.5 and 1.4 (p=0.003). Total glucose disposal was lower in the NIDDM and IGT groups (3.2, 4.2 and 5.7 mg/min/kg, p=0.0001), affecting both non-oxidative (1.6, 2.4 and 3.6 mg/min/kg, p=0.0001) and oxidative (1.6, 1.8 and 2.1 mg/min/kg, p=0.0001) glucose metabolism, but lipid oxidation was increased in the NIDDM group (0.6, 0.4 and 0.5 mg/min/kg, p=0.0008). The muscle triglyceride concentration was similar in all groups (63.4, 76.2 and 65.1 mmol/kg) but higher in normalweight (BMI 22.9) IGT subjects (62.2, n=6) than in both normalweight NGT (37.1, n=8) and NIDDM subjects (30.5, n=12) (p=0.02 and p=0.04). Considering all subjects muscle triglycerides correlated with BMI (r=0.42, p=0.0001), waist to hip ratio (r=0.24, p=0.02), total glucose disposal (r=-0.18, p=0.04), non-oxidative glucose metabolism (r=-0.19, p=0.03) but not with serum triglcyerides. In a general linear model muscle triglycerides were dependent upon BMI (p=0.0001) and inversely upon lipidoxidation (p=0.03) but not upon the glucose metabolism. In conclusion muscle triglycerides are not increased in NIDDM when taking body weight into account but may be increased in IGT.

1125

INCREASED SPLANCHNIC GLUCOSE OUTPUT AFTER ORAL GLUCOSE IN NIDDM WAS BASED ON THE SECONDARY INSULIN RESISTANCE

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The both pre and postprandial hyperglycemia in NIDDM are based on both impaired peripheral glucose clearance (PGC) and increased splanchnic glucose output (SGO). The different tissue of insulin (Ins) resistance (IR) was studied with either hyper-insulinemic euglycemic clamp combined with oral glucose (OG) (0.2-0.75g/kg) or hyperglycemic clamp with OG (0.1-0.5g/kg) with or without basal Ins replacement. The rate of SGO after OG (%SGO) was analysed. Attitude of Ins release was analysed by the delta increment of Ins after OG (75g). These studied were performed before and after glycemic control. Before glycemic control, most of non-obese NIDDM (N=66) have IR on both peripheral and hepatic tissue. Hepatic IR was well negatively correlated with the delta increment of Ins in non obese NIDDM. NIDDM with subcutaneous fat syndrome (N=12) have marked decrease of PGC and mild increase of %SGO (85-90%) (control value of 60-70%), while NIDDM with visceral fat syndrome (N=14) have marked increase of %SGO (95-100%) and mild decrease of PGC. Mitochondrial abnormal DM (N=4) had markedly increased SGO (90-96%) and mild decreased PGC. After acute replacement of basal Ins, half of increased SGO was normalized. After reaching excellent glycemic control, the DM whose delta increment of Ins was over 30 μU/ml obtained the improvement of SGO. We founded that the increased SGO was normalized by both replacement of basal Ins and improvement the daily profile of blood glucose, though the attitude of Ins secretion after OG was not normalized despite the excellent daily glucose profile.

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INOSITOL GLYCAN IN INSULIN ACTION AND RESISTANCE.

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Insulin interacts with its receptor and provokes a series of multiple intracellular events. Defects in these signaling pathways can lead to insulin resistance. The formation of low molecular weight substances termed insulin mediators is one of the products of the signaling events. We have purified and characterized one of these insulin mediators as an inositol glycan (IG). This mediator has been idenitified as 3-O-methyl-D-chiroinositol-galactosamine (IG). This pseudo disaccharide acts as potent stimulator of phosphoprotein phosphatases which control key enzymes in glucose metabolism. Several lines of evidence have demonstrated that a defect in its generation contributes to insulin resistance. IG isolated from hemodialysate, urine, and autopsy muscle is deficient in Type II diabetics when compared to control subjects. Similar data have been obtained in both rodent and primate models. Infusion of IG to streptozotocin-diabetic rats restored euglycemia without hypoglycemia. Chemically synthesized IG can mimic insulin action in vivo and in human ovarian cells. In conclusion, we have demonstrated that a IG isolated from bovine liver or synthesized chemically has insulin-like action in vivo and in vitro.

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ENHANCEMENT OF GLUCOSE TURNOVER AND OF SUPPRESSION OF HEPATIC GLUCOSE PRODUCTION WITH PORTAL INSULIN DELIVERY IN RATS WITH RENAL SUBCAPSULAR ISLET GRAFTS

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We have demonstrated decreased metabolic clearance of insulin, and insulin resistance, in rats with syngeneic renal subcapsular islet grafts (REN), that reversed streptozotocin diabetes. These abnormalities were absent with portal delivery of insulin after renal-mesenteric-vein anastomosis (RMA). In the present study, glucose turnover (GTR) was determined by ^{3}H-glucose infusion during euglycemic hyperinsulinemic clamps, with matching steady state insulin levels in similar rats with REN with or without RMA. Two months after transplantation, GTR was assessed in 4 REN and 4 RMA conscious fasted rats. After priming of 4 μCi ^{3}H-glucose, ^{3}H-glucose (0.4 μCi/min) was infused for 240 minutes and insulin (REN: 10, and RMA: 20 pmol/kg/min) was infused from 60 to 240 min with 25% dextrose infusion adjusted to maintain euglycemia. Mean values over 45 to 60 min were used for basal state; and over 180 to 240 min for insulin-stimulated steady state (INS-SS). Basal plasma glucose levels were similar (REN: 5.9±0.3, RMA: 6.2±0.1 mM); however insulin levels were higher in REN (132±18 pM) than in RMA rats (70±3), p<.005. During INS-SS, plasma glucose (REN: 4.4; RMA: 4.5 mM) and insulin levels (571±46; RMA: 574±25 pM) were similar. GTR, hepatic glucose production (HGP), and glucose infusion rate (GIR) are tabulated.

		REN	RMA	p
Basal	GTR=HGP, mg/kg/min	6.9 ± 1.2	8.7 ± 1.0	NS
INS-SS	GTR, mg/kg/min	6.5 ± 0.8	11.6 ± 1.0	0.006
	GIR, mg/kg/min	4.1 ± 1.0	15.8 ± 0.9	0.0001
	HGP, mg/kg/min	2.4 ± 0.5	-4.2 ± 0.7	0.0002

Thus we conclude that chronic systemic delivery of insulin from islet grafts results in insulin resistance with impairment of both suppression of HGP and stimulation of GTR. These abnormalities are prevented by portal delivery of insulin.

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A TYROSINE PHOSPHATASE AND PKC CONTRIBUTE TO INSULIN RECEPTOR KINASE INHIBITION BY HYPERGLYCAEMIA

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Cells incubated in high glucose develop an impaired insulin receptor kinase (IRK) activity. Several studies suggested that the activation of serine/threonine kinases might be involved in this effect. It has been speculated that an analogous mechanism is responsible for the reduced IRK activity in NIDDM. We therefore designed a study with a range of specific pharmacological inhibitors of different serine/threonine kinases to identify the crucial proteins involved in IRK modulation. As reported earlier, a relatively unspecific PKC inhibitor (H7), which also inhibits PKA, prevented the negative effect of glucose on IR autophosphorylation. We have now used three specific PKC inhibitors with inhibitory activity to different PKC isotypes: RO318220 (PKC $\alpha,\beta1,\gamma,\varepsilon$), Bisindolylmaleimide (PKC $\alpha,\beta1,\varepsilon,\gamma$) and Gö6976 (PKC δ,ε,ζ). IR tyrosine phosphorylation was reduced to about 51% in cells incubated in 25mM 2-Deoxyglucose. Pre-treatment with RO318220 (100nM) and Bisindolylmalemide (250nM) abolished or reduced this inhibition (110% and 81% of insulin stimulated (100%), whereas Gö6976 had no effect. This most likely indicates that additional PKC isotypes are inhibited by RO318220. After downregulation of PKC with TPA for 16 hrs the IRK inhibition was retained, suggesting that a TPA insensitive isoform can mediate the effect. We could not detect a reversal of the glucose effect with the PI3-kinase inhibitor Wortmannin, the PKA inhibitor H89, the Cam-kinase inhibitor KT5926 or the serine/threonine phosphatase inhibitor ocadaic acid. Treatment of the cells with the tyrosine phosphatase inhibitor vanadate (100µM) abolished the glucose induced inhibition. We also used an antibody that recognises the active form of the stress kinase p38, however, since p38 was not activated a role of this pathway can be excluded. In summary, we can demonstrate that the glucose induced IRK inhibition is regulated by at least two different mechanisms, a PKC isoform that is resistant to downregulation by TPA and a tyrosine phosphatase. We speculate that changes in the expression level or function of these proteins could contribute to the insulin resistant state in obesity and NIDDM.

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INSULIN SENSITIVITY AND PANCREATIC β-CELL RESPONSIVENESS IN NEWLY DIAGNOSED NON INSULIN DEPENDENT DIABETICS

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We have evaluated insulin sensitivity and pancreatic β-cell responsiveness in newly diagnosed non-insulin dependent diabetics (NIDDMs) and made comparison with BMI-matched normals. A frequently sampled insulin-modified IVGTT (0.3g glucose, 0.05 or 0.03 insulin per kg body weight) with minimal model (MINMOD) assessment of insulin sensitivity (S_I and S_G) was performed in 20 NIDDMs (age: 54±9 yrs, BMI: 28.5±3.6 kg/m^2, FPG: 12.6±3.5 mmol/L; mean±SD) and 20 normals (age: 47±11 yrs, BMI: 28.5±3.7 kg/m^2, FPG: 5.0±0.5 mmol/L). A standardised meal tolerance test (MTT, 75g CHO, 500 kcal) was performed in 16 NIDDMs (age: 50±9 yrs, BMI: 29.3±3.7 kg/m^2, FPG: 12.6±3.2 mmol/L) and 16 normals (age: 50±10 yrs, BMI: 29.2±3.6 kg/m^2, FPG: 5.1±0.5 mmol/L). Pancreatic sensitivity M_I (increase in C-peptide secretion per unit increase in plasma glucose concentration) and basal pancreatic sensitivity M_0 (fasting C-peptide secretion per unit fasting glucose concentration) were calculated employing a novel model of pancreatic responsiveness during MTT. In comparison with normals, insulin sensitivity S_I and pancreatic sensitivity M_I in NIDDMs were reduced by ~80% (S_I: 0.8±0.5 vs 3.8±1.8x10^{-4}/min per mU/L, p<0.001; M_I: 17.7±11.4 vs 90.0±43.3x10^{-9}/min, p<0.001; NIDDMs vs normal), glucose effectiveness S_G by ~25% (1.6±1.0 vs 2.2±0.6x10^{-2} /min, p<0.05), and basal pancreatic sensitivity M_0 by ~50% (5.4±2.2 vs 10.3±4.9x10^{-9}/min, p < 0.005). We conclude that in newly presenting NIDDM there is a 4 to 5 fold reduction in *both* insulin and pancreatic sensitivity.

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MUSCLE GLYCOGEN SYNTHASE AND PHOSPHORYLASE IN OBESE SUBJECTS WITH AND WITHOUT DIABETES

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The purpose of the present study was to verify in muscle biopsies, in obese subjects ± NIDDM, previous observations in normal subjects where a rise of plasma FFA by means of a neutral fat infusion was inducing during a glucose-insulin clamp a negative correlation between glycogen concentration [gly] and glycogen synthase activity (GS) and a positive correlation between [gly] and glycogen phosphorylase activity (GP). A euglycemic hyperinsulinemic clamp, associated with indirect calorimetry, was performed in 18 non-diabetic obese, 6 obese diabetic and 9 lean subjects taken as a control. Muscle biopsies were performed in vastus lateralis for measurements of GS, GP and [gly]. Results showed a negative correlation between [gly] and GS (p=0.007) and a positive correlation between [gly] and GP (p<0.001), both in the obese group but not in the control group (NS). The positive correlation between the rise in GS (ΔGS) and [gly] (Δ[gly]) during the clamp in the control group (p=0.05), which might correspond to stimulation of glycogen synthesis by GS, was absent in the obese groups (NS). Plasma FFA were higher (186 ± 24 vs 60 ± 9 µmol/L, p=0.004) in the obese compared to the control group, total glucose disposal (M) lower (3.67 ± 0.42 vs 7.24 ± 0.68 mg/kglbm.min, p=0.0002) and glucose storage lower (1.15 ± 0.42 vs 3.70 ± 0.64 mg/kglbm.min, p=0.004). This study suggests that the negative correlation of [gly] to GS might correspond to inhibition of GS in obese subjects (retrograde regulation of GS) while the positive correlation of ΔGS to Δ[gly], which normally correspond to the stimulation of glycogen synthesis by GS (anterograde regulation), was blunted. The resultant of these two opposite forces in obese subjects and even more pronounced in obese diabetic patients would lead to a lower rise in GS and lower glucose storage in response to the glucose-insulin infusion. The positive correlation of [gly] to GP classically represents stimulation of GP by glycogen concentration. It might possibly oppose inhibition of GP by increase in G-6-P resulting from decreased glucose oxidation. These results present a mechanism which might participate to the decrease in glucose disposal (lower M) and lead to insulin resistance in obesity and type II diabetes.

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INSULIN RESISTANCE, HYPERINSULINEMIA AND SALT SENSITIVITY IN NORMOTENSIVE, OBESE TYPE 2 DIABETICS AND NONDIABETICS.

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The aim of the study was an evaluation of the relationship between insulin resistance, hyperinsulinaemia, and blood pressure response to salt intake (salt sensitivity) in normotensive, obese (BMI>25,0) patients with and without type II diabetes. Material: 10 newly diagnosed, obese, normotensive type II diabetic patients (DM), aged 18-41 ys (x=35,7±7,2 ys) and 21 obese normotensive subjects (OB), aged 19-43 ys (x=33,1±8,1 ys) without diabetes. BMI and basal 24-h blood pressure measurements were similar in both groups. Plasma insulin levels were measured on fast and during oral glucose tolerance test (OGTT). Insulin sensitivity was estimated by steady-state plasma glucose concentrations (SSPG) achived during infusion of somatostatin, insulin, and glucose. Salt sensitivity was defined as an increase of mean arterial blood pressure > 5% after 7 days of a high vs. 7 days of low dietary Na intake. Insulin resistance was significantly higher in DM than in OB group (SSPG: 11,70±1,2 mmol/L vs. 7,9± 2,4 mmol/L, respectively, p<0,05). Fasting insulin levels were 32,8±12,0 mU/L and 19,1±11,1 mU/L, respecitvely, p<0,01; while insulin secretion measured as area under the curve (AUC) during OGGT wes similar in DM and OB groups (62,9±18,3 mU/L/min and 58,2±17,2 mU/L/min, respectively). Salt sensitivity was found in 6 of DM patients and in 10 of OB patients. SSPG levels in DM group were similar in SS and SR group (11,8±0,6 mmol/L and 11,6 ±1,9 mmol/L, respectively). Fasting insulin levels in DM were higher in SS than in SR group (38,0±8,9 mU/L vs. 25,1±8,2 mU/L, respectively, p<0,05) . In DM patients AUC of insulin during OGTT was also higher in SS than in SR group (71,3±12,1 mU/L/min and 50,2±16,0 mU/L/min, respectively, p<0,05), In OB subjects insulin resistance was higher in SS than SR group (SSPG levels 9,54±1,2 mmol/L vs. 6,82±2,4 mmol/L respectively, p<0,01) while insulin levels and insulin secretion were similar in both SS and SR groups. Conclusion: Salt sensitivity of blood pressure seems to be related to hyperinsulinaemia in normotensive, obese, type II diabetics and to insulin resistance in normotensive, non-diabetic obese subjects.

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SKIN FIBROBLAST PC-1 mRNA CONTENT AND INSULIN SENSITIVITY IN KOREAN NIDDM PATIENTS

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The pathogenesis of non-insulin dependent diabetes mellitus(NIDDM) consisted of decreased insulin secretion and insulin resistance. The cellular mechanism causing insulin resistance is not fully understood. Recently, It is reported that overexpression of PC-1 is related with insulin resistance in NIDDM patients. The present study was undertaken to determine whether skin fibroblast PC-1 is related with insulin resistance in Korean NIDDM patients. We have measured insulin sensitivity by euglycemic hyperinsulinemic clamp test and PC-1 mRNA content(arbitrary densitometric unit, ADU) of skin fibroblast in 13 Korean NIDDM patients(Age, 31.9±5.0 yr; BMI, 24.7 ±2.5 kg/m') and 8 healthy control subjects (Age, 29.4±5.2 yr; BMI, 22.4±2.2 kg/m'). Insulin sensitivity in NIDDM patients was slightly lower than that of control subjects(5.4±2.6 vs. 7.8±4.2 mg/kg/min, p>0.05). 2. PC-1 mRNA content in the NIDDM patients was slightly higher than that of control subjects(2.4±1.6 vs. 1.9±0.8 ADU, p>0.05). 3. There were significant positive correlation between BMI and PC-1 mRNA content(r=0.51, p<0.05), and negative correlation between BMI and insulin sensitivity(r=0.49, p<0.05). However, there was no significant correlation between insulin sensitivity and PC-1 mRNA content. This data suggest that skin fibroblast PC-1 is not related with insulin resistance in Korean NIDDM patients.

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INSULIN SENSITIVITY AND BODY FAT DISTRIBUTION IN A GROUP OF VARYING GLUCOSE TOLERANCE SUBJECTS.

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The body fat accumulation is associated to insulin resistance. The aim of our study was to evaluate the influence of body mass index (BMI) and of visceral fat accumulation on the insulin resistance and some associated cardiovascular risk factors. After an overnight fast an insulin tolerance test (0.1U.Kg^{-1}), in 28 subjects with varying glucose tolerance, was performed. The mean (± SD) value of the blood glucose disappearance rate (K_{ITT}) was significantly reduced in non-insulin-dependent diabetes mellitus (NIDDM: 1.53 ± 0.4 ;n=13) as compared to normal glucose tolerance (NGT: 3.68 ±1.9 n = 6; p<0.02) impaired glucose tolerance (IGT: 2.72 ± 0.9; n=5 ; p<0.02) and insulin dependent diabetes mellitus (IDDM: 3.65 ± 0.5; n = 4; p<0.001) subjects.
The visceral fat accumulation (waist to hip ratio :W/H) was greater in NIDDM (1.00 ± 0.07) versus IDDM (0.88 ± 0.07; p<0.01) and NGT (0.87 ± 0.09; p<0.01) subjects. It was no significant difference in BMI between NIDDM (28.7 ± 5.6) IGT (31.6 ± 2.1) and NGT (30.5 ± 4.2) subjects. IDDM subjects had lower BMI (21.8 ± 2.6; p<0.01) as compared to NIDDM, IGT and NGT groups. W/H was correlated to K_{ITT} (r = -0.70; n=28;p<0.001), basal insulin levels (r=0.69 ; n=13 ; p<0.05), Total cholesterol/HDL cholesterol ratio (r=0.61;n=23;p<0.01) systolic and diastolic arterial blood pressure (r=0.44; n=28; p<0.05).Between BMI and K_{ITT}, W/H, arterial pressure and lipid parameters it was no significant correlation.
Our data suggest that visceral fat accumulation is associated to insulin resistance and some vascular risk factors, independent of BMI variation.

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INSULIN RESISTANCE IN NIGERIAN SUBJECTS WTH NIDDM.

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Insulin resistance is now well documented to be closely related to the onset and course of NIDDM. Nigerians are generally thought to be very sensitive to insulin. No previous studies have looked into the insulin sensisitivity status of Nigerians. A study was thus carried out to measure insulin sensitivity (IS) in uncomplicated NIDDM subjects.. The glucose disposal rates (GDR) of the study subjects was compared with a control group. Forty-three (22 males, and 21 females) people with NIDDM were recruited. The two groups of subjects had their IS determined by short insulin tolerance tests. The mean (SEM) of the GDR (the index of insulin sensitivity). for the control subjects was 3.20 (1.06) and 3.17 (1.07) in males and females respectively; in the non diabetics GDR correlated negatively with age, BMI and WHR. The mean (SEM) values for GDR in the NIDDM subject was 1.73 (1.04) and 1.80%(1.06) in males and females respectively. The GDR in NIDDM subjects was significantly lower (p<0.05) than in the control subjects. 70% of NIDDM subjects had insulin resistance by comparison with the controls. We conclude that in Nigerians insulin resistance is commoner than generally thought among people with NIDDM.

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BASAL C-PEPTIDE IN ORALLY-TREATED, FAIRLY CONTROLLED NIDDM.

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We aim at elucidating the relationships of C-peptide and the C-peptide/glycaemia ratio with known surrogate markers for insulin resistance in NIDDM patients in good to fair control obtained without the use of exogenous insulin. To achieve such a purpose, 130 orally-treated patients with a HbA_{1c} level<7.5% have been studied. Non-stimulated C-peptide levels (RIA) and the C-peptide/glycaemia have been determined, and their relationships with the blood pressure status, blood pressure figures, estimates of adiposity, age, known duration of diabetes, current therapies, plasma lipids, glycaemic control, urinary albumin excretion rate, uric acid and creatinine have been ascertained. C-peptide levels were significantly (p<0.05) correlated with systolic (r=0.21) and diastolic blood pressure (r=0.19), BMI (r=0.21), HDL (r=-0.22) and non-HDL-cholesterol (r=0.23), apolipoprotein B (r=0.29), log of triglycerides (r=0.39) and uric acid (r=0.35); and almost significantly (p<0.1) with waist-to-hip ratio (r=0.18) and total cholesterol (r=0.17). The C-peptide/glycaemia ratio had significant correlations with known duration of diabetes (r= -0.23), diastolic blood pressure (r=0.21), BMI (r=0.22), log of triglycerides (r=0.23) and uric acid (r=0.36). Hypertensives had significantly higher C-peptide levels than normotensives (1.04±0.04 vs. 0.88±0.04 nmol/ml, respectively (mean±SE), p<0.05), and this difference remained significant after adjustment for age and known duration of diabetes, and almost significant after adjustment for the previous variables and BMI. The C-peptide/glycaemia ratio was almost significantly greater in hypertensives only after adjustment for age and known duration of diabetes. In conclusion, in well-controlled NIDDM patients not receiving exogenous insulin, both C-peptide levels and the C-peptide/glycaemia ratio have significant relationships with surrogate markers for insulin resistance.

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CLINICAL CHARACTERISTICS OF HIGH SERUM CPR LEVELS IN PATIENTS WITH NON-INSULIN DEPENDENT DIABETES MELLITUS.

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Syndrome X is defined as a concept including hyperinsulinemia, impaired glucose tolerance, lipid metabolism disorder and hypertension, and it advances atherosclerosis and then leads to ischemic heart disease. We designed that some of patients with non-insulin dependent diabetes mellitus (NIDDM) might reveal the pathogenesis similar to syndrome X. We therefore analysed clinical characteristics of NIDDM patients showing high serum CPR levels. Studies were conducted in 116 NIDDM patients (male:62, female:54, mean age:54.3 ± 2.4 y-o) therapied by diet alone or oral hypoglycemic agents (OHA). Serum CPR were measured at fasting in the morning by RIA method and then we divided into two groups as follows: one is a high CPR group with values more than 2.3 ng/ml (group A) and another is a normal or low CPR group with those less than 2.3 ng/ml (group B). All patients were measured body weight, blood pressure, and serum lipids. Twenty eight percent of all patients showed high serum CPR levels (Diet:15%, OHA:31%). There was the tendency of a positive correlation between CPR levels and the mean blood pressure. Prevalence of the complication with hypertension in group A was 38%, whereas that in group B was 23%, and therefore a significant discrepancy was observed between both groups. Body mass index in group A was obviously high compared with that in group B, and prevalence of the complication with obesity in group A was significantly higher than that in group B. In lipid, serum triglyceride levels in group A was significantly high compared with that in group B ($p<0.05$).
Conclusions: Diabetic patients with high serum CPR levels were frequently complicated with hypertension, obesity and hypertriglyceridemia, and therefore they might possess a critical risk to provoke the atherosclerosis.

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INSULIN RESISTANCE MEASURED WITH SPECIFIC INSULIN ASSAY AND MINIMAL MODELLING IN SUBJECTS WITH NIDDM

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NIDDM patients have impaired insulin sensitivity (Si) and glucose effectiveness (Sg) when estimated by minimal modelling. However insulin measurements in previous studies were performed using radioimmunoassay, therefore including biologically less active substances eg. split-32,33 proinsulin. We measured insulin action and secretion in 20 NIDDM and 10 normal subjects using a specific insulin assay measured by ELISA (BMI 24.9 (IQR 24.1-26.5) vs 24.0 (20.7-28.8) $kg \cdot m^{-2}$. Fasting glucose, insulin and lipid concentrations were measured while Si and Sg were derived from Bergman's minimal model applied to an insulin-modified frequently-sampled intravenous glucose tolerance test. NIDDM had significantly higher fasting glucose 9.7 (8.4-10.8) vs 4.9 (4.7-5.3) $mmol \cdot l^{-1}$, $p<0.001$; as well as fasting total cholesterol 5.6 (4.8-6.2) vs 4.7(4.1-5 4) $mmol\ l^{-1}$, $p<0.05$, and triglyceride levels 1.63 (0.90-2 59)] vs 0.64 (0.57-0.68) $mmol \cdot l^{-1}$, $p<0.005$. HDL-C was significantly lower 1.03 (0.90-1.21) vs 1.49 (1.27-1.66) $mmol \cdot l^{-1}$, $p<0.05$ Glucose tolerance as well as first phase insulin response were significantly impaired in NIDDM, 0.60 (0.46-0.77) vs 2.33 (1.57-2 68) $mmol \cdot min^{-1}$, $p<0.001$; 24 (12-146) vs 107 (94-599) $pmol \cdot l^{-1}$, $p<0.05$ but there was no significant difference in fasting insulin levels, 1.8 (1.0-11.8) vs 5.0 (1.4-6.7) $pmol \cdot l^{-1}$. NIDDM also had reduced Si and Sg compared to normals, 3.08 (0.98-6.52) vs 18.26 (5.93-26.90) $10^{-4} \cdot min^{-1}$ per $pmol \cdot l^{-1}$, $p<0.005$; 1.2 (0.9-1.4) vs 2.5 (2.0-2.9) $10^{-2} \cdot min^{-1}$, $p<0.001$. Impaired insulin sensitivity and glucose effectiveness have therefore been demonstrated in NIDDM using our assay for specific insulin which is more logical for modelling purposes

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INSULIN RESISTANCE, HIPERINSULINEMIA, OBESITY, HYPERTENSION, AND DYSLIPIDEMIA IN NIDDM SUBJECT IN INDONESIA, S.Ndraha, M. Oemardi, I.Subekti, P.Soewondo, S.Sugondo, S. Waspadji, A.B.Ranakusuma, S. Suyono, Metabolism and Endocrinology Division, Department of Internal Medicine, Faculty of Medicine, University of Indonesia Jakarta, Indonesia

Non Insulin Dependent Diabetes Mellitus (NIDDM) is recognized to be associated with obesity, hypertension, dyslipidemia, hyperinsulinemia and insulin resistance. This cluster of metabolic-cardiovascular disorders is known as the Insulin Resistance Syndrome. However, insulin resistance in NIDDM subjects in Indonesia has not evaluated. A cross sectional study was conducted to find it. The correlation between insulin resistance and obesity, fasting insulin level, hypertension and dyslipidemia were also evaluated.
A body mass index, blood pressure and lipid profile studies as well as euglycemic clamp technique were performed in 20 NIDDM subjects (8 women and 12 men, mean [±SD] age, 46,1 ± 3,5yrs, mean [±SD] BMI, 25,1±4,3 kg/m^2). The mean [±SD] fasting insulin level, insulin sensitivity (M) and sensitivity index (M/I) were 14 ± 6 mU/L, 6,4±2 mg/kg/min and 5,6±2, respectively. BMI had significant inverse correlation with insulin sensitivity. There was a significant correlation between M value and fasting insulin level. However, no significant correlation between systolic, diastolic blood pressure, serum triglyceride, cholesterol HDL and total cholesterol.
The result suggest that (1) fasting insulin level was lower than non diabetic Asian subjects, (2) insulin sensitivity was higher, (3) obesity was associated with insulin sensitivity (4) insulin sensitivity was related to fasting insulin level, but not related to blood pressure, triglyceride, HDL cholesterol and total cholesterol level. As conclusion, insulin sensitivity in NIDDM subjects in Indonesia was higher compared to the previous studies, and no significant correlation between insulin sensitivity and blood pressure, plasma triglyseride, HDL cholesterol and total cholesterol. However, insulin sensitivity was associated with hyperinsulinemia and obesity.

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CLINICAL REMISSION IN TYPE I DIABETIC PATIENTS RESULTS FROM ENHANCEMENT OF INSULIN SENSITIVITY

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In this study, we aimed to investigate peripheral insulin resistance (PIR) and beta cell functions in 8 Type I diabetic patients with clinical complete remission (F/M; 1/7, mean age; 23.8 ± 8.9 year BMI; 22.3 ±3.1 $kg.m^{-2}$, and Hb A_{1c}; 5.82±1.1 %). Remission was induced by multiple subcutaneous insulin injections (MSCII) and defined by no insulin requirement in spite of daytime normoglycaemic for at least 15 days. Results were compared with data of 4 healty control subjects (F/M; 3/1, mean age 21.2±6.5 year, BMI; 19.8±2.2 $kg.m^{-2}$, and HbA_{1C}; 5.6±1.2 %). PIR was assessed with hyperinsulinemic euglycaemic clamp technique and beta cell functions with first phase insulin release (FPIR) to IVGTT and C-peptide stimulation test (after 1 mg Glucagon i.v. injection). No difference was observed in glucose disposal rate (M value) between remission and control subjects (6.76±1. and 7.11±1.1 $mg.kg^{-1}. min^{-1}$). On the contrary, FPIR [(1'+3' min. insulin)-2x basal insulin] was almost completely lost in remission patients (6.01 ± 6.5 $mU.ml^{-1}$, compared to 98.19 ± 23.2 $mU.ml^{-1}$ in control group). Moreover, both basal (BCP) and stimulated C-peptide (SCP) levels in the remission group were found to be significantly lower than in the control group (BCP; 0.6 ± 1.9 and 1.9±0.5 ng/ml, $p<0.005$, and SCP; 1.9±0.7 and 3.4±0.5 ng/ml, $p<0.01$ in remission and control groups respectively). No correlation was noticed between M value and FPIR. As a conclusion, our study suggested that improvement in peripheral glucose utilization induced by removal of glucose toxicity with MSCII rather than relieved endogen insulin secretion capacity might have impact on appearance of clinical remission in Type I diabetes.

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IMPAIRED PROTEIN-INDUCED CATECHOLAMINE (CA) EXCRETION IN DIABETIC RATS.

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It was shown earlier that protein supplementation to diet results in an increase of CA excretion in normal humans and animals. However, there is no data regarding the influence of protein on CA secretion in diabetes mellitus. We investigated urine excretion of epinephrine (E), norepinephrine (NE), and dopamine (D) in 18 streptozotocin-induced diabetic (1 month duration of disease) and 18 control rats fed with chow and protein diets. CA were measured by HPLC in urine collected for 24 hours 4 days before and during protein (casein) supplementation. Results were analyzed using ANOVA-test. Basal CA levels were significantly increased in diabetic rats compared to controls. The supplementation of protein led to an increase of CA excretion in control animals with the most pronounced elevation of D excretion. However, in diabetic rats protein induced significant rise of NE excretion only. Urine D levels rose from 27026±2399 to 54275±5130 pmol/ml (mean±SEM), $p<0.01$ in controls, and from 56740±7309 to 70715±12025 pmol/ml, $p>0.05$ in diabetic rats. NE excretion changed from 4014±152 to 4598±222, $p<0.05$ in controls, and from 7404±317 to 9276±990 pmol/ml, $p<0.05$ in diabetics. E excretion did not change significantly in diabetics while it increased in controls by 29.5%. Thus, short-term diabetes mellitus is associated with diminished response of D and E excretion to protein supplementation to diet and these changes may reflect impairment of CA secretion in diabetic rats.

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Glucose Metabolism in Other Disorders, Sex Steroids

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INSULIN RESISTANCE IN CHRONIC LIVER DISEASE WITH IMPAIRED GLICOREGULATION

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The aim of this study was to investigate the action of exogenous insulin on glucose metabolism in a group of patients with liver cirrhosis (LC) and chronic hepatitis (CH) in correlation with the altered glucose tolerance (DGT/DM) and the degree of liver damage (LC/CH). **Material and method.** The study was carried out in a group of 36 patients with LC and CH with or without DM or DGT. 11 patients with LC (Child class B and C) also had DM, 4 patients with LC presented DGT after OGTT with 75g powder glucose. The 17 patients with CH, of the same etiology, presented normal oral glucose tolerance. All the patients were submitted to insulin tolerance test (ITT) with i.v. 0,1 IU insulin/Kg body weight, determining the rate of serum glucose disappearance (K-ITT) and the insulin sensitivity index (Delta Glucose/Glucose=DG/G). The area under the curve was also calculated (AUC-TAI method). The results were statistically interpreted using the ANOVA test and the Student t test, comparing the 3 groups. **Results.** 1. A significant decrease of the mean K-ITT in LC with DM was found, compared to CH without DM (2,21 vs. 3,53, $p<0,05$) and a slow rate of serum glucose disappearance. 2. Significantly modified AUC in chronic liver disease with DM, compared to that without DM or DGT (table). 3. Insulin sensitivity index (DG/G) was correlated with the degree of glucose tolerance impairment and liver damage (DG/G=0,24 in CH without DM, versus 0,40 in LC with DM, $p<0,05$). **Conclusions.** 1. Patients with LC and DM present a more marked insulin sensivity. 2. The degree of insulin resistance is correlated with level of glucose tolerance and liver function.

LC with DGT (4 patients)	2371 mg/dl/30'
LC with DM (11 patients)	4024 mg/dl/30'
CH without DM/DGT (17 patients)	2180 mg/dl/30'

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SEVERE INSULIN RESISTANCE IN A MODEL OF CRITICAL ILLNESS AND PARENTERAL NUTRITION

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During parenteral nutrition critically ill nondiabetic patients often show elevated blood glucose levels despite high doses of insulin. This is most likely due to insulin resistance caused by high concentrations of insulin-counteracting hormones. As studies in such patients are difficult to perform we tried to simulate hormonal and carbohydrate metabolism of critically ill patients and quantified insulin resistance in this model. 6 healthy volunteers received an i.v. infusion of 4 insulin-counteracting hormones (epinephrine 100 ng/kg/min, glucagon 16 ng/kg/min, cortisone 5 µg/kg/min, growth hormone releasing hormone 8 ng/kg/min) for 4h together with glucose (260 ng/kg/min) as simulation of parenteral nutrition. In a control experiment only glucose was infused. In a second study insulin sensitivity was estimated by means of a two-step hyperinsulinaemic euglycaemic glucose clamp (blood glucose 5.0 mmol/l, insulin infusion rates 2.5 and 5.0 mU/kg/min with hormone infusion; 1.0 and 2.5 mU/kg/min in the control experiment without hormone infusion). This model established a metabolic situation comparable to that seen in critical ill patients as indicated by highly elevated concentrations of the infused hormones (epinephrine 1085±89 pg/ml (mean±SEM), glucagon 1100±114 pg/ml, cortisol 1004±32 ng/ml, growth hormone 21±6 pg/ml), blood glucose concentrations (20.2±2.1 mmol/l), insulin concentrations (90±32 µU/ml), FFA (1073±159 µmol/l), lactate (5.7±0.4 mmol/l), pyruvate (188±20 µmol/l) and ß-hydroxybutyrate (143±1 µmol/l). In the control experiment blood glucose and insulin concentrations increased slightly (blood glucose 6.9±0.8 mmol/l, insulin 18±7 µU/ml, $p<0.001$ vs. hormone infusion), wheras the metabolites remained stable (pyruvate, lactate) or decreased slightly (FFA, ß-hydroxybutyrate), $p<0.05$ vs. hormone infusion, resp.). Infusion of insulin-counteracting hormones led to a app. 90% decrease of the insulin sensitivity index (0.6±0.4 vs. 4.5±1.3 ml/min x m^2 / µU/ml, $p<0.001$). This model is able to establish a metabolic situation as observed in critical illness under parenteral nutrition. The 90% decrease in insulin sensitivity explains the hyperglycaemia seen in nondiabetic critically ill patients.

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TYPE 2 DIABETES MELLITUS AND HCV-Ab+ CHRONIC ACTIVE HEPATITIS: EFFECT OF RECOMBINANT α-2b-IFN TREATMENT

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It has reported that the Interferon (IFN) treatment (tr) of Chronic hepatitis (CAH) in patients with diabetes mellitus could interfere with insulin action/secretion. **AIM**: to evaluate in NIDDM pts with biopsy-proven HCV+ CAH the effect of recombinant IFN-α-2b (*INTRON-A®, Schering-Plough*) on: 1) glycemic control, 2) autoimmune responses of islet cell antibodies (ICA), **PATIENTS**: 15 ICA- NIDDM pts (12F/3M) with mean age 58±4y, diabetes duration 9±4y. All had had serum ALT persistently > twice the upper normal limit for at least 6 months (**mo.**), ad had fasting glycemia 142±65 mg/dl, mean daily glycemia (MDG; on 7 values) 158±71, HbA1c 7.6±1% . **METHODS**: All subjects received 3 MU IFN thrice weekly (tw) for 2 mo., then 5 MU tw for 10 mo. MDG, HbA1c (Bio-Rad, n.v.<6.6), body weight (BW), ICA (indirect IF on human pancreas according to the 3rd Int. Workshop Stand. of ICA), were measured before, 6 and 12 mo. after IFN starting. IFN efficacy was assessed on persistent ALT normalization for at least other 6 mo. **RESULTS**: compared to baseline, IFN Tr produced no impairment of glycemic control (this being improved in 10 pts and unchanges in 5), MDG was -9.5±3% (141±9 vs 156±7, p n.s.), HbA1c -6.3±0.6% (7.1±0.6 vs 7.6±1, p n.s.), body weight -5±2.5% (74±7 vs 79±9, p n.s.). Apon IFN withdrawal, 4 pts were sustained responders (26.7%), 6 early rsponders with relaps (40%), and 5 non-responders (33.3%). All had no significant changes of ICA titres. **CONCLUSION**: the effect of recombinant IFN-α-2b Tr that we observed in ICA- NIDDM pts: 1) is similar to that already reported by other, 2) does not affect the glycemic control, and 3) does not induce ICA+, therefore it may be safely used in NIDDM pts with HCV+ ICA- CAH.

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INSULIN SENSITIVITY AFTER CHRONIC PREDNISONE TREATMENT

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Glucocorticosteroid treatment produces glucose intolerance combined with higher insulin levels. Peripheral hyperinsulinemia can be due to an enhanced beta-cell secretion, to a reduced hepatic extraction or to a combination of both. The simple observation of the peripheral values of the hormone does not provide further information on the level at which the impairment occurs. Aim of the study is to evaluate the beta-cell insulin production and his hepatic clearance, in normal glucose tolerance subjects treated with a long term prednisone (PN) therapy. We studied 6 subjects (2 males, 4 females; BMI 26.9±2.6 kg/m^2; 49.3±9.0 yrs), with normal oGTT, without family evidence of diabetes mellitus, not in therapy with any drugs in the last 6 weeks, with normal renal and liver function, affering to the Pneumology ward for sarcoidosys or chronic obstuctive pulmonary disease and needing for prolonged prednisone treatment (50 mg orally for 30 days). They received a Frequent Sampling Intra-venous Glucose Test (FSIGT: 0.33 g glucose/kg) the day before the beginning (BT) ant after thirty days of treatment (AT), minimal model is used. Fasting plasma glucose, insulin, C-peptide levels, glucose effectivness (SG) and basal insulin delivery rate (BDR) during FSIGT were not modified by PN treatment. whereas insulin sensitivity (SI = BT: 44.2±25.9 vs AT: 35.9±22.4 min^{-2} /(pmol/L) MEAN±DS; $p < 0.05$ Student's t test for paired data) and sensitivity of the first-phase C-peptide secretion (ΦIC = BT:106±52 vs AT: 193±62 pmol min^{-1} mg^{-1}dl; $p<0.003$) were significantly modified. These results suggest that chronic prednisone treatment induces a reduction of insulin sensitivity with higher insulin secretion and normal hepatic extraction without hyperglicaemia.

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DIABETES MELLITUS AND LIVER TRANSPLANTATION IN MALNOURISHED HCV-Ab+ CIRRHOTIC PATIENTS

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The asociation between non insulin dependent diabetes mellitus (NIDDM) and liver cirrhosis (C) has been frequently described. In addition, transient diabetes mellitus has been reported after orthotopic liver transplantation (OLT) in C as a consequence of prednisone treatment (PT). NIDDM is more frequent in decompensated than in compensated C. In turn, a close relationship is reported between liver function (LF) and nutritional status (NS), the prevalence of malnutrition (Ma) increasing as liver function deteriorates. ***AIM***: evaluate a possible relationship between changes of NS and glycometabolic equilibrium (GE) in 5 malnourished HCV+ C pts (Child C) with NIDDM before and during 24 months after OLT. Mean age pre-OLT was 57±6, and b wt 62±5 (BMI 23±3 even though with acites), diabetes duration 8±3y and cirrhosis known by 5±2y. ***METHODS***: lean body mass (cm, MAMC: mid-arm muscle circumference), triceps skinfold thickness (mm, TSF: fat deposit), muscle arm area (cm2, MAA, extrapolated) were measured and insulin dosage (IU) (U/kg b wt) was recorded. ***RESULTS***: compared with pre-OLT values, a significant increase of MAMC (23±7 vs 28±4, p<0.01), TSF (7.5± vs 10.2±4, p<0.05), and 43±8.9 vs 51.4±9.4, p<0.05) and a significant decrease of ID (0.9±0.5 vs 0.2±0.2, p<0.05) were observed in parallel with prednisone reduction; 2 out of 5 pts withdrown insulin. The correlation coefficient between the reduction of ID and MAMC was -0.738, p<0.01. ***CONCLUSION***: a significant increase in lean body mass is associated with a reduction of insulin need in C pts with NIDDM, probably due, in addition to prednisone reduction, to an improvement of insulin-resistance of malnourished and decompensated cirrhotic patients.

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INSULIN ACTION IN PRIMARY HYPERALDOSTERONISM

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The impaired insulin sensitivity has been repeatedly described in patients with essential hypertension. The aim of this study was to evaluate insulin action at receptor and postreceptor levels in hypertensive patients with primary hyperaldosteronism (PH). This diagnosis was confirmed in six patients (mean age 50 yrs, range 38-61 yrs, body mass index 28.2±2.8 kg/m^2) by clinical and laboratory test. Their blood pressure was monitored during 24 hrs by Spacelab tonometer (SBP 159±13 mm Hg, DBP 98±8 mm Hg), plasma aldosterone concentration in supine position was 344±168 pg/ml (normal value below 150 pg/ml) and serum potassium concentration 3.0±0.3 mmol/l. All patients had normal fasting plasma glucose (4.6±0.4 mmol/l) and fasting serum insulin concentration (15.5±4.3 mU/l). Isoglycaemic hyperinsulinemic clamp on Biostator (insulin infusion rate 1.0 mU/kg/min) and insulin receptors on erythrocytes were evaluated in all of them. Plasma potassium concentration was maintained within the normal limits during the clamp. Control group consisted of 7 healthy persons of appropriate age and body mass index. A decreased glucose disposal rate (21.0±6.6 vs 34.9±10.2 μmol/kg/min, p<0.01), metabolic clearance rate of glucose (4.6±1.6 vs 7.6±1.9 ml/kg/min, p<0.01) and insulin sensitivity index (23.8±9.8 vs 39.4±13.7 μmol/kg/min per mU/lx100, p<0.02) were found in patients with PH as compared to healthy persons. No changes in the insulin receptor binding characteristics were present in these patients. An inverse relationship between plasma aldosterone concentration and insulin sensitivity index (r=-0.36, p=0.05) was observed in PH patients. We conclude that primary hyperaldosteronism is accompanied by insulin insensitivity at postreceptor level. Further research will be necessary to discover possible underlying pathogenetic mechanism.

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POST TRANSPLANT DIABETES MELLITUS IN LIVE RELATED RENAL TRANSPLANT RECIPIENTS

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Post Transplant diabetes mellitus (PTDM) is a major sequelae secondary to glucocorticosteroid therapy in renal transplant recipients. The aim of this study was to determine the prevalence of PTDM in the early post transplant period in two groups of patients, in relation to dosage of steroids. Group I on a high dose (0.6 mg/kg/day) and Group II on low dose (0.3 mg/kg/day). Each group comprised of 62 patients with a renal allograft from a live related donor. The follow-up period was one year. Cyclosporin was given to both groups in the dose of 10 mg/kg/day. Each recipient was subjected to a fastmg and 2 hours post prandial blood glucose on every follow-up visit. The WHO criteria of fasting blood glucose $\geq$ 7.8 mmol/l and 2 hours post prandial blood glucose $\geq$ 11.1 mmol/l (venous plasma) was diagnostic for diabetes mellitus. Patients with doubtful values were subjected to a formal Oral Glucose Tolerance Test as recommended by WHO. Body mass index (BMI) of all cases was calculated by the formula : wt in kg / ht in m^2. Family history of diabetes was inquired and HLA-DR antigens noted. The male to female ratio (4.6 : 1 vs 4.2 : 1) and the mean age (30.2 yrs vs 29.25 yrs) was similar in both groups. Thirteen patients from Group I and 4 from Group II developed PTDM. The mean time period between transplantation and appearance of PTDM was 79 days in Group I and 108 days in Group II. The mean dose of methylprednisolone taken by PTDM subjects of Group I (36.5 mg/day) was significantly higher than those with a normal glucose tolerance (28.1 mg/day) $p < 0.001$. In Group II the mean dose in PTDM subjects was 16mg and in non PTDM 14mg daily. BMI was significantly higher in the PTDM subjects of both groups compared with non PTDM (Group I : 21.8 vs 18.4, $p < 0.01$ and Group II : 21.02 vs 17.9, $p < 0.01$). Other factors as HLA antigens, family history of diabetes and gender did not influence the development of PTDM. Satatistical analysis included Chi Square Test and Student t Test. The study concluded that steroid dose was directly related to the development of PTDM in the early post transplant period and obesity (raise BMI) was also an associated risk factor.

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CARDIOVASCULAR RISK FACTORS IN ADULT TURNER SYNDROME AND THE INFLUENCE OF SEX HORMONE SUBSTITUTION

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Turner syndrome is associated with abnormalities of glucose metabolism and epidemiological data show increased frequency of NIDDM, ischaemic heart disease and osteoporosis. Since most women with Turner syndrome are treated with sex hormone substitution we examined the impact of this treatment on cardiovascular risk factors. We performed OGGT and FSIVGTT (minimal model), as well as 24 hour ambulatory blood pressure and measurement of circulating lipids. The patients (N=26, 33.1±7.9 y (mean±SD)), were examined twice before (TB) and after (TA) sex hormone treatment, while controls (N=24, 32.7±7.8 y) were examined once. Women with Turner syndrome received either oral (O) or transdermal (T) 17-β-estradiol (17-β-E) and oral norethisterone (O-17-β-E: N=14; T-17-β-E: N=12) for 6 month after 4 month washout. AUC_{glc}(TB) from OGTT was significantly higher than in controls (7.7±2.3 vs 4.9±2.0 mmol/L*3 h, $p < 0.0005$), and increased after treatment ($p < 0.02$). AUC_{ins}(TB) from OGTT was also significantly higher than in controls (622±380 vs 441±152 pM*3 h, $p = 0.05$), while there was no difference before and after treatment. S_I(TB) was similar to controls, and unchanged after treatment. First phase insulin secretion was deficient compared with controls ($p = 0.03$) and did not increase with treatment. No change in S_G and circulating levels of cholesterol, HDL-cholesterol and triglycerides was seen. 24-hour systolic, diastolic BP and diastolic night/day (N/D) ratio were all significantly elevated in Turner syndrome before treatment compared with controls, and 24 hour diastolic BP decreased, while diastolic N/D ratio increased significantly with treatment, as did LBM ($p = 0.0005$). No differences could be attributed to route of administration. Glucose intolerance and deficient insulin secretion are prominent characteristics in Turner syndrome. Elevated blood pressure, partly normalized by sex hormones, and increased diastolic N/D ratio, suggesting that Turners are non-dippers, and a normal lipid metabolism was seen. In conclusion, women with Turner syndrome have several of the characteristics seen in the metabolic syndrome.

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DISTURBANCES IN CARBOHYDRATE METABOLISM AMONG PATIENTS WITH SHEEHAN'S SYNDROME

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Sheehan's syndrome is a clinical picture due to postpartum necrosis of anterior pituitary gland with various degree of pituitary insufficiency. Tendency to hypoglycemia is an expected situation because of counter-regulatory hormone deficiencies such as growth hormone (GH) and cortisol. Although fasting blood glucose levels and insulin values were reported as normal, impaired glucose tolerance and reduced stimulatory effect of glucose on insulin secretion were also reported in GH deficient states. In order to determine the prevalence of glucose intolerance, an oral glucose tolerance test (OGTT) was caried out and blood glucose, insulin and C peptide values were determined at 0, 30, 60, 90, 120, 150, 180, 240, 300 minutes in six incomplete (three of them have normal ACTH reserve) and 13 complete ; total 19 patients with Sheehan's syndrome. All cases were GH deficient. Average age (year) and body mass index (BMI= kg/m^2) for control group was 45.6± 9.1 and 31± 2.2 and for the patients with Sheehan's syndrome was 45.9± 7.7 and 28.1± 6.6 respectively. All patients had normal fasting blood glucose levels , however, three (15.8%) of them showed diabetic curve after OGTT. Thirty minutes blood glucose values (188.7± 16.6 ; 155.4± 33.3 mmol/l) and 150 and 180 minutes insulin values (6.0± 2.4 / 19.8± 14.4 ; 3.6± 1.8 / 22.2±28.8 nmol/l) were significantly ($p<0.05$) different in control and patient groups respectively, as determined by area under curve (AUC) at 30 minute intervals and results are expressed as means± SD. In conclusion; like GH excess, GH deficiencies, meanwhile Sheehan syndrome, may cause glucose intolerance although the underlying mechanism is not completely clear.

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ANDROGEN STEROID PATTERN IN NORMAL WEIGHT MEN WITH INSULIN DEPENDENT DIABETES MELLITUS. EWCM van Dam, EWGM Lentjes and HMJ Krans. University of Leiden, Leiden, The Netherlands.

Androgens, insulin resistance and hyperinsulinemia are supposed to be factors involved in the pathogenesis of atherosclerosis. Current literature suggests that insulin resistance and hyperinsulinemia are accompanied by decreased serum concentrations of Testosterone (T), Sex Hormone Binding Globulin (SHBG) and Dehydroepiandrosterone Sulphate (DHEAS). Persons with IDDM have increased (peripheral) insulin levels and develop insulin resistance. Therefore we compared fourty-seven men with IDDM of 2 to 15 years duration, in the age of 30 to 45 years, without complications or other diseases, with 44 sex-, age- and weight-matched controls, who have no relatives (up to third degree) with Diabetes Mellitus. The following results were obtained (t-test for independent samples):

	control (n)	IDDM (n)	p-value
$Insulin_{day}$ (U/day)	-	58.1±1.9(47)	-
$glucose_{fasting}$ (mmol/l)	4.8±.1 (44)	11.7±.8 (46)	0.000
HbA1c (%)	5.2±.1 (44)	8.1±.2 (45)	0.000
testosterone (nmol/l)	22.7±.8 (43)	25.2±.9 (47)	0.036
SHBG (nmol/l)	27.9±1.47(44)	41.7±2.42 (46)	0.000
FAI (Tx100/SHBG)	86.7±5.1 (44)	67.5±3.5 (46)	0.002
DHEAS (umol/l)	7.5±.3 (44)	6.7±.3 (47)	0.189

We found a slightly increased T, a clearly increased SHBG, a decreased free androgen index (Tx100/SHBG) and an unchanged DHEAS in IDDM. These findings suggest that 1) there is no support for a direct connection between hyperinsulinemia or insulin resistance and DHEAS 2) peripheral hyperinsulinemia and insulin resistance are accompanied by unsuppressed SHBG and 3) the FAI and thus bioavailability of T is lower in longstanding hyperinsulinemia and insulin resistance, as a consequence of a strongly increased SHBG and less increased total T. Further statistical analysis, eg logistic regression will be performed on these factors and other involved hormonal factors.

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SEVERE INSULIN RESISTANCE IN PITUITARY APOPLEXIA.

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A 27 year old non diabetic acromegalic man developed severe insulin resistance after pituitary apoplexia. Large doses of human regular insulin (700-900 U/day) intravenously were necessary for glycemic control for 3 days when the insulin doses were progressively decreased and 14 days after the acute event the glycemic levels became normal without insulin therapy . During acute phase fasting C peptide was 4.63 nM , GH 85 μg/L, IGF-1 1730 ng/ml, and insulin antibody levels negative. To investigate if a factor present in serum could be responsible for this unusual insulin resistance, TNF α was measured (bioassay) and patient's sera during acute phase (S_1) and 30 days after apoplexia (S_2)(IGF-1 905 ng/ml) were incubated during 60 minutes with rat epididymal adipose cells. To avoid the IGF-1 influence a control serum (C) in which IGF-1 was added to reach similar concentration to that in patient's serum was also incubated with the adipocytes. After incubation cells were washed five times in Krebs-Ringer III buffer. Insulin binding was determined incubating ^{125}I -insulin with the adipose cells and glucose transport by incubation with D-U-^{14}C-glucose with increasing amounts of insulin for 60 minutes.

	Glucose transport(nmoles/2x10^{5}cells)			%Binding	TNFα
Ins (μU/ml)	0	50	100	(2x10^{5}cells	IU
S1	16.6	49.6	78.2	5.0	2,560
S2	19.6	80.3	124.1	3.8	4
C	20.2	86	117	4.6	<8

Impaired glucose transport was observed in spite of increased insulin binding in the adipocytes treated with the patient's serum with high TNFα levels. However when the cytokine level became normal, glucose transport was restored. TNFα at very high concentration was probably an important factor for the insulin resistance presented by the patient.

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ALBUMIN-SEX HORMONE-BINDING CAPACITY IN IDDM

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It has been recently recognised that the bioavailable testosterone (T) includes not only the free but also 55% of the albumin-bound fraction. Forty and 60 % of T and oestradiol (E2) respectively are carried by albumin. Our aim was to investigate if the albumin-bound T and E2 will be affected in IDDM and if there will be any correlation with the degree of albumin glycation. Twelve male and 18 female IDDM patients 38.5±6.6 years (mean±SEM) with a mean duration of DM of 15.1±5.4 were compared with 23 age & sex matched controls. Fasting blood samples were withdrawn from both groups for measurement of serum albumin, serum fructosamine (as an indicator of albumin glycation), serum total and albumin-bound T in males and E2 in females. Serum albumin was similar in controls and DM. Albumin-bound T in IDDM (mean+SEM nmol/L) was 2.4±0.7 compared to 5.17±0.2 in controls ($p< 0.001$). In females, albumin-bound was similarly reduced in IDDM compared to controls; 46.99 ± 2.83 pmol/L and 120.01 ± 3.82 respectively ($p< 0.001$). Serum fructosamine showed significant negative correlation with the albumin-bound sex hormone fractions with a correlation coefficient (r) of 0.62 in males and 0.87 in females. Glycated albumin sex hormone- carrying capacity is significantly impaired in DM, with a reduction in the bioavailable fractions. The clinical relevance and impact of DM control merit further investigation.

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PLASMA INSULIN GROWTH FACTOR 1 CONCENTRATION AND INSULIN ACTION IN HEALTHY CENTENARIANS

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Independently of anthropometric characteristics healthy centenarians (HC) have been shown to have an insulin-mediated glucose uptake (IMGU) better than aged subjects (AS). Since IGF-1 affects insulin action one can not exclude that difference in plasma IGF-1 concentration might provide an explication. Thus, in 30 (13M/17F) healthy AS (mean age=78±0.7 yrs) and in 19 (8M/11F) HC (mean age=102±0.8) anthropometric characteristics were assessed by bioimpedance analysis, plasma leptin,insulin,insulin growth factor I (IGF-1) and insulin growth factor binding protein-3 (IGFBP-3) concentrations determined by radioimmunoassay and insulin action measured by euglycemic glucose clamp. Total plasma IGF-1 concentration was not different between AS and HC (82±53 vs 65±49 μg/L p=NS) while plasma IGFBP-3 concentration was greater in AS than HC (2993±1129 vs 1789±1007 μg/L $p<0.001$). Consequently, plasma IGF-1/IGFBP-3 molar ratio, expression of unbound plasma IGF-1, was greater in HC than AS (0.18±0.08 vs 0.11±0.05 $p<0.02$). Similarly, IMGU was greater in HC than AS (32.4±0.8 vs 22.4±0.4 μmol/Kg FFM x min $p<0.01$).In HC plasma IGF-1/IGFBP-3 molar ratio correlated with body fat ($r=-0.62$ $p<0.003$), fasting plasma leptin ($r=-0.63$ $p<0.004$), fasting plasma glucose ($r=-0.46$ $p<0.02$), fasting plasma triglycerides (T) ($r= -0.58$ $p<0.01$) and fasting plasma free fatty acids (FFA) ($r=-0.64$ $p<0.005$) concentrations. The correlations between plasma IGF-1/IGFBP-3 molar ratio and plasma T ($r= -0.44$ $p<0.05$) and FFA ($r= -0.53$ $p<0.03$) concentrations persisted after correction for daily carbohydrate and fat intake and IMGU. Finally, in HC plasma IGF-1/IGFBP-3 molar ratio correlated with IMGU ($r=0.64$ $p<0.005$). This latter correlation persisted after adjustment for body fat, daily carbohydrate and fat intake and daily physical activity ($r= 0.55$ $p<0.009$) but not after further adjustment for plasma FFA ($r=0.33$ $p=0.17$). In conclusion, HC have unbound plasma IGF-1 concentration greater than aged subjects. Such difference might explain a better IMGU in HC through an inhibition of glucose-fatty acids cycle.

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The roles of DHEA and DHEAS in NIDDM patients : a possible modulator of glucose metabolism

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Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) are the most abundant steroids and have been shown to ameliorate diabetes in experimental animals. Insulin seems to play a regulatory role in DHEA(S) metabolism according to the results from hyperinsulinemic clamp studies. However, the exact roles of these hormones in NIDDM have not been elucidated so far. To clarify this, DHEA and DHEAS levels were determined in various conditions in middle aged NIDDM male patients (n=21). When glycemic control was poor(FPG199±54mg/dl,mean±SD), basal morning DHEAS, but not DHEA levels were lower in NIDDM (1622 ±655ng/ml) when compared to the age and the BMI matched controls (2071±635,$p<0.001$). In the course of diabetic treatment period(29days), the DHEAS levels did not change significantly. Postprandial acute elevation of insulin levels, either endogenously or exogenously, caused a concomitant decrease in DHEA not in DHEAS. This was also observed after the glycemic control was achieved. However, there was no significant correlation between basal insulin and DHEA or DHEAS levels throughout the treatment course. In contrast, there was a significant positive correlation between DHEAS and fasting glucose both before($r=0.599$,$p=0.005$) and after($r=0.496$,$p=0.029$) treatment. In conclusion, 1) in the middle aged male NIDDM patients, the basal DHEAS levels were diminished compared to controls. 2) Acute elevation of serum insulin was accompanied with a decrease in DHEA irrespective of glucose levels. 3) There was a significant positive correlation between fasting glucose and DHEAS. From these observation,there is a difference between acute and chronic insulin action on adrenal androgen metabolism in diabetes. DHEAS may serve as a modulator of glucose metabolism in chronic hyperglycemic state.

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A RAPID RISE IN INSULIN-LIKE GROWTH FACTOR-I AND A DECREASE IN INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-1 AFTER ALCOHOL WITHDRAWAL.

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Moderate alcohol intake may improve insulin sensitivity, whereas insulin resistance has been reported in alcohol abusers. We studied the possible mechanisms behind the altered carbohydrate metabolism in alcoholics. Blood glucose, serum insulin, plasma insulin-like growth factor-I (IGF-I) and IGF-binding protein-1 (IGFBP-1) were measured in 27 male alcoholics at the end of a drinking period, and thereafter, during three consecutive days along the abstinence. A single blood sample was obtained from 26 healthy male controls. The groups were similar for age and BMI. Insulin/glucose (I/G) -ratio was higher in the alcoholics at the end of the drinking period compared with the controls (3.1±1.9 vs. 2.3±0.9, respectively, $p<0.05$), whereas IGF-I levels tended to be lower in alcoholics (8.8±2.9 vs. 10.3±4.3 nmol/L, p=NS). I/G -ratio increased by +61 %, and IGF-I increased by +41% during the alcohol withdrawal ($p<0.01$and $p=0.001$ after ANOVA, respectively). In alcoholics, an inverse correlation between insulin and IGFBP-1 ($r=-0.43$, $p<0.05$) was seen only at the end of the drinking period. Cessation of drinking induced a decrease of -59% ($p<0.001$) in IGFBP-1 levels. In conclusion, alcohol abuse is associated with decreased insulin sensitivity. Immediately after the cessation of drinking, insulin sensitivity is even more deteriorated. Acute alcohol intake has been suggested to stimulate the secretion of IGFBP-1 in the liver. Our data supports such an effect also in chronic alcohol abusers, since alcohol withdrawal is associated with a dramatic decrease in the IGFBP-1 plasma levels. A loss of the normal inhibitory regulation of IGFBP-1 by insulin during the withdrawal is suggested in the present study.

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DEHYDROEPIANDROSTERONE-INSULIN RELATIONSHIP IN MINIMAL MODEL BASED IVGTT ANALYSIS

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Dehydropepiandrosterone (DHEA) deficiency is related to aging, hypertension, obesity and diabetes. Possible DHEA-insulin interactions were investigated in 8 normal men and 6 diet treated normoglycemic type 2 diabetic men during intravenous glucose tolerance test (0.3g/kg glucose). A minimal model based calculation of insulin sensitivity (SI) was performed. A significant correlation between SI and basal DHEA level was found ($r=0.65$, $p=0.02$). Linear regression indicated that SI is linearly related to the basal DHEA level, SI= -0.534 + 0.105 x DHEA. An early DHEA elevation within first 30 minutes was observed in most patients. Using stepwise regression we obtained model where DHEA elevation area (DA) was a function of early insulin peak (IP) and early insulin peak time (IPT). DA = -159.7 + 2.885xIP -2.651xIPT. Age, BMI, SI and calculated remote insulin level failed to enter the equation. No changes in and correlation between DHEA-sulphate levels were found. We conclude that basal DHEA level is related to insulin sensitivity. DHEA response during IVGTT is induced by the first phase insulin secretion.

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ACUTE REGULATION OF FREE INSULIN-LIKE GROWTH FACTOR-I BY INSULIN.

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Exogenous insulin-like growth factor (IGF)-I suppresses insulin secretion and causes hypoglycemia by an insulin-independent mechanism. Long term insulin treatment of type 1 diabetes results in increased circulating total IGF-I concentrations. Little is known, however, about the short term effects of insulin or glucose on circulating free IGF-I. We have evaluated the effects of a single bolus intravenous injection of glucose and insulin on serum free IGF-I in 11 normal subjects (6 men and 5 women, age 27.4 ± 7.4, BMI 23.2 ± 2.3 SD). After an overnight fast, subjects had a 180-min insulin modified frequently sampled glucose tolerance test (FSIGT) with injection of glucose at time=0 and of insulin at 20 min Serum free IGF-I, total IGF-I, IGF-binding protein (IGFBP)-1 and IGFBP-3 were measured by immunoradiometric assays. IGFBP-3 remained constant during the first 30 min, then slowly increased following insulin injection to 20% above the basal value by 180 min. IGFBP-1 remained constant during the first 60 min, but fell steadily thereafter, resulting in an undershoot by 100 min. IGFBP-1 then sharply increased to 3-fold the basal levels by 180 min. Total IGF-I remained constant during the 180 min of the experiment. Free IGF-I decreased by 20% below basal levels following glucose injection and decreased further by 40% after insulin injection. After insulin, but not glucose injection, the decrease of free IGF-I was concomitant to the rise of IGFBP-3, and the percent change of free IGF-I was greater than that of IGFBP-3. In a cross-sectional analysis, free IGF-I was inversely correlated with serum glucose ($r_s=0.55$, $p<0.05$) and IGFBP-1 ($r_s=0.64$, $p<0.025$). Free IGF-I, thus, is suppressed by insulin and may play a physiological role in glucose homeostasis.

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PERIPHERAL GLUCOSE METABOLISM IN POLYCYSTIC OVARIAN SYNDROME BEFORE AND AFTER TREATMENT WITH GnRH ANALOGUE.

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The aim of this study was to investigate the peripheral glucose metabolism and the serum levels of IGF-I, IGFBP-1 and insulin in patients with polycystic ovarian syndrome before and after treatment with GnRH analogue. Eight normal (N) women and sixteen patients with polycystic ovarian syndrome (PCOS) were studied after an overnight fast (12-14 h) and during 3 hours after ingestion of 75 g of glucose. Five PCOS patients were studied before and after treatment with 1 mg of leuprolide acetate daily during 8 weeks. The study was performed using the forearm technique combined with local indirect calorimetry. The hyperinsulinemia detected in the PCOS patients after the oral glucose stimulus (AUC-N = 4836 ± 902 vs PCOS = 11151 ± 1632 µU/3 h) did not determine a proportional increase in forearm glucose uptake and utilization, which were similar to the normal levels, suggesting an insulin resistance state in PCOS. Comparing the PCOS patients before and after the treatment with the GnRH analogue, the glycemic profiles did not change significantly and the forearm glucose uptake showed no significant difference in the PCOS patients before and after treatment. However, the serum insulin levels decreased significantly (AUC: 12948 ± 3449 vs 8589 ± 1130 µU/3 h, before and after, respectively), resulting in I/G index significantly different (131.3 ± 24.6 vs 108.7 ± 25.9 $\mu U.ml^{-1}/mg.100$ ml $forearm^{-1}$, before and after, respectively). The serum levels of IGF-I did not change significantly, but the serum IGFBP-1 concentrations showed a trend to decrease during the treatment. These data suggest that the suppression of the hyperandrogenism may decrease the insulin resistance of nonobese patients with polycystic ovarian syndrome.

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THE ROLE OF GLUCAGON ON GLUCOSE INTOLERANCE IN PATIENTS WITH ACROMEGALY

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It is known that the incidence of glucose intolerance with insulin resistance in acromegaly is high. Various factors are suggested to play a role in insulin resistance in acromegaly, and plasma glucagon (IRG) may be one of those factors. We investigated the effects of IRG on glucose intolerance before and after Hardy's operation, using 75g-OGTT in 6 patients (DM pattern; 1 patient, IGT pattern; 5 patients) with acromegaly. Serum GH levels were normalized in all patients after surgery. 1 patient still remained DM pattern and 3 patients, IGT pattern, but the degree of glucose intolerance was improved, and 2 patients with IGT returned to a normal pattern after surgery. The plasma IRG concentrations during OGTT (mean $\pm$ SE) before surgery were 0': 80 $\pm$ 27, 30': 86 $\pm$ 32, 60': 79 $\pm$ 22, 120': 60 $\pm$ 16 pg/ml, which were not inhibited even though plasma glucose levels were increased. After surgery, they were 0': 62 $\pm$ 43, 30': 47 $\pm$ 23, 60': 45 $\pm$ 24, 120': 42 $\pm$ 19 pg/ml, which were inhibited through the increment of plasma glucose. In conclusion, after the removal of pituitary adenoma in 6 patients with acromegaly, glucose intolerance was improved, and plasma IRG levels and response to increment of glucose were normalized concomitantly with normalization of serum GH levels. Thus, it is suggested that hypersecretion of IRG might play a role in insulin resistance in patients with acromegaly.

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SPECIFIC INSULIN SECRETION AND INSULIN SENSITIVITY IN SUBGROUPS OF WOMEN WITH POLYCYSTIC OVARIES (PCO) AND WOMEN WITH NORMAL OVARIES.

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Hyperinsulinaemia and reduced insulin sensitivity (S_i) are recognised features of PCOS. Insulin secretion measured using RIA has been shown to be abnormal in women with anovulatory PCO. We aimed to assess insulin secretion, S_i and glucose effectiveness (S_g) in both ovulatory (ov) and anovulatory (anov) women with PCO and controls, using an assay for specific insulin. 45 women with PCO (18 ov and 27 anov) were compared to 18 (BMI matched) control subjects (N). S_i and S_g were measured with an insulin modified, frequent sampling intravenous glucose tolerance test and minimal modelling. Median [interquartile range] given. Fasting insulin levels (F_{ins}) were higher in anov compared to N but not ov (ov 11[32] pmol.l^{-1}, anov 17[33] vs N 3[16] $p<0.03$). First phase insulin area (AUC) was greater in anov but not in ov compared to N (ov 523[1112] pmol.l^{-1}, anov 572[1044] vs N 282[403] $p<0.02$). S_i was lower in anov in comparison to N and ov (anov 4.27[12.35] x10^{-4}.min^{-1}.pmol.l^{-1}. vs ov 5.98[35.49] $p<0.04$ vs N 9.09[20.82] $p<0.03$). S_g showed no significant difference between groups (ov 0.033[0.028] min^{-1}, anov 0.024[0.015], N 0.023[0.015]). Anov had higher testosterone (T) levels compared to N but not ov (ov 2.0[0.8] mmol.l^{-1}, anov 2.5[1.25] $p<0.01$ vs N 1.6[0.7]). However, anov and ov were more hirsute than N (ov 9[11] {Ferriman Gallwey score} $p<0.03$, anov 18[10] $p<0.01$ vs N 5[1]). There was no correlation between T and AUC or F_{ins} in any group. Triglyceride levels in anov (but not N or ov) were correlated to AUC ($p<0.05$) and F_{ins} ($p<0.03$). HDL levels were negatively correlated in anov and N but not ov to AUC (anov $p<0.01$, N $p<0.05$) and F_{ins} (anov $p<0.01$, N $p<0.02$). In conclusion, hyperinsulinaemia and decreased S_i were confirmed in a group of anovulatory women with PCO in comparison to women with normal ovaries. S_g however, was similar in these groups. Although the group of ovulatory women with PCO were equally hyperandrogenaemic they did not display the same degree of insulin abnormalities. In this group of women, high testosterone levels were not associated with hyperinsulinaemia.

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INSULIN RESISTANCE IN CUSHING'S SYNDROME AND ACROMEGALY - THE ROLE OF ENDOGENOUS BETA-ENDORPHIN.

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Insulin resistance and hyperinsulinemia (HI) are common features in Cushing's syndrome (CS), Cushing's disease (CD) acromegaly (A) and in human obesity with abdominal body fat distribution (ABF-D). It has been demonstrated that endogenous beta-endorphin (B-EP) may be responsible for HI in ABF-D obesity and that opioid receptors blockade by naloxone (NAL) decreases insulin (IRI) secretion and improves insulin sensitivity in obese subjects. To assess the possible influence of endogenous B-EP on the basal IRI secretion in patients with CS and CD and in A we evaluated IRI, cortisol (C), growth hormone (GH) and B-EP secretion and blood glucose concentration (as area under the 4 point's curve-AUC) during 60 min of 0.9% saline infusion (basal secretion) and during 60 min after NAL administration (0.8 mg iv.). Six patients with CS and impaired glucose tolerance (IGT), 8 persons with CD and normal glucose tolerance (NGT), 14 acromegalic patients (4 with NGT and 10 with type III diabetes) and 6 healthy subjects in similar age participated in the study. Serum or plasma hormone levels were evaluated by RIA. In all patients HI and insulin resistance were found. Patients with CS demonstrated markedly ($p=0.03$) lower basal IRI secretion than patients with CD. B-EP basal secretion was significantly higher only in patients with CD than in healthy subjects. Patients with A demonstrated lower basal IRI and C but higher basal GH secretion than patients with CD. NAL administration resulted in a marked decrease of basal IRI secretion in patients with CD and acromegalic patients with NGT (from 40.9 to 24.7 mU/l/min^{-1}, $p=0.003$ and from 27.4 to 23.6 mU/l/min^{-1} $p=0.04$ respectively) and basal C secretion (from 833.7 to 674.3 nmol/l/min^{-1}, $p=0.017$ and from 339.2 to 237.8 nmol/l/min^{-1} respectively). In all groups of patients NAL administration did not change blood glucose levels as well as basal secretion of GH and B-EP. These results suggest that endogenous B-EP may be in part responsible for HI in patients with CD and acromegaly with NGT and that opioid receptors blockade might improve insulin sensitivity in these syndromes.

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HYPERINSULINISM IN WOMEN WITH OVARIAN POLICYSTIC DISEASE ON TREATMENT WITH FLUTAMIDE.

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Hyperinsulinism has been depicted in association to policystic ovarian disease PCOD). In this study we have assessed the effects of flutamide, a non steroid antiandrogen on the glucose and insulin plasma levels on women diagnosed with hirsutism due to PCOD. A standard oral glucose tolerance test (OGTT) measuring plasma glucose and insulin every 30 minutes during 2 hours after oral administration of 75 g. of glucose was conducted in 12 nondiabetic and non obese women, BMI 22.3±2.4,aged 18 to 34, with mild to severe hirsutism secondary to PCOD, prior and after six monts of treatment with flutamide 250 mg bid. Contraception was guaranteed by means of an intrauterine device in 8 patients and by tube ligature in 4 cases. A control group of 10 women of the same age and BMI was also studied. Statistical study was done using the T-student test and the Spearman rank correlation coefficient. Plasma free testosterone which was elevated in the study group in relation to controls ($p<0.01$),significantly decreased after flutamide treatment ($p<0.01$). The basal plasma insulin levels did not change but the insulin levels at 120 minutes after OGTT significantly decreased after treatment (from 99.2±66.6 to 68.2±35.0,$p<0.05$), as well as the area under the curve of insulin (from 11875±7684.5 to 9132±3910, $p<0.05$). We found significant correlation between the plasma levels of free testosterone and the area under the curve of insulin (r :0.56, $p<0.05$). Hyperinsulinism associated to PCOD significantly decreases on treatment with the antiandrogen flutamide.

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THE PREDICTORS OF THE INCREASE OF BLOOD GLUCOSE IN HUMANS WITH NORMAL GLUCOSE TOLERANCE.

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The purpose of the study was to find the predictors of blood glucose levels (Bgl) during oral glucose tolerance test (OGTT) in insulin resistant patients (active acromegaly n=15) and in healthy people (n=30), both groups with normal OGTT according to WHO criteria. We considered fasting serum insulin (INS), C-peptide (CP), growth hormone (GH) and plasma glucagon (GG) as predictors of blood glucose in OGTT. Analysis of variance with repeated measurements was used in statistical analysis. From the above parameters fasting INS was the predictor of the higher values of blood glucose in OGTT in insulin resistant patients ($p<0.03$), but not in healthy people.

Insulin resistant group		Healthy group	
INS (pmol/l)	glucose in OGTT (mmol/l)	INS	glucose in OGTT
>59	5.4 8.6 8.7 6.9 5.0	>36	4.6 7.7 7.2 5.5 4.8
≤59	4.2 7.2 6.3 5.5 4.9	≤36	4.2 7.2 6.6 6.1 5.3

We found that interrelation among fasting molar INS, CP, GG concentrations expressed as the index = (CP/GG) / (INS/CP) occurred to be the predictor of increased Bgl in OGTT. Lower values of this index both in insulin resistant patients (<517) and in healthy people (<170) was significantly accompanied with elevated blood glucose levels in OGTT ($p<0.01$ and $p<0.056$ respectively).

Insulin resistant group		Healthy group	
Index	glucose in OGTT (mmol/l)	Index	glucose in OGTT
≤517	5.2 8.3 8.2 7.0 5.3	≤170	4.4 8.1 7.7 6.1 5.0
>517	4.1 7.1 5.5 4.8 4.5	>170	4.4 7.1 6.4 5.5 5.0

Conclusion: Proposed index allows to predict the inclination to development of impaired glucose tolerance in insulin resistant patients, furthermore indicates the group prone to the higher blood glucose in healthy people as well.

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ORAL GLUCOSE TOLERANCE TEST BEFORE AND DURING HORMONE REPLACEMENT THERAPY IN MENOPAUSAL WOMEN

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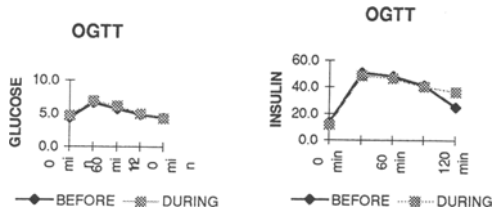
The main aim of this study was evaluating sex steroid concentration (estradiol and rogesterone) influences on glucose and insulin metabolism in menopausal women before and during hormone replacement therapy (HRT). We have examined 22 women 52±3 y's old, BMI=26.6±3, 85 kg/m^2, 2±1 year in the menopause. Standard 75g oral glucose tolerance test (OGTT), with glucose and insulin determination, was performed before and one year later during the HRT with Gynodian depot amp. I i.m. monthly. Insulin concentrations were detected by RIA. Statistics: Student's T test. Glucose and insulin, during OGTT were shown on Fig.1 & 2. CONCLUSION: There were no statistic significant differences in OGTT characteristics in menopausal women before and during HRT.



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MECHANISMS OF EFFECTS OF INSULIN AND HYPERTHYROIDISM TO MODIFY TISSUE PYRUVATE DEHYDROGENASE KINASE ACTIVITIES

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Pyruvate dehydrogenase kinase (PDHK) catalyses the phosphorylation (inactivation) of the pyruvate dehydrogenase complex (PDHC). PDHK activity in rat hepatic and cardiac mitochondrial extracts is increased ≈ 2 fold by the induction of hyperthyroidism by tri-iodothyronine (T3) administration. Studies with cardiomyocytes and hepatocytes have shown direct tissue effects of T3 to enhance PDHK activity which are opposed by insulin and by inhibition of mitochondrial β-oxidation. The aim was to determine whether increased PDHK activities elicited by hyperthyroidism are due to changes in enzyme concentration or specific activity, and thereby to gain insight into mechanisms by which insulin might suppress PDHK activity. Antibodies were raised to purified recombinant PDHKII. The antiserum was specific to PDHKII, with negligible cross-reaction to PDHKI as assessed by Western Blots. E.l.i.s.a. assays of PDHKII were conducted over a range of PDH activities (from 0.03 to 4 m-units/well). The amount of mitochondrial immunoreactive PDHKII in heart was unaffected by hyperthyroidism; however, the amount of mitochondrial immunoreactive PDHKII in liver was significantly increased (by 1.5 fold; $P<0.001$) together with a 2.2 fold increase in PDHK activity (1.7±0.1 min^{-1} to 3.6±0.3 min^{-1}; $P<0.001$). Total PDHC activity was unaffected. In conclusion, hyperthyroidism increases cardiac PDHKII specific activity, but has a long-term action to increase PDHK concentration in liver. Since the increase in hepatic PDHK concentration and activity occurs without a change in PDHC total activity, the results demonstrate that the expression of individual PDHC components are regulated independently. Additionally, hepatic PDHK can be added to the cohort of gene products whose expression is regulated by insulin and thyroid hormone.

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POSTMENOPAUSAL ESTROGEN REPLACEMENT THERAPY MAY IMPROVE GLUCOSE TOLERANCE?

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Previous studies concerning the effect of estrogen replacement therapy (ERT) on glucose metabolism have suggested that non-oral delivery of estrogen might be more beneficial due to the bypass of the first-pass effect of liver, but the results have been controversial. To compare the effects of peroral estradiol valerate 2 mg/d with percutaneous estradiol gel 1mg/d, (Divigel, Orion Pharma), 79 hysterectomized, non-diabetic postmenopausal women aged 48 to 62 years were randomized to a double-blind, double-dummy trial. After overnight fast glycosylated hemoglobin ($GHbA_{1c}$) and standard 75 g oral glucose tolerance test including fasting and postchallenge blood glucose, serum insulin and C-peptide (at 30, 60 and 120 minutes) measurements, were determined at baseline and after six months of ERT. Area under the curve (AUC) was calculated for parameters of glucose tolerance test. Minor weight gain, +0.6 kg, and increased body mass index, +0.21 kg/m^2, were noticed in percutaneous estrogen group, but not in peroral therapy. Both percutaneous and peroral estrogen reduced $GHbA_{1c}$, mean change -0.15 % ($p<0.01$) and -0.17 % ($p<0.01$), respectively. The mean decrease of AUC for C-peptide (μg/l·h) was 0.95 ($p<0.05$) in gel group and 1.0 ($p<0.01$) in peroral group. No other significant changes were noticed in fasting or postchallenge glucose and insulin levels or AUCs for glucose and insulin. In conclusion, after six months of ERT both regimens reduced $GHbA_{1c}$ and AUC for C-peptide, but glucose and insulin levels remained unchanged. No differences between treatments were noticed. Decreased $GHbA_{1c}$ suggests long-term beneficial effects on glucose and insulin metabolism, which may be obtained by lower endogenous insulin (C-peptide) secretion as suggested by reduced AUC for C-peptide.

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RELATION BETWEEN THYROID FUNCTION AND CYTOKINE LEVELS IN DIABETIC PATIENTS.

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In non-thyroidal illness including diabetes mellitus, euthyroid sick syndrome or low T3 syndrome is sometimes found. Recent reports demonstrated that several cytokines may modulate thyroid function. In an atempt to elucidate the mechanisms of abnormalities of thyroid hormone metabolism in diabetic patients, we have studied the relationship between thyroid function parameters and cytokines.

Subjects and Methods: Peripheral blood was obtained from 39 diabetic patients who had no endocrine disease including thyroid and autoimmune diseases. Serum TSH, free T4 (FT4), free T3 (FT3) and reverse T3 (rT3) were measured by radioimmunoassay kits. Plasma interleukin (IL) -1β, IL-6, IL-1 receptor antagonist (IL-1ra) and tumor necrosis foctor-α (TNF) were measured by ELISA kits. Results: TNF levels were positively correlated with HbA1c levels ($p<0.05$). TNF levels were neither correlated with thyroid hormones nor TSH. IL-6 levels were positively correlated with rT3 ($p<0.01$). IL-1ra levels were positively correlated with rT3 ($p<0.05$). IL-6 and IL-1ra were not correlateded with any diabetic control markers. IL-1β levels were not correlated with thyroid hormones and diabetic control states. All the cytokines measured were not parallelly associated with FT3 or FT4.

Conclusion: Our results suggest that IL-6 and IL-1ra may increase serum rT3 levels in diabetic patients.

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EFFECT OF TESTOSTERONE REPLACEMENT THERAPY ON INSULIN SENSITIVITY AND OTHER CARDIOVASCULAR RISK FACTORS IN MALES WITH IDIOPATHIC HYPOGONADOTROPHIC HYPOGONADISM (IHH)

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Background: Women in child bearing age have 2-3 times lower incidence of coronary heart disease (CHD) compared to men; the rates equalise after menopause, and, with hyperandrogenism. It is not clear whether testosterone is predisposing or oestrogen's are protective to CHD. **Aim:** to look at the role of testosterone in development of insulin resistance and other cardiovascular risk factors. **Design:** prospective, "before-after" study on ten IHH males pre- and post-testosterone replacement therapy; *outcome measures:* anthropometry, lipoprotein profile and M value (whole body glucose disposal rates on standard hyperinsulinaemic euglycaemic clamp; insulin infusion rate: 40mU/m^2). **Results:** Base line upper segment (77.8 ± 1.73cm), was less than lower segment (96.3±1.73); body span was 182 ± 2.96cm; mean weight was 62.1kg; BMI was 20.5 ± 1.86kg/m^2; waist-hip ratio was 0.865 ± 0.25; none had anosmia. Pre-treatment serum testosterone was 0.43 ± 0.24 ng/ml, LH was 1.29 ± 0.72IU/L and, FSH was 1.42 ± 0.79 IU/L. None had glucose intolerance. After replacement testosterone levels increased to 9.3 ng/ml ($p=0.0005$), weight increased by 5.0 kg ($p=0.140$), BMI increased to 21.7 ± 1.79 kg/m^2 ($p=0.285$), no change was seen in WHR (0.863 ± 0.266); skin fold thickness decreased (triceps to 24.3± 2.92 vs. 22.3 ± 2.84mm, $p=0.85$). There was no change in testicular volume $p=0.14$. M-value (mg/kg/min) did not change after testosterone therapy (5.72 ± 1.58 vs. 5.33 ± 1.26, $p=0.766$). Insulin levels (mU/L) achieved during the clamps were 89.5 ± 6.7 before and 146 ± 10.3 after androgen therapy ($p=0.127$). There was no change in glucose area under curve (mg.min.dL^{-1}) 14406 ± 39.8 vs. 12557 ± 51.1, $p=0.312$). On testosterone replacement therapy total and LDL cholesterol levels declined (123.1 ± 6.5 vs. 91.6 ± 3.94, $p=0.007$; 65.9 ± 31.7 vs. 39.4 ± 23.7, $p=0.045$); Ratio of total cholesterol to HDL ratio also decreased significantly ($p=0.05$). No changes were seen with triglyceride ($p=0.247$), HDL cholesterol levels ($p=0.198$). **Conclusions:** Testosterone replacement therapy of IHH was associated with decrease in total cholesterol, LDL cholesterol, and cholesterol to HDL ratio. There was no change in HDL and triglycerides. Insulin sensitivity did not decrease on testosterone replacement. The gender difference in coronary heart disease has to be explained by some mechanism other than testosterone causing insulin resistance.

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THE RELATIONSHIP BETWEEN THE GLYCEMIC CONTROL AND THE HYPOTHALAMUS-PITUITARY-THYROID AXIS IN DIABETIC PATIENTS

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Diabetes mellitus, similar to other nonthyroidal illness, is associated with circadian rhythm abnormalities of serum TSH and thyroid hormones.

In this study, we investigated the effect of good and poor metabolic control on the nocturnal serum TSH peak and the TSH response to TRH stimulation in diabetic patients. 32 diabetic patients (insulin dependent,n=9; noninsulin dependent,n=23;18 men,14 women ; the mean age :45.8±10.5 yrs) with either poor glycemic control (n=22) or good glycemic control (n=10) were enrolled in this study. The nocturnal serum TSH peak (22^{30}-02^{00}h) was abolished in the poor glycemic control group ,whereas there was statistically significant peak in the other group($p=0.0001$).The morning serum TSH value in diabetics with the poor glycemic control group did not differ from that in the other group, but the serum TSH and TT4 were significantly higher in the good glycemic control group than the other group and no differences were found in the increase of the serum FT4,TT3 and FT3 levels between the two groups after TRH stimulation.The morning serum TT4 and TT3 levels were significantly higher in the good glycemic control group than the other group ($p=0.04$,$p=0.007$) whereas the morning serum FT4, FT3 and TSH values did not differ in the two groups.The increase of the serum TSH and TT4 levels after TRH stimulation were significantly higher in the good glycemic control group than the other control group whereas there were no difference in the increase of the serum TT3, FT4 and FT3 levels between the two groups.

In conclusion,the metabolic control affects the hypothalamus-pituitary-thyroid axis and the metabolic decompensation in diabetic patients leads to the impairment of TSH secretion, thyroid hormones secretion their response to TRH.

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GENDER DIFFERENCE IN THE RELATIONSHIP BETWEEN INSULIN RESISTANCE AND SHBG LEVELS IN SUBJECTS AT INCREASED RISK OF DEVELOPING NIDDM.

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Decreased plasma SHBG levels predicts noninsulin dependent diabetes, frequently accompanied with insulin resistance, in women but not in men. Thus the relationship between insulin resistance and SHBG may be different in men and in women. However, there has been few data in vivo on the relation of insulin resistance to SHBG in men. To clarify this issue, 119 subjects (56 men and 63 women) with borderline glucose tolerance, proposed by Japan Diabetes Society and proven to be at increased risk of developing NIDDM, received 2hr of euglycemic hyperinsulinemic clamp studies under primed and continuous infusion of insulin at a rate of 40 mU/m^2/min. Circulating SHBG levels were measured before and 2hr after the beginning of the clamp studies. Glucose infusion rate during the last 30 minutes of clamp studies (GIR) and basal SHBG levels correlated positively both in men ($r=0.40$, $p<0.01$) and in women ($r=0.45$, $p<0.01$). As expected, age, BMI and WHR correlated with SHBG and with GIR in men and/or in women. Multiple regression analysis, selecting SHBG as dependent variable and age, BMI, WHR and GIR as independent variables, disclosed that GIR related to SHBG independently in women but not in men. Thus the positive correlation between GIR and SHBG in men may be an epiphenomenon. On the other hand, during the clamp procedure, circulating SHBG levels decreased significantly only in women (60±39 nmol/L→50±34, M± SD, $p<0.025$) but not in men (30±14→28±12, $p>0.05$). These results indicate that the relationship between insulin resistance and SHBG, and acute effect of insulin on circulating SHBG levels are different in men and in women, which could explain the gender difference in the role of decreased SHBG predicting the development of NIDDM .

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INSULIN SENSITIVITY IN MALNUTRITION DUE TO ANOREXIA NERVOSA - RELATIONSHIP BETWEEN HORMONAL DISTURBANCES.

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In obese persons with abdominal body fat distribution (ABF-D) and in some subjects with anorexia nervosa (AN) impaired insulin sensitivity and hyperbeta-endorphinemia were found. It has been demonstrated that endogenous beta-endorphin (B-EP) may be in part responsible for hiperinsulinemia (HI) and insulin resistance in ABF-D obesity. To assess the possible relatonship between insulin resistance and hormonal disturbances in patients with anorexia nervosa we evaluated basal serum/plasma insulin (IRI), glucose (G), growth hormone (GH), B-EP and cortisol (C) concentrations and their response to oral glucose load (OGTT). Eight patients with AN and normal glucose tolerance (NGT), 10 AN patients with impaired glucose tolerance (IGT) and 6 healthy subjects at similar age participated in the study. Serum or plasma hormone levels were evaluated by RIA and blood glucose by oxidase method. Patients with AN and IGT demonstrated markedly (p=0.03) higher basal IRI secretion than patients with NGT but insulin response to OGTT in both groups did not differ. In patients with AN and IGT insulin resistance assessed as IRI/G ratio in basal condition showed significant linear correlations with basal B-EP (r=0.76; p=0.01) and cortisol (r=0.68; p=0.03) plasma levels. In this group of patients basal plasma B-EP concentrations correlated with basal serum IRI levels (r= 0.70; p=0.01). In AN patients with NGT such correlations were not found. Both groups did not differ in terms of basal B-EP, GH, cortisol and glucose levels as well as BMI. These results suggest that increased opioid sensitivity and hypercortisolemia may be in part responsible for insulin resistance in some patients with anorexia nervosa.

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SEX DIFFERENCES IN GLUCOSE DISPOSAL IN NIGERIANS

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There appears to be a dearth of studies on glucose disposal rate in Nigerians. This study was carried out to assess the effect of sex and anthropometric indices on glucose disposal rate, an index of beta-cell function. One hundred and two non-diabetic subjects (50 males and 52 females) aged 13 - 47 years were tested. Age, sex, body mass index, and waist hip ratio were recorded. Each subject had an intravenous glucose tolerance test using 25g of a 50% dextrose solution, and the decay in plasma glucose levels was utilised in deriving the glucose disposal rate. Females had a significantly higher mean (SD) glucose disposal rate [2.14 (0.56) % per minute in females, 1.9 (0.49) per minute in males, $p < 0.001$). Glucose disposal rates decreased with age ($r = -0.25$ in males, $p > 0.05$, $r = -0.17$ in females, $p > 0.2$); with body mass index ($r = -0.15$, $p > 0.05$ in males, $r = -0.29$, $p < 0.05$ in females). There was no correlation between glucose disposal rate and waist hip ratio. This study shows a significant sex difference in glucose disposal rates and that Nigerian females dispose of glucose more efficiently than the males.

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ACQUIRED INSULIN RESISTANCE IN EARLY HIV AND ASYMTOMATIC AIDS

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HIV-infected individuals are susceptible to opportunistic infections as their immune system deteriorates in the face of increasing viral burden. It is now clear that there is a prolonged latency period of HIV infection with progressive deterioration of immune function prior to the onset of AIDS. Separate metabolic and endocrine evaluations of HIV-infected individuals at different stages of immune may allow for the characterization of processes that result in increased morbidity and mortality. Thus, we have performed anthropometric measurements and assayed for various endocrine and metabolic factors in HIV-infected individuals with various clinical manifestations. Five cohorts of male participants were established as follows; Controls (HIV seronegative individuals, N=4, BMI=24.8, Age=38), Early HIV(infected individuals with CD4 counts > 500 cells/mm3, N=5, BMI=23.7, Age=41), Asymtomatic AIDS (infected individuals with CD4 counts < 200 cells/mm3, N=9, BMI 24.4, Age 42), AIDS with wasting (N=3, BMI=19.5, Age 48), and AIDS with wasting and active opportunistic infection (N=4, BMI=18.6, Age 45). As compared to age and BMI matched controls, fasting plasma insulin levels [142.08±43.2 pmol/L vs 40.44±1.8 pmol/L ($p<.0055$)] and percent body fat [24.5±1.2% vs 17.9±2.6% ($p<.049$)] were significantly greater in Asymtomatic AIDS patients. In Early HIV individuals, insulin levels and percent body fat (52.8±12 pmol/L and 20.4±2.3 %, respectively) were increased compared to controls, but not significantly. Insulin levels were increased in both AIDS with wasting and AIDS with wasting and opportunistic infection cohorts despite smaller BMI and lower percent body fat as compared to controls, however these differences also did not reach statistical significance. Fasting blood glucose and triglyceride levels, and basal and stimulated cortisol and ACTH levels were similar among the five cohorts. The results of this small study suggest that fasting hyperinsulinemia/insulin resistance is observed among HIV-infected individuals with decreasing immune function. This acquired insulin resistance before the onset of wasting with and without opportunistic infection appears to be associated with increased percent body fat without an increase in weight. We speculate that progressive HIV infection and the resultant deterioration of immune function rather than increased glucocorticoid levels account for insulin resistance. We conclude that with new anti-retroviral therapy that increase long-term survival among the HIV-infected population, the acquisition of insulin resistance may present significant increases in glucose intolerance and cardiovascular disease.

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RELATIONSHIP BETWEEN DHEA-S AND INSULIN SENSITIVITY IN PATIENTS WITH NON-INSULIN DEPENDENT DIABETES MELLITUS.

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The circulating level of dehydroepiandrosterone-sulfate(DHEA-S) which is thought to have anti-atherogenic action has been reported to be inversely related to death from any cause, especially cardiovascular disease in men. Little was known about the mechanism of the effect of DHEA-S on insulin sensitivity in the patients with NIDDM. We, therefore, attempted to investigate the effect of *DHEA-S on insulin sensitivity and lipid metabolism* in patients with NIDDM. Blood levels of DHEA-S and various lipids were estimated before hyperinsulinemic euglycemic glucose clamp(HEGC) in *158 patients with NIDDM*. Those subjects were divided to two groups according to the serum level of LDL-cholesterol(LDL-C) and HDL-cholesterol(HDL-C) as follows: 81 diabetics showed high level of LDL-C *more than 120mg/dl(H-LDL) and 77 cases did low level of LDL-C less than* 120mg/dl(L-LDL); 71 subjects showed high level of HDL-C more than 50mg/dl(H-HDL) and 87 cases did lwo level of HDL-C less than *50mg/dl(L-HDL)*. *M values during HEGC which are reflecting insulin* sensitivity in whole body were significantly higher in H-HDL than in L-HDL. It was also observed that serum levels of DHEA-S was significantly *decreased by aging. There was significantly a positive correlationship* between M values and serum DHEA-S levels in both of H-LDL(*) and L-LDL(*) groups, while there was not observed in L-LDL and H-HDL groups. *There was a significant and positive correlation between serun levels of* total cholesterol and DHEA-S in H-LDL(*) and L-HDL(*) groups. (*$p<0.05$). In conclusion, DHEA-S may play a crucial role in the regulation of insulin *sensitivity and lipid metabolism in diabetics.*

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DIFFERENCE IN BONE DENSITY ACCORDING TO SEX IN NIDDM

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Recently, study on relationship between diabetes mellitus and osteoporosis is gaining background due to increased interest in osteoporosis among general population. The authors achieved following results by comparatively analyzing bone mineral density (BMD) and number of factors considered to have influence on BMD in 137 participants. BMD measured by dual x-ray absorptiometry at lumbar spine. Among 24 men and 113 women, 8 men and 79 women had normal glucose tolerance but 16 men and 34 women had NIDDM. Men with NIDDM had BMD level similar to those men with normal glucose tolerance, whereas women with NIDDM had significantly higher BMD levels than age matched control group. The mean BMD in women with NIDDM compared with normoglycemic women was $1.08\pm0.1 g/cm^2$ vs $0.91\pm0.2 g/cm^2$ at lumbar spine. Both group and sex had similar body mass index and their mean age was 57.2 ± 9.9years. Mean estrone, SHBG, free testosterone level in women with NIDDM compared with control group are 129.13 ± 78.1pmol/L vs 95.8 ± 92.8pmol/L, 53.7 ± 30.5 nmol/L vs 81.5 ± 36.4nmol/L, 0.039 ± 0.013nmol/L vs 0.037 ± 0.009nmol/L, respectively. But the difference had no statistical significance. In female NIDDM patients, BMD and SHBG showed negative correlation. In conclusion, older women with NIDDM or hyperglycemia had better BMD than women with normal glucose tolerance but no difference in bone density by diabetic status were observed in men. Further study is suggested to clarify the relationship between sex hormone and BMD through extended study and appropriate analysis.

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Rare Types of Diabetes, TNFα, Melatonin, Amylin, Antioxidants

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THYROID FUNCTION IN SUBJECTS WITH MALNUTRITION RELATED DIABETES MELLITUS

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Patients with malnutrition related diabetes mellitus (MRDM, as defined by WHO Study Group), although clinically euthyroid, have earlier been shown to have significantly lower levels of both serum T_3 and T_4 and higher levels of TSH as compared to age-matched Control. NIDDM patients of similar age group showed only lowered levels: T_3, T_4 and TSH were similar to Control. The present study aimed to assess the thyroid function of MRDM patients by measuring the functionally important fractions of thyroid hormones (FT_3 and FT_4) and also to elucidate whether any change in these hormones are related to the serum levels of thyroxine binding globulin (TBG). Seventeen newly diagnosed MRDM (9 PDDM, 8 FCPD), 24 NIDDM and 20 Control subjects were studied. Serum C-peptide was measured by ELISA; T_3, T_4, FT_3, FT_4 and TSH by microparticle enzyme immunoassay (MEIA); TBG by radial immunodiffusion (RID), and HbA_{1c} by HPLC methods. T_3 in all diabetic groups were lower as compared to Control (NIDDM 1.88 ± 0.05, PDDM 0.99 ± 0.33, FCPD 0.66 ± 0.32 and Control 2.06 ± 0.34 nmol/l, M±SD). However MRDM subgroups showed the lowest values which differed significantly from Control as well as from NIDDM ($p<0.05$) patients. NIDDM group had values of FT_3 and FT_4 similar to Control, but MRDM subgroups still showed significantly deficient thyroid function (*FT3, pmol/l, M±SD*: Control 4.43 ± 0.54; NIDDM 4.33 ± 0.84; PDDM 2.68 ± 0.68; FCPD 2.56 ± 0.62; $p<0.05$, Bonferroni t-test and *FT4, pmol/l,M±SD*: Control 14.53 ± 2.30; NIDDM 16.96 ± 3.00; PDDM 12.30 ± 3.60; FCPD 13.08 ± 2.25; $p<0.05$). TBG was similar in all the groups (TBG, mg/l, M±SD: Control 8.93 ± 2.40; NIDDM 8.78 ± 3.00; PDDM 8.36 ± 2.50; FCPD 6.80 ± 1.60) showing that abnormalities of this transport protein may not account for the thyroid dysfunction in these patients. Serum T_3 and T_4 in all diabetic subjets showed a significant negative correlation with the degree of hyperglycemia as measured both by glucose and HbA_{1c}. Fasting serum C-peptide showed a positive correlation ($r=0.034$, $p<0.05$) with T3 in the diabetic subjects. The results suggest an abnormality of thyroid function in MRDM patients which may be related to their severe hyperglycemia and deficient insulin secretory capacity.

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AMYLIN INHIBITS PENTAGASTRIN-STIMULATED GASTRIC ACID SECRETION AND PROTECTS AGAINST ETHANOL-INDUCED GASTRIC MUCOSAL DAMAGE IN RATS

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Amylin, a peptide hormone secreted from pancreatic β-cells in response to nutrient stimulation, is a physiological regulator of gastric emptying. To investigate whether amylin exerted other gastric actions, we examined its effects on pentagastrin-stimulated acid secretion and on ethanol-induced gastritis in fasted Sprague Dawley rats. To study acid secretion, rats chronically implanted with gastric cannulae were injected subcutaneously with 125μg/kg pentagastrin. Gastric contents obtained by flushing the cannulae every 10 min were titrated to measure acid production. 40 min after pentagastrin, rats were injected subcutaneously with rat amylin and the aggregate acid secreted over the next 60 min measured. Amylin suppressed acid secretion by 93.3% from that in saline treated rats (60 min after amylin; $P<0.001$; n=5,6). The ED_{50} for this effect was 0.027μg/rat ± 0.07 log units, a dose calculated to result in physiological changes in plasma amylin concentration. To study a potential gastroprotective effect of amylin, rats were injected subcutaneously with amylin 20 min before gavage with 1mL absolute ethanol and sacrificed 30 min later. The stomachs were excised, washed and everted for grading of injury by observers blinded to the treatment. Amylin reduced the injury score by up to 67%. The ED_{50} for the gastroprotective effect of amylin in this experimental system was 0.036μg/rat ±0.4 log units, similar to that for inhibition of gastric acid secretion. We propose that endogenously secreted amylin may play a role in restraining gastric acid secretion and the protection of gastric mucosa.

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MECHANISM OF KETOSIS RESISTANCE IN MALNUTRITION RELATED DIABETES MELLITUS

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To understand the mechanism of charactersric ketosis resistance in malnutrition ralated diabetes mellitus we have studied the ketogenic responses of to oral fat in 17 MRDM subjects (FCPD 7 and PDDM 10) along with 15 age-matched NIDDM and 10 control subjects. Overnight fasting subjects were fed with a dairy fat 'Ghee' (1g per kg body weight) and serum was collected at 0, 120 and 240 min. Although the BMI of FCPD (Mean 13.7) and PDDM (16.2) patients were markedly lower than the NIDDM (21.4) and Control (21.6) and although FBG of FCPD (16.9±4.2, mmol/l, M±SD) and PDDM (18.1±3.1) patients were considerably higher than those of NIDDM (12.8±4.9) and Control (5.4±1.5), the fasting S β-HBA levels did not vary significantly among the groups (Median β-HBA, mg/dl: Control 1.5, FCPD 2.2, PDDM 1.6, NIDDM 1.5). β-HBA level was maintained in spite of very low S C-peptide in FCPD (0.4±0.3 ng/ml, M±SD) and PDDM (0.7±0.1) subjects (NIDDM 2.1±0.8 and Control 2.2±0.5). The S glucose response to oral fat was almost flat in Control, but there was a gradual rise at 120 min and gradual fall at 240 min in the 3 diabetic groups. The S C-peptide paralleled the rise and fall of glucose only in NIDDM; in FCPD and PDDM there was almost flat responses, and in Control there was a rise and fall of C-peptide. The β-HBA response in the Control clearly differed from the 3 diabetic groups with a sharp rise of levels at 120 min (about 62% increase) and equally sharp fall near the baseline at 240 min. In contrast β-HBA continued to rise at 240 min in the 3 diabetic groups which had similar levels and the values differed significantly ($p<0.05$) from that of Control. TG levels showed a similarity among all the 4 groups at all time points. Conclusions: a) Depleted body fat store do not lead to decreased fasting ketone bodies in MRDM, b) The effect of NEFA (from oral fat) on glucose metabolism varies between nondiabetic and diabetic subjects and MRDM subjects do not show any peculiarity in this respect, c) The β-cell function MRDM subjects is severely compromised, and d) Re-esterification and ketone body synthesis pathways seem to be comparable among MRDM and NIDDM and, thus, factor(s) other than these probably contribute to the development of ketosis resistance in MRDM subjects.

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STUDIES USING NEUTRALIZING ANTIBODY AND THE ANTAGONIST AC187 REVEAL THAT ENDOGENOUS AMYLIN INHIBITS GLUCAGON SECRETION.

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The ß-cell hormone, amylin, like its partner hormone, insulin, inhibits pancreatic glucagon secretion. We have recently published that infused amylin can inhibit L-arginine-stimulated glucagon secretion by over 60% with an EC_{50} of 18 pM ±0.3 log units. To examine whether endogenous amylin tonically inhibits glucagon secretion, we measured plasma glucagon while blocking the effects of endogenous amylin by either neutralizing the ligand (amylin) by infusion of a specific amylin monoclonal antibody, or by neutralizing the receptor using the potent and selective competitive amylin antagonist, AC187. For the monoclonal antibody studies, we injected male Harlan Sprague Dawley rats intraperitoneally with 3mg of specific amylin monoclonal antibodies (n=5) or nonspecific antibodies (n=5) 24 hours before testing. For the amylin antagonist studies we infused with either AC187 at 3mg/h (n=8) or with saline (n=30) during testing. Testing comprised measurement of plasma glucagon in overnight-fasted halothane-anesthetized rats before and after 60 min of euglycemic clamp (glucose 5.8mM, primed/continuous insulin infusion of 12mU + 5mU/kg/min). In animals administered the specific (25-27) anti-amylin antibody the previous day, plasma glucagon levels were 61% higher throughout the clamp procedure than those in animals administered the non-specific (40-6) antibody (93±14 vs 58±5pM, $P<0.03$, t-test, Welch correction for different SD's). In animals infused with the amylin antagonist, AC187, or saline, plasma glucagon concentrations were similar before the clamp (82±9 vs 80±7pM, respectively, $P=0.9$). After 60 min, plasma glucagon had doubled in the AC187-infused group (160±30pM, $P<0.03$ vs t=0) and were higher than in the saline-infused group (103±9pM, $P<0.02$ between groups). Blockade of amylin action with a specific monoclonal antibody or with a selective amylin antagonist, AC187, increased glucagon secretion. Together, these data support the idea that endogenous amylin regulates glucagon secretion.

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HYPERINSULINAEMIC EUGLYCAEMIC CLAMP STUDIES IN 'MALNUTRITION RELATED DIABETES MELLITUS'.

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Background: High insulin requirements have been proposed as a diagnostic criterion for malnutrition related diabetes mellitus. This has been taken as a marker of insulin insensitivity.

Aim: to demonstrate whether these subjects have insulin insensitivity

Design: case control; *Cases:* ten ketosis resistant young onset (<30 years at onset) diabetic subjects; *controls:* six young onset classical ketosis prone diabetic subjects. *Outcome measure:* M value on hyperinsulinaemic euglycaemic clamps (40 $mU.m^{-2}$, intended insulin concentration 100$uU.ml^{-1}$) (mean c.v. for plasma glucose 60-120 min of clamp: 3.58%+/-0.38)

Results: The cases and controls were matched for age (23.4 ± 1.8 vs. 19.8 ±2.5 years) and BMI (16.7±0.6 vs. 18.5 ±0.6kg/m2). HbA1 (12.1± 0.5 vs 10.5± 1.2%), serum triglyderides(147±13 vs 110±9mg/dL) and cholesterol (145±11 vs 167±21mg/dL) of the cases and controls were comparable.With adequate euglycemia the glucose disposal rate (M value, mg.kg-1.min-1) were 7.06±0.80 in cases and 7.43 ± 1.34 in controls (p=0.67).

Conclusions: ketosis resistant diabetes of young (malnutrition related diabetes mellitus) is not associated with insulin resistance.

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COMPARISON OF EFFECTS OF AMYLIN, GLUCAGON-LIKE PEPTIDE-1 AND EXENDIN-4 TO INHIBIT PENTAGASTRIN-STIMULATED GASTRIC ACID SECRETION

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Amylin, a peptide hormone secreted from pancreatic ß-cells, is a physiological regulator of gastric emptying. Exendin, a component of the venom of the Gila monster, is as potent as amylin in regulating gastric emptying, while glucagon-like peptide-1 (GLP-1) is ~20-fold less potent. Here we compared the effects of amylin, GLP-1 and exendin on pentagastrin-stimulated gastric acid secretion in rats. Acid production from the stomachs of chronically cannulated fasted rats was measured by titration of contents flushed every 10 min. Forty min after subcutaneous injection of 125µg/kg pentagastrin, rats were injected subcutaneously with rat amylin, human GLP-1[7-36]NH_2, or exendin-4 and the aggregate acid secreted over the next 60 min measured. Amylin potently inhibited gastric acid secretion with an ED_{50} of 0.027µg ±0.07 log units. GLP-1 was 180-fold less potent (ED_{50} 4.9µg ± log units). Exendin-4 failed to inhibit acid secretion at any dose. Thus, the order of potencies for inhibition of gastric acid secretion by amylin, GLP-1 and exendin (amylin>>GLP-1>>exendin) differed markedly from that previously obtained for gastric emptying (exendin≥amylin>>GLP-1). This result suggests that structures responsible for exendin-inhibition of gastric emptying are distinct from those responsible for inhibiting gastric acid secretion. Structural distinctions could include differences in access to receptors mediating gastric emptying vs. acid secretion, and could even indicate different receptor subtypes mediating these responses.

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SERUM AND ERYTHROCYTE MAGNESIUM IN NEWLY DIAGNOSED MALNUTRITION RELATED DIABETES MELLITUS SUBJECTS

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Hypomagnesemia is implicated in the pathogenesis of insulin resistance in diabetic patients. Recent data from our group suggest that patients with Protein Deficient Diabetes Mellitus (PDDM) [a subgroup of Malnutrition Related Diabetes Mellitus (MRDM), the third major class of diabetes recognized by the WHO Study Group] suffer from a more severe degree of hypomagnesemia compared to NIDDM ones. Since these patients presenting with severe degree of hyperglycemia (serum glucose usually >16 mmol/l) need a relatively large amount of insulin for controling diabetes. It is important to study their Mg metabolism in a greater detail for a possible link with insulin resistance. The Erythrocyte (E) Mg content is a more specific marker of total body Mg compared to serum levels and this has been measured in the present study in 15 PDDM, 10 NIDDM and 12 age-matched nondiabetic Control patients. RBCs were washed twice with 300 mM ice-cold sucrose to get rid of extracellular Mg and the content of the element in the homogenized cells was measured by an enzymatic-colorimetric method. Protein content of the cells was measured by a detergent compatible Kit using spectrophotometric technique. Although NIDDM patients had lower S Mg compared to control (S Mg, mmol/l, M±SD, 0.89±0.04 in Control vs 0.86±0.76 in NIDDM) the two group did not differ statistically. However the level in PDDM Group (0.84±0.05) was significantly lower compared to Control (p= 0.004). The Mg content of RBC demonstrated the Mg deficiency in diabetic patients more unambiguously. The PDDM and NIDDM subjects showed similar values which were significantly lower than those of Control (E Mg, µmol/mg protein, M±SD, Control: 0.16±0.02, PDDM: 0.12±0.05, and NIDDM: 0.12±0.0). The closely similar E Mg values in PDDM and NIDDM patients were maintained in spite of marked difference in their S glucose values (S Gl, mmol/l, M+SD, Fasting : 18.17 in PDDM vs 10.62+3.79 in NIDDM; Postprandial : 26.34±8.01 in PDDM vs 19.27+4.54 in NIDDM). The results suggest the following: a) E Mg is a more sensitive marker of Mg-deficiency in diabetic subjects; b) Mg-deficiency may be related to the need for high deses of insulin in MRDM subjects and c) the theory is that hypomagnesemia caused by sequestration of Mg within RBCs as a consequence of hyperglycemia may not be compatible with the present findings.

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PLASMA AMYLIN CONCENTRATIONS IN RATS: UTILITY OF A MONOCLONAL IMMUNOENZYMOMETRIC ASSAY

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Reported values for plasma amylin concentrations in the rat vary widely. Some of this variation may be attributable to concentrations that are artifactually elevated, a difficulty often inherent in polyclonal antibody-based competitive RIA. We have measured basal and stimulated plasma amylin concentrations in a variety of rat strains using a monoclonal antibody-based fluorescent immunoenzymometric assay (IEMA). In the IEMA the capture antibody binds to the N-terminal and the detection antibody (alkaline phosphatase/4-methylumbelliferyl-PO4) to the C-terminal ends of amylin, and both have < 0.5% cross-reactivity to calcitonin or calcitonin gene-related peptide. The standard curve (0, 1.25, 2.5, 5.0, 10 pM) was fit by SD-weighted linear regression: relative fluorescent units (RFU)=pM*17.57+6.66, R^2=0.989. The zero standard (11.9±1.2 RFU (±SEM); n=53) was distinguishable from the 1.25 pM standard (22.4±3.1 RFU; n=12; P<0.001), enabling measurement into this range. Amylin concentrations (pM) in plasma obtained by cardiac puncture were lower in fasted vs fed anesthetized rats of different strains: Harlan Sprague Dawley (HSD): 1.40±.17 (n=8) vs 4.81±0.56 (n=8); P<0.0001: Lewis: 1.02±0.09 (n=11) vs 3.08±0.34 (n=8); P<0.0001: Wistar: 1.63±0.15 (n=9) vs 7.70±0.80 (n=9); P<0.0001. Plasma amylin concentration was significantly strain-dependent (ANOVA, P<0.01 for fasted animals; P<0.0001 for fed animals). Plasma amylin concentration increased by 1.88±0.28pM in fasted anesthetized HSD rats (n=9) after intravenous injection of 5.2mmol/kg of glucose (P<0.0001 vs saline-infused controls; n=8) and by 4.02±0.83pM in fed rats (n=6; P<0.005 vs controls; n=6). In conclusion, a sensitive monoclonal antibody-based IEMA revealed plasma amylin concentrations that were lower than values that have been reported using some other less sensitive assays, but which are internally consistent in regard to patterns of β-cell secretion after food or glucose stimulation of fasted or fed rats.

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NON DIABETIC SUBJECTS FROM MATERNAL INHERITANCE, DIABETES AND DEAFNESS FAMILIES DEMONSTRATE INSULIN RESISTANCE ON I.V. GLUCOSE TOLERANCE TESTING

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Conflicting reports exist as to the precise pathogenetic mechanism of diabetes mellitus in subjects with the A3243G tRNAleu $^{(UUR)}$ mutation (the 3243 mutation) of mitochondrial DNA with some studies favouring islet cell failure and others suggesting insulin resistance. We aimed to determine whether nondiabetic subjects with the mutation demonstrated any abnormalities of insulin sensitivity (Si), insulin secretion (first phase, phi1) or glucose effectiveness (SG) as a precursor to the diabetic state. Initially we performed a 75g oral glucose tolerance test (GTT) in 12 subjects from four kindreds (all subjects carried the 3243 mutation) and twelve age, sex, BMI and lean body mass matched controls with no family history of NIDDM. We found subjects with the 3243 mutation had identical glucose and insulin responses compared with controls in the first 30 mins of the oral GTT but were more hyperinsulinemic and hyperglycemic from 90-180 mins. We used the IVGTT with minimal model analysis in six 3243 mutation subjects and their controls to determine the relative contributions of insulin secretion, Si and SG to overall glucose tolerance (Kg). There was no difference in Kg between the 3243 mutation and control groups (1.61±0.65 and 1.51±0.62, x 10^{-2} min^{-1} respectively; NS). The 3243 mutation group had reduced Si c.f. controls (5.98±3.99 vs 11.7±7.14 , x 10^{-4} min^{-1} per mU/L; p=0.05). Fasting insulin, however, was not increased in the 3243 mutation group (6.6±2.2 vs 6.7±2.3, mU/L,NS) nor was phi1 altered (4.0±2.0 vs 3.4±2.5 mU/L min^{-1} per mg/dL, NS). SG was increased in the 3243 mutation group but was not significant (2.03±1.05 vs 1.12±0.65, x 10^{-2} min^{-1}, p=0.15). Using regression analysis, Phi1 was strongly associated with Kg in the 3243 mutation group (regression coefficient -0.003, 95%CI -0.004 to -0.002, p=0.004, R^2 =0.83) as was SG (regression coefficient -0.50, 95% CI -0.1 to -0.9, p=0.02, R^2=0.83). There was no significant association between SG and Phi1 in the 3243 mutation subjects (R^2= 0.41, p=0.11) although there was in the controls (R^2= 0.75, p=0.03). Lastly, there was no difference in insulin mediated glucose disposal between the two groups (Si x Phi1, p=0.8). We conclude alterations in glucose disposal are present in nondiabetic subjects with the 3243 mutation. Abnormalities include insulin resistance and increased glucose effectiveness but normal insulin secretion. We conclude insulin resistance plays a pathogenic role in the development of DM in subjects with the 3243 mutation.

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ISLET AMYLOID FORMATION IS ASSOCIATED WITH DIMINISHED β-CELL FUNCTION IN FAMILIAL NON-INSULIN DEPENDANT DIABETES.

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Islet amyloid is found in 96% of subjects with NIDDM at postmortem. The aim of this study was to try to determine the relationship between amyloid deposition and the physiological changes which take place in the development of NIDDM. As part of a longitudinal study of families with NIDDM, 5 male siblings underwent physiological testing using a 1 hour infusion of glucose in 1984 and again in 1994. At baseline the eldest sibling (D.Y.)-was already diabetic. The other siblings showed typical characteristics of the Metabolic Syndrome with obesity, hypertension, hyperinsulinaemia and dyslipidaemia. Mean fasting glucose was 4.7mmol/l(±0.16), mean %S was 48(32-78) and mean %β was 150(130-180). An esimate of %S and %β was made using CIGMA-(Continuous Infusion of Glucose with Model Assessment). During the 10 year follow-up period, D.Y. died and pancreas was obtained at postmortem. His identical twin E.Y. developed NIDDM in 1989. At follow-up in 1994 a third sibling R.Y. was also found to be diabetic. In 1994 %β had markedly declined in the two siblings who had developed NIDDM(<30%) but %S remained unchanged. Fasting proinsulin levels in the diabetic siblings were markedly elevated(>60pmol/l) compared with the nondiabetic siblings(<20pmol/l). Fasting IAPP (islet amyloid polypeptide)was raised in the newly diagnosed diabetic R.Y. but was below normal in E.Y. who was on sulphonylurea treatment. Sibling E.Y. subsequently died and pancreas was obtained at postmortem. Islet amyloid immunoreactive for islet amyloid polypeptide was found in all islets of postmortem pancreas in both diabetic subjects. In conclusion islet amyloid deposition is associated with a decline in β-cell function and elevated proinsulin secretion; IAPP levels appear to be elevated in the early stages of NIDDM.

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INSULIN RECEPTOR BINDING KINETICS IN PATIENTS WITH MYOTONIC DYSTROPHY.

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Myotonic dystrophy (MD) is a multisystemic disease where endocrine abnormalities are found, and may be associated to insulin resistance. We studied the dissociation kinetics and the effect of pH upon insulin binding to lymphocytes transformed with Epstein-Barr virus in 6 patients with MD (BMI:17-22 kg/m^2; basal plasma insulin:8-78 µU/ml). Results were compared with those from healthy subjects (n=4, BMI:21-23 kg/m^2. basal plasma insulin:8-24 µU/ml). Either, maximal specific ^{125}I-insulin binding (34±2.7% /10^7 cells, Mean±SEM), maximal insulin binding capacity (0.8±0.3 nM/10^7 cells) and affinity (0.8±0.2 nM) in EBV-lymphocytes were within the normal range. Also, all the insulin binding parameters were within the normal range in partially purified receptors. The ^{125}I-insulin dissociation rate in the absence or in the presence of 500 or 100,000 ng/ml of insulin (20.7±2.1; 69.4±2.4; 37.2±1.2 %/5 min) was similar to that observed in control subjects. The ^{125}I-insulin dissociation rate at pH 6.8 and 6.0 (26.2±7.6 and 59.7±1 %/ 5 min at 4°C) was in the normal range. Our results suggests that the characteristics of the interaction between insulin and its receptor in MD patients is not different from normal subject and therefore the insulin resistance associated with this disease is due to defects located at post-receptor levels.

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COMPARISON OF CENTRAL AND PERIPHERAL ADMINISTRATION OF AMYLIN ON REDUCTION OF FOOD INTAKE IN RATS.

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Amylin, a 37 amino acid peptide co-secreted with insulin from pancreatic β-cells, is reported to decrease food intake in rodents. Amylin binds to certain areas within the central nervous system, including the area postrema and nucleus accumbens, and some of amylin's actions may be centrally mediated. To investigate the site of amylin's effect on food intake, we compared inhibition of food intake following intraperitoneal (IP) or intracerebroventricular (ICV) administration of rat amylin in Sprague-Dawley rats (weight 300-350g) in which lateral ventricular guide cannulae had been implanted. At least ten days after implantation, rats were injected just before the onset of the dark cycle with 0-10µg of rat amylin via either the ICV route (2µL/dose; n≥5/dose) or IP route (n=4/dose). Food intake was measured by the difference in food weight 2 hours after presenting it to the injected rats. ICV amylin totally inhibited food intake at the 10µg dose, and the ED50 was 0.19µg/rat ± 0.07 log units. The 10µg IP dose inhibited food intake by 34%, compared to saline-injected controls. Assuming that total inhibition of food intake could have eventually been obtained with a high enough IP dose, the ED50 for the IP route of administration was 10.2µg/rat ± 0.18 log units. Thus, ICV amylin was 53-fold more potent than IP amylin in inhibiting food intake. This result supports the idea that amylin may regulate food intake via a central structure in rats.

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Study of Insulin Receptor Gene Variations in Patients with Acanthosis Nigricans and Close Relatives

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Insulin resistance clearly exists in patients with acanthosis nigricans(AN) so it is importamt to find thd defect in insulin receptor(IR) gene in the patients and their pedigrees. We used technique of PCR-SSCP and DNA direct sequencing to examine the mutations and polymorphisms in the exons 17 and 20 of IR gene in 9 patients with AN and their 23 first degree relatives. Among them, 7 patients with diabetes, 13 associated with obesity, 6 with hypertriglyceridemia, and 6 with hypertension. In total, 14 variant SSCP patterns were detected. Direct sequencing revealed eight point mutations and six silent polymorphisms. Seven mutations appeared not mentioned in the literatures, such as Val to Met 983 (GTG→ATG) Gln 1004 to Lys (CAG→AAG), a homozygous for Stop to Glu 1185 (GAA→TAA), a heterozygous for Arg to Gly 1185 (GGG→AGG), polymorphisms in Gly 1008(GGC→GGT), Glu 1034 (GAG→GAA) and Cys 981 (TGC→TGA), homozygous Ile to Met 1153 (ATG→ATC), missense mutations Gln 1004 → Lys (CAG→AAG), Glu 1035 to Stop (GAG→TAG), Lys 1004 for Gln (AAG for CAG), Gly 1023 → Lys (GAG →AAG)

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THE EFFECT OF 300 AND 600 MG OF VITAMIN E ON INSULIN ACTION IN OBESE TYPE 2 DIABETIC PATIENTS

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Increased insulin sensitivity was described previously after pharmacological doses of vitamin E. We evaluated the effect of two doses of vitamin E administered in obese Type 2 diabetic patients in which the insulin resistance was documented. Diabetic patients on oral agents or on dietary regimen alone were treated 3 months either by 300 mg of vitamin E (Group A, n=6, BMI: 40.3±8.9 kg/m^2, age 50±8 yrs) or by 600 mg of vitamin E (Group B, n=11, BMI: 31.4±3.7 kg/m^2, age 46±9 yrs).Control group consisted of 12 healthy non-obese persons. Isoglycaemic hyperinsulinemic clamp on Biostator (insulin infusion rate I mU/kg/min) was performed during 90 min after previous 60 min stabilization phase in all of them before and after vitamin E treatment. The insulin receptor characteristics on erythrocytes were evaluated too. No significant changes of diabetes control expressed by fasting plasma glucose or glycated hemoglobin and of metabolic parameters like glucose disposal rate (M), metabolic clearance rate of glucose (MCRG) or insulin sensitivity index (M/I) were observed in Group A using 300 mg of vitamin E. On the contrary, 600 mg of vitamin E caused a decrease of basal serum insulin concentrations (29±15 vs 19±8 mU/l, p<0.01), glucose disposal rate (29.5±11.0 vs 22.6±9.0 umol/kg/min, p<0.02) and metabolic clearance rate of glucose (3.7±1.7 vs 2.9±0.8 ml/kg/min, p<0.05). An increase of insulin receptor binding capacity observed in Group A (268±65 vs 325±116 pmol/l, p<0.02) contrasted with a significant decrease of insulin binding capacity in Group B (284±84 vs 171±95 pmol/l, p<0.01).We conclude, that 300mg of vitamin E had no significant influence on metabolic parameters in obese Type 2 diabetic patients whereas further worsening of insulin action was demonstrated in patients treated with 600mg of vitamin E.

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INSULIN RECEPTOR DEFECT IN INDIAN WOMEN WITH TYPE A INSULIN RESISTANCE.

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The study aims to identify erythrocyte insulin receptor defects in Type A insulin resistance using reticulocyte count to correct for changes in red cell age. Six Indian women with hypothalamic disorders and Type A phenotype (Acanthosis Ni gricans, Hirsuitism, Hyperandrogenism) and six normal women were given oGTT. Il25-Insulin binding to erythrocytes was measured.Subjects with Type A pheno type had impaired glucose tolerance, hyperinsulinemia and higher free fatty acid levels (680 ± 180 Vs 182 ± 28umol/L, $p<0.05$ and 783 ± 160 Vs 381 ± 97umol/L) in GTT. Maximal specific insulin binding was 6.46 ± 0.75%/3.5 x 10^9 erythrocytes in controls. Displacement curves revealed marginal reduction in Type A subjects when data are expressed in terms of red cell count. However, when binding was normalised for reticulocytes, a greater, 50% decrease was seen. Scatchard analysis revealed the presence of 180 receptors/cell with KD 5.5 x 10-9 M in controls. Subjects with Type A phenotype exhibited 60% decrease in receptor number with similar KD (3.1 x 10^{-9}M). The data suggest the presence of fewer receptors in erythrocytes of Type A subjects identified more precisely using reticulocyte count. Reduced receptor number and defective lipolysis are likely causes of syndrome "X" observed in these subjects.

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ANTIOXIDANT VITAMIN LEVELS IN WELL CONTROLLED TYPE 2 DIABETES MELLITUS

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Oxidative stress may effect the development of the late complications in diabetes mellitus. Antioxidant vitamins, despite of their relatively low concentrations in plasma, play important role in protection against oxidation. The aim of the study was to find out weather the plasma level of the antioxidant vitamins (A, E and C) was different in well controlled type 2 diabetics from that in control subjects. The vitamin levels of 78 type 2 diabetic subjects (39 men and 39 women, mean age: 59,0±10,04 y. o.) were tested and compared with that of 44 healthy controls (19 men and 25 women, mean age 38,3±14,4 y. o.). The measurements were carried out by HPLC method. HbA1c levels were 4,66±0,04% in the control and 6,86±0,19% in the diabetic group. The patients were divided into four groups on the basis of lipid parameters: control, hyperlipaemic control, diabetes mellitus, diabetes mellitus with hyperlipaemia. Vitamin E levels were significantly increased in the diabetic group (Control: 28,55±1,388μM vs. DM: 32,14±0,941μM, $p<0.05$). Vitamin A concentrations were also higher in diabetic subjects (Control: 1,954±0,075μM vs. DM: 2,383±0,100μM, $p<0.01$) and in men. Vitamin E/cholesterol, Vitamin E/LDL, Vitamin A/cholesterol, and Vitamin A/LDL ratio did not differ in the two groups. In diabetics with hyperlipaemia both Vitamin E, and A levels were significantly elevated. A significant positive correlation could be observed between serum cholesterol, triglyceride, and the levels of the lipid soluble vitamins. The difference in Vitamin C levels (Control: 37,59±4,392μM vs. DM: 29,64±4,027μM) did not reach significancy. No correlation could be detected between parameters reflecting carbohydrate metabolism (fasting glucose and HbA_1c) and Vitamin A, E, C concentrations. We assume that the increased lipid soluble antioxidant vitamin concentrations in diabetes - due to the higher lipid levels - show only apparent relation with an increased antioxidant defence.

This work was supported by the PhD fellowship grant of Semmelweis University.

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DIFFERENT PATHWAYS OF INSULIN ACTION IN LEPRECHAUNISM

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The insulin sensitivity of glucose and protein metabolism, and of circulating FFA, potassium (K) and c-peptide concentrations, were investigated in a leprechaun female (age 12 yr, weight 24 kg, height 130 cm). The subject has two mutant alleles of the insulin receptor gene, that inhibit the post-translational processing and the transport of the receptors to the plasma membrane. The mutations do not affect the insulin affinity and the insulin-stimulated phosphorylation of the receptors. The subject was studied with a primed-continuous infusion of [6,6-$^2H_2$] glucose and [1-^{13}C]leucine during a basal period followed by two steps of insulin infusion (1 and 10 mU/kg/min) of two hours each. The glucose concentration decreased from 131 mg/dl in the basal period to 116 and to 93 during the 1 mU and the 10 mU insulin steps respectively. Glucose flux was almost unaffected by insulin, decreasing from 3.84 to 3.62 and to 3.40 mg/kg/min. The endogenous leucine flux, an index of proteolysis, was completely insensitive to insulin (from 182 to 189 and 180 μM/kg/h). The FFA concentration changed from 1135 to 799 and 917 μM/l. In contrast, the concentration of K dropped from 4.1 to 3.2 and to 3.3 mEq/l during the 10 mU insulin step, and an infusion of 20 mEq/h of KCl was necessary to prevent further hypokalemia. Finally, the c-peptide concentration was suppressed from 1.85 to 0.97 and 0.29 pmol/ml. In summary 1) the insulin action on proteolysis is completely absent in leprechaunism, 2) the effect on glucose and FFA metabolism is severely impaired, 3) the insulin sensitivity of K concentrations is maintained and 4) the feed-back inhibition of insulin on its own secretion is still effective. These data suggest two possibilities: 1) the activation a minimal number of receptors is sufficient to elicit the insulin effects on circulating K and on insulin feed-back inhibition, but not sufficient to affect the intracellular signaling pathways for protein and glucose metabolism, 2) insulin can affect circulating K and can suppress its own secretion using pathways alternative to the activation of its own receptor.

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ANTIOXIDANT DEFENSES ARE REDUCED DURING OGTT IN NORMAL AND NIDDM SUBJECTS.

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Free radical production has been reported to be increased in patients with diabetes mellitus and it has been suggested that hyperglycemia may directly contribute to the generation of an oxidative stress. In this study an OGTT was performed in 10 NIDDM patients (7 males and 3 females; age 55.1±1.7 years, mean±SE; duration of diabetes 10.0±1.5 years; BMI 25.8±1.1 Kg/m^2) and in 10 healthy normal subjects (6 males and 4 females) matched for age (56.1±1.8 years) and body mass index (25.9±1.6 Kg/m^2). In diabetic patients, during OGTT, SH groups ($p<0.03$-0.005), and vitamin C ($p<0.01$) were significantly decreased at 30 to 120 min, and uric acid at 60 to 120 min ($p<0.05$-0.01).Total plasma radical-trapping activity (TRAP), which evaluates plasma antioxidant capacity due to known and unknown antioxidants present in the plasma as well as their mutual cooperation, was also significantly reduced at 30 to 120 min ($p<0.001$). Vitamin E was not affected. In normal subjects, during OGTT, TRAP ($p<0.05$-0.005), SH groups ($p<0.001$) and vitamin C ($p<0.05$) were significantly decreased at 30 to 120 min, uric acid ($p<0.01$) at 90 and 120 min, while vitamin E did not change. Adding glucose to a pooled plasma sample did not interfere with plasma TRAP assay (before: 827.5±45.2 μmol/l; after: 836.4±51.7 μmol/l).This finding supports the hypothesis that hyperglycemia may, even acutely, induce an oxidative stress.

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ACUTE EXPRESSION OF TNFα mRNA IN SKELETAL MUSCLE AND THE HEART: RELATION TO ENDOTOXIN-INDUCED INSULIN RESISTANCE

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TNFα is an established insulin-antagonist, which may contribute to insulin resistance in both obesity and acute infections. We determined whether endotoxemia induced by lipopolysaccharide (LPS) acutely induces expression of TNFα in skeletal muscle and the heart. Chronically catheterized awake rats received an intavenous bolus injection of either LPS (1 mg/kg, LD_{10}, n=8) or saline (n=8) at 0 min. Insulin sensitivity was thereafter followed for 2 hours using the euglycemic insulin clamp technique (insulin infusion rate 18 mU/kg·min). At 2 hours, abdominis and heart muscles were freeze-clamped and analyzed for TNFα mRNA concentrations using a competitive reverse transcriptase PCR technique. Serum TNFα concentrations increased from 38 ± 23 pg/mL to a maximum of 16043 ± 2154 pg/mL at 60 min in the LPS rats but remained unchanged in the control rats (19 ± 17 vs 32 ± 23 pg/mL, 0 vs 60 min, NS). Whole body glucose uptake during hyperinsulinemia was 42 % lower in the LPS (90 ± 6 μmol/kg·min) than in the saline (156 ± 8 μmol/kg·min, p < 0.01) rats, and was inversely related to changes in circulating TNFα concentrations. In the heart, TNFα mRNA, normalized to actin mRNA, was 10-fold higher in the LPS than in the saline (TNFα/actin mRNA ratio 20±1 vs 2±0.1, LPS vs saline rats, p<0.001) rats. In abdominis muscle, TNFα/actin mRNA was 25-fold higher in the LPS (140 ± 37) than the saline (6 ± 2, p<0.001) rats. Immunohistochemical staining using a rabbit anti-ratTNFα antibody showed intense staining in Kupffer cells in the liver while no positive staining was observed in heart or skeletal muscle. These data demonstrate that TNFα mRNA is expressed acutely within 2 hours in skeletal and heart muscles during endotoxemia. During this time frame, whole body insulin resistance is, however, more likely to be due to circulating than locally produced TNFα.

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EVALUATION OF OXIDATIVE STRESS IN STREPTOZOTOCINE DIABETES MELLITUS IN RATS

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The aim of this study was to evaluate some indexes characterizing the oxidative stress in streptozotocine (STZ)-induced diabetes mellitus.

Methods: Diabetes mellitus was induced with a single dose of STZ 60 mg/kg b.w. in 21 Wistar rats aged 2 months, killed sixty days after the onset. Total tissue homogenates from the heart, liver and kidneys were prepared at pH 7.4. Total protein, superoxide dismutase (SOD) and total antioxidative capacity (TAOC) in the serum and homogenates were investigated. Controls were 21 animals at the same age.

Results: The blood glucose, serum fructosamine and HbA_1 values were significantly higher in diabetic rats compared to the controls. Serum SOD was 27,3±6,5 U/l in diabetic rats and 46,8±8,7 U/l in controls (p<0,001). There was a significant difference in SOD in kidney tissue homogenates between diabetic rats (37,8±2,8 U/g prot) and controls (51,6±8,7 U/g prot, p<0,05), while SOD in the livers and hearts tissues homogenates were similar in both groups. TAOC in the serum of diabetic rats was 29,2±7,1 $\mu mol/H_2O_2/ml/min^{-1}$ and 58,1±17,0 $\mu mol/H_2O_2/ml/ min^{-1}$ in controls. TAOC in kidney tissue homogenates was 82,7±23,3 U/g prot in diabetic rats and 145,2+25,6 U/g prot in controls (p<0,001). TAOC levels in livers and hearts homogenates were similar in both groups.

Conclusion: Antioxidative defense capacity in STZ-diabetes mellitus in rats is diminshed according to the SOD and TAOC levels in the circulation and kidneys. The similar SOD and TAOC levels in livers and hearts homogenates in diabetic rats and controls may be due to an adaption and mobilization of antioxidative defense systems.

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ENDOTOXEMIA CAUSES A GLUT 4 TRANSLOCATION DEFECT IN RATS.

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Significance: Acute infection is associated with decreased insulin sensitivity. Our current study evaluates the hypothesis that the mechanism of decreased insulin sensitivity during infection is due to decreased function of the insulin-responsive glucose transport protein, GLUT 4. Methods: In order to test our hypothesis, 17 chronically catheterized, freely moving rats were injected with either lipopolysaccharide "LPS",(*S. typhimurium*, LD10, 1mg/kg i.v.) or saline "SAL". A hyperinsulinemic euglycemic (insulin 18mU/kg min) clamp (120min) was started immediately after the injection. Blood samples were withdrawn at 0,30,60, and 120 min for insulin concentrations. At 120 min, the animals were sacrificed. Heart (H) and rectus abdominis (A) were dissected and immediately freeze-clamped for subsequent determination of GLUT 4 translocation. To assess subcellular GLUT 4 localization, membrane subfractions were prepared using sucrose gradients and immunoblots. Radionucleotide counting was employed to determine GLUT 4 quantity in each subfraction. Results: Whole body glucose uptake (M) was decreased in LPS compared to SAL (LPS=88±5, SAL=135±23; p<0.01). Insulin stimulation resulted in decreased GLUT 4 in the 35% sucrose fraction (intracellular)and increased GLUT 4 in the 30% (intermediate) and 25% (plasma membrane) fractions to a greater degree in SAL than in LPS in both A and H (%GLUT 4 in layer 25:A SAL=13%,LPS=2%;p= 0.02; H SAL=18%, LPS=9%;p= 0.03). In LPS, recovery of GLUT 4 in sucrose layer 35 was higher than in SAL by 25% in H and 20% in A. In both LPS and SAL, GLUT 4 content in the 25% fraction was positively correlated with maximal glucose uptake (H:r=0.55, A:r=0.57). Summary: 1) GLUT 4 in the plasma membrane enriched sucrose fraction correlated with in vivo insulin responsiveness in both SAL and LPS; 2)When compared to SAL, LPS rats had less GLUT 4 in the plasma membrane enriched sucrose fraction and more GLUT 4 in the intracellular dense sucrose fraction indicative of an endotoxemic-specific abnormality in GLUT 4 trafficking. Conclusion: Defects in GLUT 4 translocation and trafficking in skeletal muscle may contribute to insulin resistance associated with acute infection.

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NITRIC OXIDE INVOLVEMENT IN IMPROVEMENT OF GLUCOSE TOLERANCE WITH A CYTOKINE INDUCER IN KK-AY MICE

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Nitric oxide (NO) produced in the vascular wall was indicated to be involved in insulin sensitivity. Diminished NO response to insulin is a component of insulin resistance. Therefore we investigated whether NO is involved in improvement of glucose tolerance with cytokine inducers, complete Freund's adjuvant (CFA). We had found that CFA significantly improved glucose tolerance without affecting insulin secretion, indicating improvement of insulin resistance. In addition, long-term treatment with CFA inhibited development offatty liver and renal glomerular lesion in KK-Ay mice. However the mechanism remains unknown. We performed intraperitoneal glucose tolerance tests (2 g glucose/kg) of the mice which were treated 1 day before with or without CFA and/or NO synthase inhibitor (5 mg/kg of N-monomethyl arginine; NMMA) or saline for control. Postload 2h-glucose levels of the mice treated with saline, NMMA & CFA, CFA were14.0 ± 1.2, 8.6 ± 2.9, 4.7 ± 0.6 (mmol/L), respectively, significantly differing each other by ANOVA (P<0.001) . In addition, weekly CFA injections for a month recovered cyclic guanosine monophosphate (cGMP) production in the liver and muscle. cGMP contents in the liver & muscle of control KK-Ay, CFA-treated KK-Ay and Balb/c mice were19.4 ± 14.0 & 20.3 ± 6.4, 48.2 ± 32.8 & 35.0 ± 29.2, 132.6 ± 85.1 & 68.4 ± 62.8 (fmol/mg protein), respectively; significantly differing each other by ANOVA (P<0.05). In conclusion, CFA improve insulin resistance, which is reflected in glucose tolerance, partly by involvement of the sequential NO production.

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A TNF-α GENE POLYMORPHISM IS RELATED WITH INSULIN RESISTANCE, PERCENT BODY FAT AND INCREASED LEPTIN

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The aim of this study was to investigate whether the NcoI polymorphism of the TNF-α gene influences the relationship between insulin resistance, percent body fat and serum leptin levels. A sample of 26 subjects [(8 lean subjects, 4 females, age 33.6 ± 2.0; BMI 22.9 ± 2.0, range 19.1-25), and 18 obese, 10 females, age 37.1 ± 1.9; BMI 32.5 ± 0.6, range 30.1-37.9)] was divided into two groups on the basis of the NcoI genotype. Fifteen subjects were (+/+) homozygotes for the presence of the NcoI restriction site, 9 subjects were (+/-) heterozygotes and 2 (-/-) homozygotes for the absence of the restriction site. TNF-1 (+/+) and TNF-2 (+/- and -/-) groups of subjects were comparable in sex, age, proportion of lean/obese subjects, BMI, waist/hip ratio, and several skinfold measurements. Basal serum insulin was slightly greater (14.5 ± 2 vs 10.1 ± 0.8 mU/L, p=0.05) in the TNF2 group in the presence of comparable serum glucose concentration. The integrated area under the curve of serum insulin concentrations after oral 75 g glucose, and the percent body fat were significantly increased in TNF-2 subjects (276.6 ± 44.7 vs 170.9 ± 16.7 mU/L, p=0.021; and 37 ± 3.3 vs 26.3 ± 2.6%, p=0.01, respectively). TNF-2 subjects also showed a decreased insulin sensitivity index as determined by the frequently sampled intravenous glucose tolerance test with Minimal Model Analysis (1.6 ± 0.2 vs 3.13 ± 0.4 min^{-1}/mU/L, p=0.04). Paralleling the known relationship between insulin and leptin levels, serum leptin concentration was clearly increased in the TNF-2 group (22.3 ± 4.3 vs 12.3 ± 2 ng/mL, p=0.03). Therefore, (+/-) heterozygotes and (-/-) homozygotes may be more susceptible to develop insulin resistance and increased percent body fat. Results of the present study suggest that TNF-α-NcoI polymorphism may exacerbate the alterations in leptin levels normally found among insulin-resistant subjects.

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In vivo Studies of Nitric Oxide (NO) using S-Nitroso-Glutathione (GSNO) in Dog, Animal Model.

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This study investigated the pharmacological effects of GSNO (a carrier of NO) on blood glucose levels in ten mongrel dogs using the glucose oxidase method, and measured the plasma nitrate concentration(the oxidative product of NO) using an automated method. Each dog acts as its own control. Then results are expressed as Mean ± S.E., with $p<0.05$ as statistically significant. GSNO elicited a dose-dependent increase in blood glucose level which was parallel with an increase in plasma nitrate concentration (35-100%). Post-prandial blood glucose levels(after administration of 35mg/kg of GSNO and glucose load) after 2hrs and 2.5 hrs were significantly higher than in controls(7.2±0.9mmol/l and 7.1±0.7mmol/l. vs 5.3±0.3mmol/l and 4.6±0.2mmol/l, $p<0.05$). Post-prandial levels after administration of 50mg/kg of GSNO were 8.6±0.9 and 9.2±0.7mmol/l, $p<0.05$). The basal plasma nitrate concentration was 12.4±0.4µmol which increased on administration of 35mg/kg and 50mg/kg of GSNO to 16.8±1.0µmol and 24.0±0.5µmol respectively ($p<0.05$). Co-administration of Ascorbic Acid and GSNO enhanced the hyperglycaemic effect of NO(9.2±0.7mmol/l and 9.3±0.3mmol/l, $p<0.05$). This data provides evidence that GSNO caused persistent hyperglycaemia in normal dogs which is associated with an increase in plasma nitrate concentration.

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INSULIN RESISTANCE SYNDROME AND ENDOTHELIAL DAMAGE - ROLE OF ADIPOSE TISSUE DERIVED PROINFLAMMATORY CYTOKINES.

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Insulin resistance clusters not only with hypertension and dyslipidaemia, but also with fibrinogen, an acute phase protein. We have tested the hypothesis that circulating levels of proinflammatory cytokines, tumour necrosis factor-α and interleukin 6, are related to insulin resistance. Circulating concentrations of insulin, lipids, fibrinogen, C-reactive protein and cytokines, blood pressure and albumin excretion rate, were measured in 107 non-diabetic subjects. Concentrations of von Willebrand factor, thrombomodulin and cellular fibronectin were measured as markers of endothelial dysfunction. A sum score of insulin resistance variables was closely correlated to that of acute phase markers ($r=0.59$, $p<0.00001$), but more weakly to an endothelial dysfunction score ($r=0.32$, $p=0.0008$). There were correlations of each of the components of the insulin resistance cluster with levels of both TNF-α and C-reactive protein, and more weakly with IL-6. Moreover von Willebrand factor and fibronectin concentrations were related to levels of C-reactive protein and TNF-α. In pursuing the source of the cytokines, these levels and those of C-reactive protein were strongly related to measures of global and central obesity ($r=0.19$-0.51) but not (or weakly) to titres of Helicobacter, Chlamydia and cytomegalovirus. Adipose tissue expresses both TNF-α and interleukin-6. We have recently shown production of interleukin-6, but not TNF-α, by subcutaneous human adipose tissue in vivo, and this cytokine is largely responsible for hepatic production of C-reactive protein. Both cytokines increase lipolysis and may influence insulin signalling, as well as producing endothelial dysfunction. We propose that proinflammatory cytokines originating from adipose tissue may be responsible for the association of insulin resistance, endothelial dysfunction and cardiovascular risk.

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GLUTATHIONE EFFECTS ON INSULIN RESISTANCE IN NON-INSULIN-DEPENDENT DIABETES MELLITUS.

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Insulin resistance and increased oxidative stress are features of non-insulin-dependent diabetes mellitus (NIDDM). To investigate the relationship between insulin resistance and oxidative stress we evaluated the effect of an antioxidant agent, glutathione, on insulin action. We studied 10 lean NIDDM patients (7 M and 3 F; mean age 58,8±8,5 yrs; HbA1c < 7%). After an overnight fast, patients underwent a three hour euglycaemic clamp; during the last hour glutathione (1,35 g/m^2 Tationil, Boeringher Mannheim) was infused. After one week, a second euglycaemic clamp was performed with placebo (isotonic saline 0,9% NaCl) infusion in five patients, randomly selected from the above group. During each clamp, insulin sensitivity and erytrocyte GSH/GSSG ratio was evaluated at baseline, 120 min and 180 min. Total glucose uptake after glutathione infusion was significantly increased (M from 4,5±1,6 at 120 min to 5,5±1,8 at 180 min; $p<0,0001$; percentage increment 24,5±11,8 $p<0,002$ vs placebo group), consistently with an improvement in insulin sensitivity. No difference was found after placebo (M from 4,7±1,5 at 120 min to 5,1±1,7 at 180 min; p=ns; percentage increment 4,8±2,6). Moreover a significant erytrocytes GSH/GSSG ratio percentage increment after GSH infusion was found (42,9±22,5 $p<0,005$ vs placebo). Our data demonstrate that glutathione improves cellular redox status and insulin action in NIDDM patients supporting a link between oxidative stress and insulin resistance.

1202

DESYNCHRONIZATION OF CIRCADIAN RHYTHMS OF MELATONIN, ENDORPHINE, ACTH AND CORTISOL BY TRANSMERIDIAN FLIGHTS IN DIABETIC AND HEALTHY PASSENGERS.

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In order to test the influence of desynchronisation of circadian hormones by time shift on the blood glucose metabolism of type-I diabetic patients (D) we transported 14 D and 14 normals (N) from Tübingen to Los Angeles - 9 hours western time shift - and to Tokyo - 8 hours eastern time shift -using airliners for an 11 hours non-stop flight. The hormones were analyzed by radio immuno assays, statistic analysis by MANOVA. In east-west direction it took one day for melatonin to adapt to the new time zone that is low values during sun light and high values at night. Endorphine, ACTH and cortisol are desynchronized to the new time zone until the 4th day but are in resonance to eachother. After flighing in eastern direction endorphin, ACTH and cortisol are synchronized on the 2nd day, but melatonin showed a disturbed rhythm during the hole 7 days stay at Tokyo with low values at night and high values in the morning. There were no clinically significant differences between the well controlled D (HbA1c 7,1%) and the N. Blood glucose(BG) control showed larger excursions in D during the first 4 days in the new time zone compared to the control period. The adaptation of NPH insulin to the time shift on the travel days prooved feasable. The reduction of normal insulin was necessary to prevent an increase of the the percentage of BG below 3,9 mmol/l (20% of 3812 measurements). The frequency of BG values over 13,9 mmol/l increased from 6 to 18%. We conclude that the adaptation of circadian rhythms is similar in diabetic and healthy persons. An east-west time shift with a prolongation of daylight is inducing a quicker adaptation of melatonin to the new time zone whereas the other hormones take longer to adapt to the new local time. A west-east flight with a shortening of the night provokes a distortion of the melatonin rhythm for at least 6 days while the other mentioned hormones are in resonance to the new local time in 2 days. The distorsion of internal rhythms is accompanied by a labilization of BG control in D which can be managed by adaptating the insulin dosage to the time shift.

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PIOGLITAZONE AMELIORATES INSULIN RESISTANCE IN NIDDM.

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To evaluate the effect of pioglitazone on the organ-specific insulin resistance in NIDDM, a double-blind placebo-controlled trial was carried out. By using euglycemic (5.2 mmol/l) hyperinsulinemic (1200pmol/l) clamp method combined with oral glucose load (Kawamori R et al : Diab. Res. Clin. Pract. 23 : 155, 1994), insulin-stimulated hepatic glucose uptake (HGU) and peripheral glucose uptake (PGU) were examined. Twenty-one subjects received 30 mg pioglitazone and nine subjects received placebo, once daily for 12 weeks. Patient background (mean age 58.5 yr, BMI 22.7) showed no significant differences between the two groups. Pioglitazone group showed ; 1) an increase in HGU from 28.53 $\pm$19.37 to 59.35$\pm$27.12 %, $p=0.010$; and 2) an increase in PGU from 8.23$\pm$2.23 to 9.20$\pm$1.99 mg/kg $\cdot$ min, $p=0.003$. Placebo group showed no significant change in either HGU or PGU. The increment of HGU was significantly greater in the pioglitazone group than in the placebo group ($p=0.042$), whereas no significant difference between the two groups in the increment of PGU was observed. The results indicate that pioglitazone is effective in ameliorating insulin resistance in NIDDM by enhancing hepatic glucose uptake after meal-intake as well as peripheral glucose uptake.

1204

THE ACARBOSE TREATMENT OF TYPE 2 DIABETES MELLITUS ASSOCIATED WITH LIVER CIRRHOSIS

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The association between type 2 diabetes mellitus (**NIDDM**) and liver cirrhosis (**LC**) with hyperinsulinemia and insulin-resistance is well known. Acarbose (**Ac**) is an unabsorbable inhibitor of intestinal glucosidase activity, lowering both post-prandial hyperglycemia and hyperinsulinemia. ***AIM*** of the study was to evaluate the safety and efficacy of Ac treatment in a series of patients with NIDDM and well compensated LC. ***PATIENTS*** were 18F and 12M, with mean age 57±8 years; 19 were in Child's class B and 11 in class A; NIDDM duration was 7±5 years, without significant complications related to NIDDM. A doublle blind cross-over ***PROCEDURE*** was followed with two alternative two-month treatment schedules: **A)** Ac 100mg/ thrice daily, and **B)** placebo thrice daily (**Pl**). Pts with irritable bowel disease were excluded. At baseline and at 2-month intervals, assays of the blood levels of Hemoglobin A1c (**HbA1c**), fasting (**FG**) and post-prandial glucose (**PG**), postprandial C-peptide (**PC**), and routine serological tests of liver function (**RST**) were measured. ***RESULTS***: compared with Pl, Ac treatment significantly reduced HbA1c (-0.1% vs -0.9%, $p<0.05$), FG (-3% vs -11%, $p<0.01$), PG (-5% vs -19%, $p<0.001$), PC (-0.2 vs -9%, $p<0.05$) and, surpisiling, blood ammonia and clinical signs of enchephalopathy. No significant variations in RST were observed in both Pl and Ac treatment groups. ***CONCLUSION***: since Ac treatment improves glycemic control in NIDDM cirrhotics and reduces the intestinal ammonia-producers bacteria, and is neither absorbed by the bowel nor metabolized by the liver, we propose its use in the treatment of NIDDM in patients with liver cirrhosis.

1205

TROGLITAZONE IMPROVES ADIPOSE INSULIN RESISTANCE IN NIDDM RATS. S. Tagami, T. Honda, H. Yoshimura, S. Sakaue, K. Aoki, Y. Furuya, J. Ishii, J. Hirokawa and Y. Kawakami. First Department of Medicine, Hokkaido University School of Medicine, Sapporo, Japan

Several recent studies have provided evidence that protein-tyrosine phosphatases (PTPases) have integral role in the regulation of insulin signal transduction. In diabetes mellitus, PTPase activities in liver and skeletal muscle were well-investigated. However, PTPase activities in the adipose tissue are not well-known.

In the present study, the change of PTPase enzyme activities in adipose tissue from OLETF (Otsuka Long-Evans Tokushima Fatty) rats were investigated. Cytosol and particulate adipose fractions were prepared from visceral and epididymal, subcutaneous tissue of OLETF and their lean control rats. Troglitazone was given as a food admixture (0.2 %) for a week.

Cytosol PTPase activities of the viceral tissue (5.99 ± 1.03 vs 34.46 ± 2.80 mUnits/mg protein, mean ± SD) and the epididymal tissue (12.39 ± 2.01 vs 17.04 ± 2.71 mUnits/mg protein) were decreased in the diabetic tissue in comparison with normal subjects. Particulate PTPase activities of the visceral tissue (32.70 ± 1.90 vs 6.68 ± 0.75 mUnits/mg protein) and the epididymal tissue (16.44 ± 1.86 vs 8.60 ± 0.83 mUnits /mg protein) were adversely increased in the diabetic tissue in comparison with normal subjects. Troglitazone treatment restored these changes towards normal. Cytosol and particulate PTPase activities in the subcutaneous tissue were not changed. These results indicated that troglitazone normalizes the PTPase activities in the visceral and epididymal tissue.

1206

EFFECT OF ACARBOSE ON ADDITIONAL INSULIN THERAPY OF TYPE II DIABETICS WITH LATE FAILURE OF SULPHONYLUREA THERAPY

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The aim of this 24-week study was to investigate the effects of acarbose on the primary success rate of a combination therapy with insulin and sulphonylurea (SU), and on the insulin requirements of patients with type II diabetes who had late-term failure of SU therapy. Forty-eight patients entered the study, which was double-blinded and placebo-controlled for acarbose. All patients had to be ≥ 40 years of age, with a duration of diabetes ≥ 5 years, SU therapy ≥ 3 years, a HbA_{1c} > 8.5%, fasting blood glucose ≥ 160 mg/dl, and 1-hour postprandial blood glucose ≥ 250 mg/dl. Morning fixed combination therapy with insulin (regular/NPH) was started, together with acarbose or placebo, the daily dose of which was increased in a standardized manner from 100 mg up to 600 mg. The main efficacy parameters were the number of responders and the daily insulin dose in these responders after 24 weeks. Responders had a final HbA_{1c} < 8%, or a HbA_{1c} level at least 15% below the patient's baseline value. There were significantly more responders in the acarbose-treated group compared with the placebo-treated group (20 out of 24 patients *vs* 10 out of 19 patients; $p < 0.05$). The decrease in mean HbA_{1c} did not differ between the acarbose-treated group (from 10.9 ± 1.0% to 8.5 ± 1.2%; mean ± SD) and the placebo-treated group (from 11.0 ± 1.2% to 8.6 ± 1.0%). Daily insulin requirements at the end of the study, however, showed a difference of borderline significance between the acarbose-treated group compared with the placebo-treated group (9.9 *vs* 16.5 IU/day; $p = 0.07$). These results suggest that acarbose may have utility in improving the response to additional therapy with insulin in patients with type II diabetes and SU failure, perhaps even at a lower dose of insulin.

1207

BRL 49653 PREVENTS PANCREATIC ISLET β-CELL DEGENERATION IN ZUCKER DIABETIC FATTY RATS.

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The Zucker Diabetic Fatty (ZDF) rat is an animal model for NIDDM, developing hyperglycemia, glycosuria and secondary complications such as nephropathy and cataract formation. To investigate the possibility that the PPARγ agonist, BRL 49653, may change the course of this disease process, the drug (~10 umol/kg body weight daily) was given in the diet to ZDF rats, aged 6 weeks (Prevention group; n=8) or 21 weeks (Intervention group; n=7) until termination at aged 28 weeks. Untreated ZDF (n=16) and lean (ZL; n=10) rats served as controls. At aged 28 weeks, the pancreata of ZDF controls, compared with ZL rats, contained fewer and smaller islets with a disorganised architecture involving changes to β-cell morphology, apoptotic β-cells (in the centre of some islets), a clear decline in insulin content and increased prominence of α-cells. The β-cell morphological changes comprised hypertrophy with a compartmentalization of the cytoplasm into a perinuclear, slightly acidophilic zone, and a peripheral basophilic region containing most of the residual insulin. In ZL rats, α-cells were confined to the periphery of the islet, but in ZDF controls these cells were located randomly in prominent apparently hyperplastic clusters, perhaps indicating an inward collapse of islet structure resulting from apoptosis of β-cells. Pancreata in ZDF control rats showed similar changes at aged 21 and 28 weeks. In the Prevention group, the islets displayed a normal morphology and were indistinguishable from those in ZL rats, but late Intervention treatment with BRL 49653 failed to influence the established changes. The results demonstrate that early treatment of ZDF rats with BRL 49653 prevents the series of events which results in pancreatic islet β-cell degeneration.

1208

ACARBOSE CAN ELIMINATE THE NEED FOR BETWEEN-MEAL SNACKS IN TYPE I DIABETES.

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This study investigated whether acarbose, taken before breakfast, can obviate the need to split morning carbohydrate demands between a main breakfast and a mid-morning snack in patients with type I diabetes. Twenty-six patients on standard insulin regimens were randomized in a double-blinded, cross-over manner to receive either acarbose, 100 mg, or placebo with one of two meal regimens. Patients were studied on 4 days, each separated by a 1-week wash-out period. On days 1 and 2, patients received a single breakfast; while on days 3 and 4 the breakfast was split, with a third of the calorific value being taken 120 minutes after the first intake. Acarbose or placebo was taken before the first meal. Insulin doses were constant for the 4 study days and given 30 minutes before the first meal. Plasma glucose and insulin concentrations were measured at baseline (before the insulin dose); before the first meal ($t = 0$); and at 30-minute intervals for 210 minutes after the first meal. Comparison of the $AUC_{0-3.5\ h}$ for patients receiving a split meal plus placebo, with those receiving a single meal plus acarbose revealed no statistically significant difference between regimens (-3.84 mmol.h/l *vs* +0.35 mmol.h/l, respectively, $p = 0.062$). Comparison of the change in plasma glucose level between baseline and 90 minutes after the first meal, showed that acarbose induced a decrease (-1.09 mmol/l from 7.0 mmol/l baseline) in patients given a single meal, while an increase was seen in patients given placebo with a split breakfast (+1.05 mmol/l from 8.72 mmol/l). This difference between treatment groups was statistically significant ($p = 0.0034$). These results indicate that acarbose can eliminate the need for a split breakfast in these patients and prevent hyperglycaemia when breakfast is taken as a single meal.

1209

EVIDENCE FOR TROGLITAZONE, A NOVEL ANTI-DIABETIC AGENT, ACTING AS AN INSULIN ACTION ENHANCER.

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Insulin resistance is a key feature of type 2 diabetes which preceds and predicts its development in the non-diabetic population. Troglitazone represents a new class of compounds effective in improving metabolic control in type 2 diabetes primarily by enhancing the insulin sensitivity of the peripheral sites to insulin. Evidence in support of this was obtained from studies in euglycaemic healthy subjects. Using a double-blind, placebo-controlled, parallel-group study design, eighteen healthy adult male subjects (age 19-40yr, weight 64-92Kg) were randomised to receive troglitazone 200mg (n=12) or matching placebo (n=6), given three times daily with the relevant meal for 13 days. On days 0 and 13 blood samples for glucose and insulin were taken just prior to, and over the 3 hours following each of breakfast, lunch and dinner. The weighted mean and peak values, and ratio of weighted mean insulin to weighted mean glucose following each meal, were calculated. Analysis was performed on log transformed data using analysis of covariance allowing for effects due to baseline (covariate) and treatment. On day 13, no effect was observed for any glucose parameter but was associated with a clear trend for weighted mean and peak insulin, and the weighted mean insulin to weighted mean glucose ratio to be reduced. This suggests that insulin sensitivity was improved resulting in less insulin required to maintain euglycaemia. Results are presented as % change compared to placebo on day 13 (95% CI):

Time period	Wt. mean insulin	Peak insulin	Wt. mean insulin/ Wt. mean glucose
Breakfast	-13% (-32, 12)	-10% (-39, 32)	-9% (-26, 12)
Lunch	-3% (-27, 29)	-4% (-29, 30)	-8% (-28, 18)
Dinner	-20% (-43, 14)	-25% (-52, 18)	-20% (-38, 4)

Conclusions: these results in euglycaemic subjects provide evidence that troglitazone acts to improve insulin sensitivity and suggest that in type 2 diabetes the drug acts on the underlying pathophysiology of the disease to improve metabolic control.

1210

VOGLIBOSE DOES NOT MODIFY PHARMACODYNAMICS OR PHARMACOKINETICS OF WARFARIN IN HEALTHY MAN.

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Voglibose (AO-128) is a new and potent inhibitor of disaccharidases used for treatment of diabetes mellitus. Since voglibose increases gastrointestinal motility, the aim of this study was to investigate its influence on the anticoagulant effect and the pharmacokinetics of warfarin under steady state conditions.

12 healthy male volunteers were given individually adjusted doses of warfarin over 15 days to achieve stable Quick's values of 30-40 % of normal. Between day 9 and 15, warfarin doses were kept constant. From day 11 to 15, 5 mg voglibose t.i.d. was administered additionally. Quick's values from days 15 and 16 (test) were compared to those of days 10 and 11 (reference). Pharmacokinetics of S- and R-warfarin of day 15 was compared with that of day 10. Lack of interaction was concluded if the point estimate and 90% confidence interval of the ratios (test/reference) for Quick's value, AUC and C_{max} was within the range of 0.8-1.25.

Mean (and 90% confidence interval) of ratio (test/reference):

Quick's value	S-Warfarin AUC	S-Warfarin C_{max}	R-Warfarin AUC	R-Warfarin C_{max}
0.97 (0.90-1.04)	1.05 (1.00-1.10)	1.08 (1.00-1.16)	1.01 (0.97-1.05)	1.04 (0.97-1.11)

It is concluded, that voglibose had no influence on the pharmacodynamics and the pharmacokinetics of warfarin enantiomers.

1211

EFFECT OF TROGLITAZONE ON INSULIN-INDUCED VASODILATION AND ENDOTHELIAL FUNCTION IN INSULIN-RESISTANT OBESE SUBJECTS

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Insulin-induced vasodilation is nitric oxide dependent and is diminished in type 2 diabetes and obesity and, might reflect endothelial dysfunction. Troglitazone, improves insulin resistance and may thus improve insulin-dependent and/or endothelium-dependent vascular function in insulin resistant obese subjects. Therefore, 14 obese subjects (8M:6F, age 37.2±4.9 yr, BMI 31.6±3.0 $kg \cdot m^{-2}$) were treated with troglitazone 400 mg od or placebo for 8 weeks, in a randomised, double-blind cross-over design. Forearm vasodilator responses [venous-occlusion plethysmography] to intra-arterially administered acetylcholine and sodium nitroprusside, insulin sensitivity and insulin-induced vasodilation [euglycemic hyperinsulinemic clamp] and vasoconstrictor responses to L-NMMA were investigated. Ambulatory 24-hour blood pressure (ABPM) was also measured. Baseline data (placebo) were compared with that of a matched group of 13 lean individuals (BMI 21.9±0.6 $kg \cdot m^{-2}$). Obese subjects were insulin resistant when compared with leans (whole body glucose disposal: 26.9±3.2 versus 53.9±4.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $p<0.001$). Troglitazone improved insulin sensitivity (to 31.8±3.6 $\mu mol \cdot dL^{-1} \cdot min^{-1}$, $p<0.05$). Insulin-induced vasodilation was blunted in obese subjects (increased flow 11.3±12.3%, versus 66.5±23.0% lean, $p<0.05$), but did not improve during troglitazone (to 2.2±4.9%, $p=0.71$). Vasodilator responses to acetylcholine and sodium nitroprusside, and vasoconstrictor responses to L-NMMA did not differ between the obese and lean group, nor between placebo and troglitazone. During troglitazone, ABPM systolic blood pressure remained unchanged, diastolic decreased (84.4±1.3 to 81.1±1.8 mmHg, $p<0.05$). Conclusion: whilst endothelial function was normal in insulin resistant obese subjects, insulin-induced vasodilation was impaired. Troglitazone did not affect vascular reactivity. These data do not support an association between insulin resistance and endothelial function.

1212

α-GLUCOSIDASE INHIBITOR (VOGLIBOSE) SAVES ENDOGENOUS INSULIN AND IMPROVES INSULIN SENSITIVITY

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To protect diabetic patients from cardiovascular diseases, management of blood glucose alone is not sufficient. Relief of insulin resistance is also important. We investigated the effect of Voglibose (α-glucosidase inhibitor) on the endogenous insulin secretion and insulin sensitivity in NIDDM patients. Twenty-seven patients (21 men and 6 women, aged 40-71 years) whose control of blood glucose was not sufficient with diet therapy alone or with sulfonylurea were given 4-6 mg daily of Voglibose for 6 months, and the fasting plasma glucose (FPG), HbA1c, fasting serum IRI, lipids, DHEA-S and urinary excretion of C-peptide were measured before and at the end of the study. FPG decreased from 9.3 mmol/L to 8.7 mmol/L, while decrement of HbA1c was very small. Fasting IRI decreased from 60 pmol/L to 43 pmol/L. U-CPR decreased from 28.2 nmol/day to 23.4 nmol/day. In male patients, DHEA-S, which is thought to be a marker for insulin sensitivity in male, significantly increased at 3 months but the increment was not significant at the end of the study (2.65, 3.13, 2.89 μmol/L respectively). Mean fasting TG level also significantly decreased. TG levels decreased in all 8 patients whose initial TG were high. The reduction of fasting IRI level as well as decrement of urinary excretion of CPR in the face of improvement of blood glucose control indicate that treatment with Voglibose saved endogenous insulin secretion and improved insulin sensitivity in glucose metabolism. Increase of serum DHEA-S and decrease of fasting TG levels also support the improvement of insulin sensitivity.

1213

TROGLITAZONE IMPROVES INSULIN SENSITIVITY THROUGH INCREASED MUSCLE GLYCOGEN CONTENTS IN ZUCKER OBESE RATS

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Recent studies have demonstrated that troglitazone has the capacity to improve insulin resistance. The present study was undertaken to determine the effect of troglitazone on in vivo insulin action, the activities of pyruvate dehydrogenase (PDH) complex and 3-hydroxyacyl-CoA dehydrogenase (HADH) in muscle, and muscle GLUT4 and glycogen contents in Zucker obese and lean rats. Rats were fed normal chow diet with and without troglitazone as a food admixture(0.3%). In vivo insulin action was measured by the sequential euglycemic clamp technique at two different insulin infusion rates, 6.0 (L) and 30.0 (H) mU/kg•min. Plasma insulin concentrations during the L and the H insulin infusion were 100-150 μU/ml and 1,000-1,400 μU/ml, respectively, and blood glucose was clamped at fasting levels by periodic adjustment of the *i.v.* glucose infusion rate. After the clamp studies, the activities of PDHcomplex and HADH and GLUT4 and glycogen contents in red gastrocnemius muscles and heart were determined. Troglitazone treatment produced a significant rise in glucose disposal rate (GDR) during the L insulin clamp study (14.7±2.2 vs 7.1±1.1mg/kg•min, P<0.05)in obese rats, but not in lean rats (26.5± 0.3 vs 29.0 ± 1.7 mg/kg•min). No effects of Troglitazone on GDR during the H clamp study, the activities of PDH complex and HADH or muscle GLUT4 contents were observed in obese and lean rats. However, Troglitazone increased muscle glycogen contents in obese rats (6.8±0.4 vs 5.4±0.4mg/g, P<0.05). These results suggest that troglitazone improves insulin sensitivity through increased muscle glycogen contents.

1214

ACARBOSE IN AMBULATORY TREATMENT OF NIDDM ASSOCIATED TO IMMINENT SULFONYLUREA FAILURE.

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To asses the efficacy and safety of acarbose as an adjunct to high sulfonylurea (SU) doses, a randomised, multicentric, 6 month double-blind, parallel and placebo-controlled trial was performed in primary health care. NIDDM patients aged>40 year-old, BMI<35 kg.m^{-2}, HbA1c levels between 8-12% (N: 4-6%) and more than 3 years of diagnosed diabetes were included. After a month placebo run-in period, all patients were randomly allocated in two groups of treatment (acarbose 100 mg tid vs placebo). HbA1c levels, lipid profile, fasting and postprandial blood glucose levels were performed and adverse events were recorded. SPSS/PC as well as ANCOVA analysis were used. The study included 65 patients and 48 of them were randomized (22 for acarbose and 26 for placebo). No statistical differences were found on age (60.3 vs 61.7), BMI (28.6 vs 27.5) and SU doses (14.0 vs 14.5 mg glybenclamide/day). Acarbose-treated pacients significantly reduced HbA1c levels (9.1/8% vs 8.6/8.6%; p<0,01), based upon a marked decrease in mean postprandial plasma glucose levels (11.7/9.2 vs 12.6/11 mmol.l^{-1}; p=0,07). No significant differences among fasting plasma glucose (9.6/8.4 vs 10.7/10 mmol.l^{-1}), total cholesterol (5.7/5.5 vs 5.9/5.6 mmol.l^{-1}), triglycerides (2.1/1.6 vs 2.2/2 mmol.l^{-1}) and HDL cholesterol (1.5/1.5 vs 1.4/1.4 mmol.l^{-1}) were detected. Thirty patients (46.1%) of the total group presented adverse events, 10 (15.4%) placebo-treated patients and 20 (30.7%) acarbose-treated patients. In 14 (21.4%) patients of the second group it was probably or possibly due to acarbose, although no one was reported as severe event. Only 7 (10.7%) patients, 6 (9.2%) with acarbose and 1 (1.5%) with placebo, withdrew the study because of the adverse events. Thus, despite the side effects, acarbose seems to be a useful option in order to improve HbA1c levels in NIDDM with imminent SU failure.

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OVERVIEW OF THE SAFETY PROFILE OF TROGLITAZONE, AN INSULIN ACTION ENHANCING AGENT

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Troglitazone is a new insulin action enhancing agent, under development for the treatment of type 2 diabetes. Troglitazone (10-800 mg) has been investigated at once, twice and three times daily doses in more than 7706 patients to date in Europe / USA and 1058 patients in Japan. This abstract presents adverse event data collected from four phase II studies conducted in 1066 patients throughout Europe (age 39 - 85 years) for up to 16 weeks.

	Placebo [P] $n = 210$	Troglitazone [T] (all doses) $n = 856$
Patients with any adverse events	126 (60%)	405 (47%)
Patients with any drug-related adverse events	56 (27%)	230 (27%)

The incidence of adverse events was similar in the troglitazone and placebo populations. Most adverse events were mild to moderate in severity. The most common adverse events were malaise (P 11%, T 7.9%), thirst/fluid intake (P 8.1%, T 1.2%), and polyuria and diuresis (P 6.2%, T 0.5%). Adverse events, including drug-related serious adverse events (T 1% , P 1%), showed no age, sex, dose frequency or dose dependency. Although there have been three reports of symptomatic hypoglycaemia (1 P, 2 T) these were not verified by blood glucose measurements. In conclusion, troglitazone is well tolerated, with a good safety profile which supports the further investigation of troglitazone for long-term treatment of type 2 diabetes.

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Effect of metformin on impaired glucose tolerance patients

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In order to evaluate the effect of metformin on glucose metabolism, insulin sensitivity and conversion rate of DM in IGT patients , a total of 70 subjects with IGT were given metformin in 33 or placebo in 37 each for one year period in a double-blind, placebo-controlled study. The dose of metformin was 0.25g, three times daily.

The results showed that 1 IGT patient converted into DM(3.13%) , 3 remained unchanged(9.38%) and 28 became normal(87.50%) one year later in metformin group. While in placebo group the above data were 7 (21.21%), 8(24.24%) and 18(54.55%), respectively (P<0 .05). Metformin treatment was associated with improvement of FBS (from 6.89±0.15 mmol/L to 6.23±0.15mmol/L, p<0.05) and insulin sensitivity (IAI) (from -3.27 to -3.01, P<0.01) at 3 months. At 6 months, both AUCg (from 19.67±0.53 to 17.75±0.51, P<0.05) and AUCi (from 42.66×/÷ 0.32 to 36.31×/÷0.29, P<0.01) were decreased significantly. After 12 months of metformin treatment, the FBS, IAI, AUCg and AUCi were further improved and the UAE was decreased from 5 .89× /÷0.36ug/min to 5.01×/÷0.34u g/min , WHR from 0.854±0.01 to 0.840±0.01 and BMI from 26.08±0.40 kg/m^2 to 25. 04 ± 0. 39 kg/m ^{2} with statistical significance(P<0.05) compared with placebo group.

Conclusion: Our results indicated that metformin, for one year treatment, could reduce the conversion rate of DM in IGT patients and could improve glucose metabolism, insulin sensitivity , BMI, WHR and UAE in the IGT patients. Therefore, metformin might be some benefit for intervention of IGT patients.

1217

ACUTE NON-INSULIN-LIKE STIMULATION OF GLUCOSE TRANSPORT BY TROGLITAZONE IN ISOLATED RAT MUSCLE.

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Investigation of direct and acute action of troglitazone on glucose metabolism of rat soleus muscle strips revealed insulin-independent stimulation of glucose transport (cpm ^{3}H-2-deoxy-glucose/mg/h: control, 307±22; *vs.* 3.25 μM troglitazone, 380±32; p<0.01). Although an acute insulin-mimetic potential of thiazolidinediones has been suggested by others, our findings indicate that troglitazone mimicks the effects of hypoxia and muscle contractions rather than those of insulin in that: (i) Troglitazone-induced glucose transport was accompanied by increased glycolysis (μmol lactate released/g/h: control, 7.1±0.5; *vs.* 3.25 μM troglitazone, 12.3±0.7; p<0.01), but not glycogenesis (μmol glucose incorporated into glycogen/g/h: control, 1.40±0.09; *vs.* 3.25 μM troglitazone, 1.32±0.09; n.s.) demonstrating intracellular glucose handling in a catabolic non-insulin-like fashion beyond glucose transport. (ii) While insulin retained its stimulatory effect on glucose transport in hypoxia-stimulated muscle (cpm/mg/h: hypoxia, 852±77 *vs.* hypoxia + 100 nM insulin, 1229±75; p<0.01), troglitazone failed to increase glucose transport under hypoxic conditions (cpm/mg/h: hypoxia, 789±40 *vs.* hypoxia + 3.25 μM troglitazone, 815±28, n.s.) suggesting that hypoxia and troglitazone trigger glucose transport via an identical non-insulin-like mechanism. (iii) No differences of troglitazone *vs.* hypoxia were identified in respective interactions with insulin. It remains to be elucidated, whether such potential of troglitazone to acutely stimulate muscle glucose metabolism in a non-insulin-like fashion is instrumental for its long-term antidiabetic action *in vivo* or is to be regarded as an independent pharmacologic effect.

1218

REACTION OF METFORMIN WITH REDUCING SUGARS AND DICARBONYL COMPOUNDS

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The reaction between reducing sugars and amino structures in proteins (also called Maillard reaction or glycoxidation) has been shown to play a role in the development of the characteristic tissue pathology of diabetes. Guanidine compounds such as aminoguanidine, inhibit this process by blocking the reaction of amino groups with glucose or the dicarbonyl compounds derived from glucose. Despite the fact that the antidiabetic metformin is a guanidine-like compound, it has never been investigated for possible inhibitory effects on the formation of advanced glycation end products (AGEs). Metformin was incubated in the presence of various reducing sugars and dicarbonyl compounds (glyoxal and methylglyoxal). Reaction kinetics were assessed by analysis of the spectrometric changes, and reaction products analyzed by TLC. The results showed that metformin reacts with glucose, fructose or glucose 6-phosphate. The metformin reactivity is 400-times higher with glyoxal (one of the main intermediates of the Maillard reaction), and even more strongly with methylglyoxal which is increased in diabetes mellitus. Reaction of metformin with glucose was oxygen dependent whereas glyoxal reaction was not. Glycoxidation of albumin by dicarbonyl compounds in the presence or absence of metformin was also studied. Albumin glycoxidation either by glyoxal or methylglyoxal, was decreased by about 30 % and 50% respectively, in the presence of 1mM metformin. These results suggest that besides its known antihyperglycaemic effect, metformin could also decrease AGE formation by reaction with reducing sugars or glycoxidation intermediates.

1219

COMPARATIVE STUDY OF TROGLITAZONE WITH SULPHONYLUREAS : NO EFFECT ON CARDIAC MASS

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Troglitazone (TR) is a thiazolidinedione under development for the treatment of type 2 diabetes. In clinical studies TR reduces hyperglycaemia and is well tolerated. In animal studies compounds of this class have been associated with reversible increases in cardiac mass. Echocardiography data from early clinical studies indicated that TR had no adverse cardiac effects. Here, magnetic resonance imaging, a highly accurate method of determining anatomical and functional parameters of the heart, was performed to assess cardiac mass and function in patients receiving TR or a sulphonylurea (SU). Six month interim data from a 1 year study is presented. This study is a randomised, open, parallel-group design in which 34 type 2 diabetic patients, stable on glibenclamide or gliclazide either continued on pre-existing SU treatment (n=18) or switched to TR 600mg once daily (n=16) for one year. Left ventricular (LV) mass, right ventricular (RV) wall thickness and ventricular function parameters are being measured at baseline, 2, 6 and 12 months. A 6-month interim analysis of the LV mass index (LV mass corrected for body surface area) was performed using analysis of covariance allowing for effects due to treatment and baseline values.

	LV mass index (g/m^2)	
	TR (n=12)	SU (n=15)
Baseline mean	79.9	77.1
6 month adjusted mean	79.7	80.5

The difference between LV mass index for TR and SU was not statistically significant (95% CI: -7.8, 6.2). TR was well tolerated during this period. These data clearly demonstrate that, over the 6-month period, there was no difference in LV mass index between patients treated with TR and those taking a sulphonylurea. Changes in RV wall thickness or adverse effects on ventricular function are therefore not anticipated.

1220

DOUBLE-BLIND, PLACEBO-CONTROLLED, DOSE-RESPONSE STUDY OF METFORMIN HYDROCHLORIDE COMPARED TO PLACEBO IN TYPE II DIABETES MELLITUS D.Cryer,M.D.;J.Rohlf,M.D.;D.Mills,BSN; A.Garber,M.D.,Ph.D.; Princeton, N.J. and Houston, TX

Although metformin (M) has been used to treat type II diabetes for more than 30 years, a dose-response study was never undertaken. Following a three-week lead-in period, four hundred fifty-one patients were randomized to receive either placebo (P) or a single dose level of M for 11 weeks. The M doses used were 500, 1000, 1500, 2000, 2500 mg/day. Lab evaluations included FPG at baseline and wks 1, 2, 3, 7 and 11; and HbA_{1C} at baseline, and wks 7 and 11. Adverse event (AE) monitoring was done at each visit. Results: Compared to P, treatment with M resulted in dose-dependent decreases in FPG and HbA_{1C} at wks 7 and 11. Maximal reductions of FPG and HbA_{1C} were achieved at 2000 mg M and averaged -80.2 mg/dL and -2.01% at wk 11, respectively ($p<0.001$). There was no statistical difference between metformin and placebo in the number of patients reporting an AE. The most frequently reported AE was G.I. related symptoms which occurred 8% more frequently in M vs. P treated patients ($p=0.20$). Also, M treated patients exhibited a 12% increase of diarrhea vs P treated patients ($p=0.02$). Conclusion: All dose levels of M tested were well-tolerated and demonstrated statistically significant and clinically important decreases in FPG and HbA_{1C}.

1221

TROGLITAZONE HAS NO EFFECT ON THE RED CELL MASS OR OTHER ERYTHROPOIETIC PARAMETERS

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Troglitazone (TR) is a novel treatment for type 2 diabetes. In large scale clinical trials TR reduced hyperglycaemia and was well tolerated. Minor, reversible decreases in red blood cell indices were observed, consistent with animal studies where this effect was thought to be associated with an increase in plasma volume. To formally assess these effects, 24 healthy male subjects (age 19-26yrs, weight 64-90kg) were randomised into 3 groups to receive TR 200mg or 600mg, or placebo once daily for 6 weeks in a double-blind, parallel-group study. Red cell mass, determined by dilution of radiochromium-labelled red blood cells and plasma volume, determined by dilution of radioiodinated albumin, were measured pre-study and after 6 weeks of treatment. Additional haematological parameters were also measured. Statistical analysis was performed using analysis of covariance allowing for effects due to baseline and treatment. Results showed no changes in haemoglobin, erythrocyte count or haematocrit. The red cell mass was not reduced, with adjusted mean values of 27.0 ml/kg (200mg group), 25.6 ml/kg (600mg group) and 25.1 ml/kg (placebo). These data, combined with a lack of effect of TR on reticulocyte count, erythropoietin or soluble transferrin receptors indicate that TR does not affect erythropoiesis. There was no evidence of increased red cell destruction or haemolysis. The adjusted mean plasma volume increased by 5.7% (2.5ml/kg, 95% CI -1.6, 6.6) in the 200mg group and by 7.8% (3.4ml/kg, 95% CI -0.8, 7.6) in the 600mg group compared to placebo (p=NS for both). **Conclusion:** Troglitazone had no effect on red cell mass. Dilutional effects, related to modest increases in plasma volume may explain the haematological changes seen in clinical studies.

1222

THE HYPOGLYCAEMIC EFFICACY OF METFORMIN. A META-ANALYSIS.

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Background: The results of randomized, controlled trials of the effects of metformin on blood glucose regulation and body weight varies. In order to get a systematic overview a metaanalysis of the efficacy of metformin was performed by comparing metformin with both placebo and sulfonylurea.

Methods: All randomized controlled trials published since 1957 were selected by searching Current List of Medical Literature, Cumulated Index Medicus, Medline and Embase. Meta-analysis was performed using weighted mean differences in fasting blood glucose, glycosylated haemoglobin and body weight.

Results: Eight randomized, controlled trials comparing metformin with placebo and eleven with sulfonylurea were identified. The weighted mean difference between placebo and metformin in fasting blood glucose was 3,1 mmol/L (95% Cl-3.7;-2.6) and in glycosylated haemoglobin 1.3% (95% Cl -1.6; -1,0). Body weights were not different. Sulfonylurea and metformin lowered blood glucose haemoglobin equally while there was a lower weight (3 kg) after metformin treatment (95% Cl -4.1; -1.9).

Conclusions: Metformin lowers blood glucose and glycosylated haemoglobin significantly compared with placebo. Metformin and sylfonylurea have an equal effect on fasting blood glucose and glycosylated haemoglobin but the body weight was significantly lower after metformin.

1223

SUPPRESSIVE EFFECT OF TROGLITAZONE ON DEVELOPMENT OF TYPE I DIABETES INDUCED BY MULTIPLE LOW-DOSE STREPTOZOTOCIN INJECTION IN MICE.

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Troglitazone is a new class of antidiabetic agent that ameliorates hyperglycemia in NIDDM patients by enhancing insulin action. In the present study, the effect of troglitazone on development of type I diabetes and cytokine-induced pancreatic B cell dysfunction was investigated both in vivo and in vitro. Type I diabetes was induced by injecting DBA/2 mice with multiple low doses of streptozotocin(40 mg/kg/day for 5 days). In one group of diabetic mice, troglitazone was administered for 3 weeks as a 0.2 % food admixture(290 mg/kg/day) from the start of streptozotocin treatment. The second group received no additional treatment(control diabetic mice). Four weeks after the final streptozotocin injection, control diabetic mice had elevated plasma glucose(615±8 mg/dl, n=8) and their pancreatic B cells were infiltrated by lymphocytes. Troglitazone treatment markedly reduced hyperglycemia in diabetic mice(232±30 mg/dl, n=8) by suppressing lymphocyte infiltration. In vitro studies using hamster insulinoma cell line(HIT T-15 cells) revealed that TNF α (10 pg/ml) and IL-1 β (1 pg/ml) treatment for 7 days decreased insulin secretion and mitochondrial activity (as estimated by MTT assay). Troglitazone addition(0.3 μM) during cytokine treatment with TNF α and IL-1 β increased insulin secretion by 1.6 fold and mitochondrial activity by 1.5 fold compared with cytokine treatment. These findings suggest that troglitazone prevents development of type I diabetes by suppressing lymphocyte infiltration into pancreatic B cells and the subsequent TNF α and IL-1 β-induced B cell damage.

1224

METFORMIN IN INSULIN-DEPENDENT DIABETES MELLITUS WITH INSULIN RESISTANCE

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Metformin potentiates the insulin action (sensitivity) in IDDM patients, hyperinsulinemia is probably a risk factor of atherosclerosis. Aim: To examine the effect of metformin added to established treatment of insulin-dependent diabetes mellitus (IDDM) patients with high daily insulin dose and poor glycemic control. Methods: In 20 young IDDM patients (Cpeptide<0,01nmol/l) with normal body weight (BMI=24), normal cholesterol and triglycerides level treated with intensified insulin therapy (IIT) (4-6 insulin doses daily), an euglycemic hyperinsulinemic clamp (insulinemia 100mIU/l) was performed together with measuring of energy expenditure by indirect calorimetry. A dosage of 850 mg of metformin twice a day was added to the established IIT. Second clamp with indirect calorimetry was repeated 3 month later. Results: There was a significant decrease of insulin dose (by 22%) in metformin treated patients, prandial requirement of insulin especially. The decrease was directly dependable on insulin dose at the beginning of the study. The glucose uptake was significantly higher (M=3.9±1.6, resp. 5.1± 2.1 $mg.kg^{-1}.min^{-1}$) which represented increase by 29%. There was insignificant decrease of body weight, no change in HbA1c, triglycerides, cholesterol, free fatty acids. The basal insulinemia remained unchanged as well as the energy expenditure. Patients reported lower frequency of hypoglycemia in the last month of the therapy compared to previous treatment. Only 3 patients did not tolerate the treatment well - diarrhea, dyspeptic problems. The level of lactate was not significantly changed. Conclusions: The treatment of IDDM patients with IIT and metformin is safe and it leads to decrease of insulin dose, mainly the prandial one.

1225

EFFECTS OF TROGLITAZONE ON BONE METABOLISM IN ZUCKER DIABETIC FATTY(ZDF) RATS.

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Although the concept that IDDM is a risk factor for osteopenia is well established, little is known about the effects of NIDDM on bone metabolism. The aims of the present study were 1) to determine if Zucker diabetic fatty (ZDF) rats, a new animal model of NIDDM characterized by insulin resistance, have any disorders in bone metabolism; and 2) if so, to examine the effects of troglitazone, a novel antidiabetic agent, on their bone metabolism. When compared at 19 weeks of age, ZDF rats had significantly lower femoral bone mineral density (BMD)(157.0±2.2 g/cm^2, n=6) than control Zucker lean (Lean) rats(187.4±1.5 g/cm^2, n=6). Serum BGP, a marker for bone turnover, was also significantly lower in ZDF rats compared to Lean rats(8.4±2.2 vs.50.0±3.0 ng/ml). Treatment with troglitazone as a food mixture (132 mg/kg/day) for 13 weeks completely prevented diabetes onset and significantly increased femoral BMD and serum BGP in ZDF rats, while no changes in these parameters were observed in Lean rats. In contrast, pioglitazone, another antidiabetic agent assumed to share its mode of antidiabetic action with troglitazone, in a dose (53 mg/kg/day as a food mixture), which suppressed diabetes onset, markedly lowered femoral BMD both in ZDF and Lean rats after a 13-week treatment period. Pioglitazone slightly increased serum BGP in ZDF rats, but not in Lean rats. Histologically both agents promoted conversion of hematopoietic to fatty marrow in the femur of ZDF and Lean rats. With respect to the degree of the conversion, pioglitazone was far more potent than troglitazone. These results demonstrate that ZDF rats have osteopenia, which is ameliorated by troglitazone, but further aggravated by pioglitazone, indicating that these structurally similar antidiabetic agents have completely opposite effects on bone metabolism.

1226

METFORMIN IMPROVES METABOLIC CONTROL IN NON-INSULIN-DEPENDENT DIABETICS WITH ACARBOSE MONOTHERAPY

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Acarbose has been proven to be an effective adjunct in NIDDM insufficiently treated with sulfonylureas or metformin alone. So far no data exist from prospective studies where metformin was used as the second antihyperglycemic agent in combination with acarbose. The rational of this treatment is a synergistic action of metformin on hepatic gluconeogenesis resulting in reduction of fasting blood glucose whereas acarbose mainly reduces postprandial hyperglycemia. We therefore tested in a double blind placebo-controlled prospective trial with NIDDM subjects successfully treated with acarbose as a first line drug whether addition of metformin could help to optimise metabolic control. Inclusion criteria were: response to acarbose pre-treatment $\geq$ 0.6 % points decrease in HbA_1 with $\geq$ 100 mg tid, prevalue > 8.5 % HbA_1. Major exclusion criteria: contraindications for metformin. Examination schedule: subjects with at least 12 weeks acarbose monotherapy, 6 weeks 100 mg acarbose tid plus 850 mg metformin or placebo. If after 6 weeks the HbA_1 level was still > 9.0 % the dosage of metformin was increased to twice 850 mg or placebo for the following 6 weeks. Evaluation: HbA_1, fasting blood glucose and 90' after a test meal, serum insulin, C-peptide, lactate, safety parameters, compliance and adverse events. 49 subjects were randomised, 47 of them were valid for the intention to treat analysis. Metformin reduced HbA_1 from 10.3 % to 9 % ($p < 0.001$). Placebo: 10.1 $\rightarrow$ 10.4 % after 12 weeks. Fasting blood glucose was decreased from 9.1 to 8.2 mmol/l but remained unchanged with placebo (9.8 vs. 9.7 mmol/l). The corresponding levels for pp BG were 11.4 and 9.6 with metformin and 12.3 vs. 12.6 mmol/l with placebo. Serum insulin and C-peptide levels were not affected. Neither metformin nor placebo changed average lactate levels. In three cases with metformin plus acarbose we observed gastrointestinal side-effects. There were no serious adverse event or drop out in both groups nor relevant changes in safety parameters. In conclusion: in Pat. with NIDDM and unsatisfactory acarbose monotherapy perfect metabolic control can safely be achieved by a combination with metformin.

1227

TROGLITAZONE IMPROVES ALBUMINURIA IN TYPE 2 DIABETIC PATIENTS

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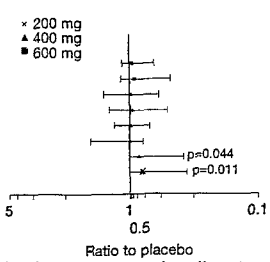
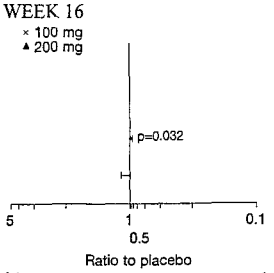
Albuminuria is a risk marker for cardiovascular and renal disease in type 2 diabetes. Troglitazone is an insulin action enhancing agent in late phase III development for the treatment of type 2 diabetes. It has a therapeutic dose-range of 200-600 mg once daily. Week 12 urinary albumin levels from 219 type 2 diabetic patients taking troglitazone 200-600 mg daily or placebo in 3 dose-ranging, double-blind, placebo-controlled studies were collected. Urinary albumin to creatinine ratio was calculated for data collected after 16 weeks from 231 patients in a double-blind, placebo-controlled study of troglitazone 100 or 200 mg daily added to a sulphonylurea (SU). Adjusted geometric means are presented here as ratio to placebo (95% CI):

ALBUMINURIA, TROGLITAZONE MONOTHERAPY, WEEK 12



URINARY ALBUMIN TO CREATININE RATIO, TROGLITAZONE ADDED TO SU, WEEK 16



The improvements in albuminuria with troglitazone 200-600 mg daily compared to placebo provide further evidence that troglitazone may be of value in the prevention of complications associated with type 2 diabetes.

1228

EFFECTS OF STOPPING METFORMIN IN CHINESE NIDDM PATIENTS ON INSULIN-ORAL HYPOGLYCAEMIC AGENT COMBINATION THERAPY FOR SECONDARY DRUG FAILURE

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Recent evidence suggests that in NIDDM patients with secondary drug failure, insulin-oral hypoglycaemic agents (OHA) combination therapy can achieve similar glycaemic control at a lower insulin dose and less weight gain when compared to insulin therapy alone. The importance of metformin in insulin-OHA combination therapy, however, remains unestablished. As a second phase of a randomized, open, parallel trial, we examine the effects of metformin in Chinese NIDDM patients stable receiving combination therapy for secondary OHA failure. 45 subjects (20 on half maximal sulphonylurea and 25 on maximal sulphonylurea-for details see other abstract), after a 20-week insulin re-adjustment phase, entered into a second phase whereby subjects who were previously on metformin stopped the metformin (n=26) and were compared with subjects who had not been treated with metformin (n=19) for a duration of 5 weeks without any adjustment of insulin or other OHA. At baseline, the No-metformin group had a shorter duration of OHA therapy (9.9±4.9 vs 13.5±3.8 years, p=0.007) and a higher HbA_{IC} (8.8±1.2 vs 8.2±0.9%, p=0.039) despite using a larger dose of bedtime insulin (21.9±8.3 vs 13.3±6.7 U p<0.001). At the end of 5 weeks, the group stopping metformin showed a significant deterioration of glycaemic control when compared to the No-metformin group (ΔFPG 3.85±2.95 vs -0.58±1.75 mmol/l, p=0.0001; ΔHbA_{IC} 1.59±0.93 vs -0.02±0.44%, p=0.0001). We conclude that in insulin-OHA combination therapy for secondary failure, patients not on metformin need a significantly larger dose of insulin to maintain glycaemic control than those patients with metformin. Regardless of whether patients were using full or half maximal dose of sulphonylurea, stopping metformin induces a significant deterioration in glycaemic control within 5 weeks. Thus metformin is a useful and important component of insulin-OHA combination therapy regimes.

1229

TROGLITAZONE IMPROVES GLYCAEMIC CONTROL IN PATIENTS ON SULPHONYLUREAS ALONE

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Troglitazone is an insulin action enhancer which improves metabolic control of type 2 diabetic patients as monotherapy. This 16-week, multicentre, parallel-group, double-blind, placebo-controlled study investigated the addition of troglitazone (T) 100 and 200 mg once daily to sulphonylurea (SU). 259 type 2 diabetes patients (mean age 60 yrs, range 34-85 yrs) with fasting capillary glucose ≥ 7 and ≤ 12 mmol/l and taking a stabilised SU regimen were randomised. Median daily doses of SUs were not different between treatment groups. After 16 weeks', fasting blood concentrations (adjusted geometric mean) were:

	n	HbA_{1c} (%)	Glucose (mmol/l)	Insulin (μIU/ml)	Proinsulin (μIU/ml)
SU alone	79	8.2	11.5	9.1	15.2
SU+T100 mg	79	7.7*	10.4**	8.0*	14.0
SU+T200 mg	84	7.4***	9.2***	8.0*	12.5*

*p<0.05, **p<0.01,***p<0.001 vs. placebo

T was well tolerated. Symptoms of hypoglycaemia were not markedly different between the 3 groups (SU 12%, SU+100 mg 7%, SU+200 mg 15%, p=NS). Thus troglitazone added to a SU improved metabolic control without adversely influencing the safety profile.

1230

TROGLITAZONE IS SUPERIOR TO METFORMIN OR INSULIN IN ANTIDIABETIC EFFICACY AND ARRESTS PROGRESSION OF NIDDM IN ZDF RATS

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Antidiabetic efficacies of Troglitazone (Trog), metformin (Mtfn) and insulin (Ins) were evaluated in 9-week-old male Zucker diabetic fatty (ZDF) rats. Glucose (glu)-matched groups were treated twice daily for 14 days. Seven groups (n=10/group) received Trog (50, 150 or 500 mg/kg), Mtfn (50, 150 or 250 mg/kg; LD_{50}=1000 mg/kg/day) or vehicle (Veh; 0.5% methylcellulose) by gavage and 2 groups (n=16/group) received Ins or NaCl (154 mmol/l), s. c. Metabolic variables were monitored weekly and blood samples for fed glu, insulin, triglycerides (tg), NEFA, lactate and drug levels were obtained on days 0, 7 and 14. Unless noted otherwise, all values (mean ± SEM) given here are for day 14, expressed as % change from day 0, and significant at p<0.01. In Veh and NaCl groups, glu, tg and NEFA remained elevated while insulin levels declined (by 71 ± 4% due to loss of pancreatic β cell function). Trog at 150 and 500 mg/kg decreased glu (by 43 ± 10 and 69 ± 1%), tg (72 ± 8 and 90 ± 1%) and NEFA (39 ± 10 and 80 ± 2%). In contrast, Mtfn at 250 mg/kg decreased glu by 24 ± 8% (p<0.05) on day 7, but by only 5 ± 12% (NS) on day 14; tg and NEFA remained elevated while lactate increased by 108 ± 28%. Serum insulin declined in Trog and Mtfn groups similar to Veh group. Ins treatment lowered glu by 72 ± 3% and NEFA by 26 ± 9% but raised insulin levels by 635 ± 128% and did not lower tg. Histology revealed a normalization of pancreatic islet and renal morphology and an absence of cataracts with Trog or Ins, unlike all other treatments. Elevated tg with Ins treatment, despite decreased lipolysis in adipose tissue, is likely due to enhanced *de novo* hepatic lipogenesis. These findings suggest that derangements of glucose and lipid metabolism in the ZDF model of NIDDM are normalized by Trog but not by Mtfn or Ins and that Trog may reverse or at least arrest the progression of NIDDM by novel mechanisms which are manifest *in vivo* as enhanced glucose and lipid (meal) tolerance and increased insulin sensitivity as assessed by the glu/ins ratio.

1231

Troglitazone Restores Free Fatty Acids-Induced Insulin Resistance in Rat Muscles.

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Effects of troglitazone on the peripheral insulin resistance were studied. Rats were maintained on the chow with/without 0.2 % troglitazone for 2 weeks. The hindquarter was isolated and re-perfused for 30 min (6cycles), using perfusate containing 15 mM glucose, 0-1000 μU/ml insulin, 0 or 1.0 mM palmitate. Under the palmitate-free conditions, glucose uptake by the hindquarter was similar between the control and the troglitazone-treated rat at each insulin level. The addition of palmitate markedly suppressed the glucose uptake in the control rat hindquarter, but did not reduce that in the troglitazone-treated rat hindquarter (58 ± 8 vs 101 ± 12 μmol at 1000 μU/ml, P<0.01). It is concluded that troglitazone restores the FFA-induced insulin resistance in the peripheral tissues.

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WEIGHT LOSS IN DIABETIC PATIENTS TREATED WITH METFORMIN.

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The availability of metformin in the USA allowed patients previously treated with insulin or sulfonylureas alone to be transferred to combination sulfonylurea and metformin therapy. A control group of non-insulin-dependent diabetes patients who remained on twice-daily mixed insulin were identified. Over an average of 10 months there was a loss of 0.3 kg (p=0.53) in the control group (C), a loss of 1.6 kg (p=0.03) in the group with metformin added to sulfonylureas(B) over 8 months and 4.7 kg (p=0.0001) in the group transferred from twice daily mixed insulin to combined sulfonylurea and metformin therapy(A) over 6 months. When subjected to multivariable analysis allowing for age, initial weight and length of follow-up there was still statistically significant weight loss in group A compared with groups B and C (p=0.0001). Weight gain was seen in 24 of 57 patients in group C, 15 of 46 in group B, and 6 of 56 in group A. Conclusion: Conversion from insulin to a metformin-utilizing regimen results in significant weight loss in the patient with non-insulin-dependent diabetes.

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PHARMACOKINETIC AND PHARMACODYNAMIC PROFILE OF TROGLITAZONE IN NIDDM PATIENTS WITH GLIBENCLAMIDE

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A randomised, double-blind, double dummy, 3-way cross-over study was conducted in 26 NIDDM patients. After co-administration of troglitazone (T) and glibenclamide (G) safety, tolerability, pharmacokinetics and pharmacodynamics were assessed following daily oral doses of 200 mg T and 2 x 1.75 mg G alone or in combination for 12 days. The plasma pharmacokinetic profiles of T and G and insulin profiles were determined after the last dose of each treatment period and blood glucose concentrations were evaluated during treatment. All treatments were well tolerated with no major differences in ADRs. Those occured were mild or moderate in severity. The ratios of the mean $AUC_{0\text{-}24h}$ ($\mu g.h.ml^{-1}$), C_{max} ($\mu g.ml^{-1}$), T_{max} (median, h) and $t_{½}$ (h) were: T/T+G 7.5/7.2, 0.97/0.85, 2.0/1.0, 22.8/29.9; G/T+G 0.60/0.57, 0.22/0.21, 1.0/1.5, 2.4/3.1. The 90% confidence intervals (CI) caculated for the ratio of T+G/G and T+G/T for AUC and C_{max} were all within the acceptance ranges (ANOVA). Mean blood glucose profiles, including post prandial blood glucose concentrations were 20-30% higher after multiple doses of T alone, compared to G alone and G+T, whose values were similar. Compared with co-administration, plasma insulin concentrations were up to ca 30% lower after T alone and up to ca 30% higher after G alone with the greatest differences occurring up to *ca* 3.5 hr post dose. Overall, co-administration of T and G was well tolerated with no or little change in the pharmacokinetics of either drug. Administration of 3.5 mg G appeared to override the action of 200mg T during co-administration in accordance with the putative of both drugs, ie T increases the sensitivity of the insulin receptors resulting in a lower requirement for insulin to improve the metabolic status of the patient, whereas G stimulates insulin production by the pancreas.

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EFFECTIVENESS OF METFORMIN TREATMENT IN OBESE TYPE II DIABETIC PATIENTS

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The aim of the study was to evaluate the effectiveness of metformin in the treatment of obese type II diabetic patients, who had been previously unsuccessfully treated with diet and glibenclamide. The study was performed in a group of 23 patients, aged 34 -72 years, 16 female and 7 male, with mean diabetes history 3.9±2.9 years. The model of treatment in the group was changed from diet and glibenclamide 3 times daily to diet and metformin given twice daily. Before and after 6 month of the study body mass index (BMI), blood pressure (BP), fasting plasma glucose (FPG), postprandial plasma glucose (PPG), HbA_{1c}, total cholesterol, HDL cholesterol, triglycerides (TAG) and fibrynogen (Fg) were estimated. We observed the blood pressure normalization, significant body mass reduction (BMI: 34.02±5.46 vs 32.91±5.14 kg/m^2, $p<0.05$), improved metabolic control of diabetes (no glycosuria, FPG: 10.66±1.96 vs 6.77±0.84 mmol/l, $p<0.05$, PPG: 11.70±2.35 vs 9.37±1.78 mmol/l , $p<0.05$, HbA_{1c}: 8.24±0.79 vs 6.71±0.52 %, $p<0.05$, Ch.: 6.16±1.16 vs 5.99±0.86 mmol/l , $p>0.05$, HDL: 1.05±0.20 vs 1.17±0.21 mmol/l , $p<0.05$, TAG: 2.73±1.00 vs 2.39±1.11 mmol/l , $p>0.05$) and decrease in Fg concentration from 4.36±0.91 to 4.14±0.94 mg/dl, $p<0.05$. The study performed indicates, that in obese patients with type II diabetes and short history of the disease metformin allows to reduce body mass and to reach good metabolic control of diabetes.

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TROGLITAZONE IS A PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR γ AGONIST: A NOVEL MECHANISM FOR AN ANTIDIABETIC AGENT

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Aim: To determine whether non-thiazolidinedione antidiabetic drugs activate Peroxisome Proliferator Activated Receptor γ (PPARγ). PPARγ is a member of the super family of ligand-activated nuclear receptors. It is most abundant in adipose tissue and plays a key role in adipocyte differentiation and in the expression of a number of genes involved in fuel metabolism. Recently we showed that the thiazolidinedione class of antidiabetic drugs were PPARγ agonists and that the potency of PPARγ agonism in vitro correlated with their antidiabetic action in vivo. These data suggest that PPARγ is the relevant biochemical target for the thiazolidinediones and that activation of PPARγ is responsible for the enhancement of insulin action seen with them *in vivo*. The most clinically advanced thiazolidinedione, troglitazone, is a PPARγ agonist with a Ki for binding to PPARγ of 3.0 μM, an EC_{50} of 0.43 μM for activation of PPARγ in a transient transfection assay and an EC_{50} of 0.48 μM in an adipogenesis assay. A number of alternative chemical classes of molecules with antidiabetic activity in NIDDM patients (or in rodent models of NIDDM) have been evaluated for potential PPARγ agonism. These include agents with known and unknown (e.g; glibenclamide (K_{ATP} Channel) and metformin, respectively) biochemical targets. The following drugs were found to have *no* effect on PPARγ (either in binding or *in vitro* functional assays) at 10 μM: sulfonylureas: glibenclamide, tolbutamide, glipizide; biguanides: metformin, phenformin; β_3 agonist: CL316243; miscellaneous: M16209, BM130907. Conclusion: When compared with a number of structurally different antidiabetic drugs, troglitazone and other thiazolidinediones are unique in their ability to activate PPARγ.

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COMPARATIVE ASSESSMENT OF THE EFFECTS OF METFORMIN AND SULPHONYLUREA.

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The purpose of the study is to compare the metabolic effects of Metformin with Sulphonylurea (Glipizide, Gliclazide). In a randomized parallel-group trial 57 obese type 2 diabetic patients are allocated to receive Metformin (29) or Sulphonylurea (28: Glipizide - 13, Gliclazide - 15) and are followed for six months. The inclusion criteria are: age ≤ 70 years, body mass index > 27 kg/m^2 (men) and ≥ 26 kg/m^2 (women), preprandial blood glucose ≤ 10 mmol/l, lack of contraindications for biguanides or sulphonylurea. After six months of follow-up the mean change in the body mass index, the levels of glycated hemoglobin, lipids (total and HDL cholesterol, triglycerides), liver enzymes (ALAT, ASAT), creatinine and lactate are compared across the two groups (two sample t-test or non parametric Mann-Whitney test are used). We find that Metformin has greater potential to reduce body weight: 1.25 v/s 0.5 kg/m^2 decrease in the body mass index ($p < 0.05$). The Metformin's effect on glycemic control seems to be less pronounced: glycated hemoglobin (Hbac) 8.91% v/s 7.87% ($p = 0.05$). No significant difference across the two groups is observed in the other characteristics.

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EFFICACY AND SAFETY OF ACARBOSE IN THE TREATMENT OF TYPE II DIABETES: A 2-YEAR SURVEILLANCE STUDY.

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The aim of the study was to assess the efficacy and safety of acarbose in patients with diabetes over a 2-year period, focusing particularly on patients with type II diabetes. A total of 2035 patients were enrolled in the study; approximately 95% were classified as having type II diabetes. Physicians had sole control of the doses of acarbose prescribed to their patients. Efficacy and safety were evaluated by assessing changes in fasting blood glucose levels, 1-hour and 2-hour postprandial glucose levels, HbA_{1c} or HbA_1, glucosuria, and a range of other clinical parameters, such as blood cell counts and liver enzyme levels. Patients were ideally evaluated every 3 months. Over the 2-year study period, in patients with type II diabetes, the mean fasting blood glucose level decreased by 2.40 ± (SEM) 0.11 mmol/l, while mean 1-hour and 2-hour postprandial blood glucose levels both decreased by 3.57 ± (SEM) 0.17 mmol/l. There were also decreases in HbA_1 and HbA_{1c} values, of 2.0 ± (SEM) 0.44 and 1.1 ± (SEM) 0.14 percentage points, respectively. The drop of HbA_1/HbA_{1c} was sustained throughout the 2 years. These effects were generally achieved at relatively low, and hence well-tolerated, doses. The incidence of adverse effects and withdrawals was low, at 7.5% and 2.5%, respectively. There were no sustained adverse changes in laboratory parameters. The results of the study indicate that acarbose has a good efficacy and safety profile when used long-term in the day to day treatment of patients with type II diabetes.

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EFFECT OF 6 WEEKS TREATMENT OF METFORMIN ON INSULIN SENSITIVITY IN GLUCOSE INTOLERANT OBESE PATIENTS

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The biguanide metformin is still considered as the first choice treatment in non-insulin-dependent diabetes (NIDDM) associated with excess weight. Insulin sensitivity and blood lipid profile could be improved, as well as weight reduction. If this improvement could be applied to glucose intolerant patients, progression to overt diabetes mellitus which occurs in 30% of these patients after 10 years could be prevented.

19 obese subjects (BMI 36.5±1.3 kg/m2) aged 47±3 years, glucose intolerant (OMS criteria), received metformin (850 mg 2x/day) in a double blind study, cross over with placebo.

Insulin sensitivity was measured by a modified insulin suppression test and glucose oxidation rate by indirect calorimetry. Fasting plasma glucose improved significantly after metformin treatment (6.1±0.1 mmol/l vs 5.8±0.1 mmol/l; p = 0.02). This improvement was not due to an improvement in insulin sensitivity (13.4±0.7 mmol/L placebo vs 13.1±0.8 mmol/l metformin).

Body weight, blood lipid profile, basal plasma insulin and blood pressure did not improve after treatment. However, the systolic blood pressure was significantly improved after the metformin treatment ($p<0.01$).

In conclusion, 6 weeks metformin treatment improve significantly fasting plasma glucose in obese glucose intolerant. This effect was not due to an improvement in insulin sensitivity. Further studies are required to demonstrate if improvement in fasting plasma glucose with metformin could prevent, in obese glucose intolerant sujects, the progression to overt diabetes mellitus.

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EFFECT OF ACARBOSE ON GLUCOSE INTOLERANCE IN NIDDM PATIENTS. K. Noda. Harasanshin Hospital, Japan.

We evaluated the effect of acarbose, an α - glucosidase inhibitor, on glucose intolerance in patients with NIDDM. Acarbose was given orally (300 mg/day) for 24 weeks to 20 NIDDM patients. Data in 75 g OGTT were evaluated before and after 24 weeks of treatment using principal component analysis (PCA), a statistical method that can be used to reveal the general properties of multiple variables. Acarbose administration significantly reduced the postprandial plasma glucose during 24 weeks of treatment. PCA indicated an index of insulin response and insulin resistance and these indices separated our patients into responders (n = 14) and nonresponders (n = 6). The responders had a significantly smaller initial BMI than the nonresponders. There was a significant improvement of fasting and postprandial glucose level after 12 and 24 weeks of treatment in the responders, but not in the nonresponders. Plasma glucose level in the 75 g OGTT significantly improved after 24 weeks of treatment in the responders (Hotelling T^2 value = 47.098, P = 0.022500), but not in the nonresponders. The IRI level did not change in either group. Results thus indicated that acarbose improved insulin resistance in the responders.

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METFORMIN DECREASES BODYWEIGHT AND INSULIN LEVELS BUT NOT CIRCULATING LEPTIN LEVELS IN NON-DIABETIC SUBJECTS.

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Background: Metformin reduces bodyweight and insulin levels in obese type-2 diabetic as well as in obese non-diabetic subjects. Leptin, a recently discovered hormone produced in fat cells, has anorectic and weight reducing effects which are probably exerted at the hypothalamic level. Circulating leptin levels correlates well with BMI as well as fasting insulin levels. **Aim of the study:** To investigate whether metformin treatment influences circulating leptin levels. **Design:** Sixty non-diabetic men with coronary heart disease were treated with diet and lifestyle advice and lovastatin 40 mg/day during a four week run-in period. At week 0 metformin was added for twelve weeks in half of the subjects while lovastatin treatment was continued both in the metformin group (M) and control group (C). The mean daily metformin dose at week 12 was 1759 mg. **Results:** In non-obese subject (BMI < 27 kg/m^2) weight was unchanged both in the M and C group. In obese subjects (BMI > 27 kg/m^2) weight was reduced by 2.3 ± 0.6 kg ($p<0.005$) in the M group while in the C group weight increased insignificantly by 0.8 ± 0.5 kg. Fasting insulin decreased by 16.5% ($p=0.07$) in the M group while it increased insignificantly by 3.9% in the C group. However, metformin treatment did not affect leptin levels (means ± SEM; ng/ml):

Subjects	Treatment group	Change from week 0 to 4	P-values for difference from week 0	Change from week 0 to 12	P-values for difference rom week
All	C	-0.49 ± 0.34	ns.	-0.06 ± 0.49	ns.
	M	-0.34 ± 0.34	ns.	-0.10 ± 0.43	ns.
BMI < 27	C	-0.38 ± 0.34	ns.	0.12 ± 0.26	ns.
	M	-0.12 ± 0.37	ns.	-0.09 ± 0.32	ns.
BMI > 27	C	-0.60 ± 0.59	ns.	-0.24 ± 0.95	ns.
	M	-0.49 ± 0.52	ns.	-0.10 ± 0.71	ns.

Both in the M and C group changes in leptin levels correlated with changes in fasting insulin levels. Only in the C group BMI correlated with changes in leptin levels. During the study 55 to 75 % of variations in leptin levels was explained by BMI and fasting insulin (multiple linear regression analyses). **Conclusion:** Despite that metformin lowers bodyweight and fasting insulin levels, this treatment do not affect circulating leptin levels.

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LONG-TERM EFFECTS OF VOGLIBOSE IN NIDDM PATIENTS

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We investigated clinical benefits of long-term treatment with Voglibose, α-glucosidase inhibitor, in NIDDM patients.

Subjects; Ninety nine NIDDM patients at out-patient clinics of Shizuoka General Hospital undergoing diet and exercise therapy(59 male, 40 female). The average age was 58.1.

Methods; Voglibose(0.9mg/day) was administered for one year. BS, HbA1c, TC, HDL-C, TG, BMI were measured before and 3, 6, 12 months after the beginning of treatment. The subjects were divided in terms of age (65), HbA1c (8.5%), BMI (23) or combination (+/-) and the statistical analysis was performed between these groups.

Results; HbA1c and TG levels were decreased to the similar extent in not only younger group (age<65) but also elderly group (age>=65). The decrease rate of HbA1c level at 12M was greater in the higher goup of HbA1c levels (>=8.5%) than the lower group (<8.5%) (-22% vs -6.2%). In the obese group (BMI>=23), lower HDL level and higher TG level were observed and the decrease rate of HbA1c level in this group was similar to the non-obese group (-9.7% vs -10.7%). The difference in improvement of HbA1c level between Voglibose alone and combined with SU or insulin was not significant (-6.7% vs -13%). Nineteen cases dropped out.

Conclusion; In NIDDM patients, one-year treatment with Voglibose was very effective on not only glycemic control but also improvement of serum lipids levels despite of age, glycemic control levels, obese or non-obese and alone or combination.

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METFORMIN IMPROVES GLUCOSE OXIDATION IN NON-INSULIN-DEPENDENT DIABETES MELLITUS

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Although the metabolic effects of Metformin are still not fully understood it has been shown to improve insulin sensitivity. Metformin decreases hepatic glucose output by inhibiting gluconeogenesis and at the same time it increases the rate of lactate oxidation. The aim of our study was to investigate the effect of metformin on glucose oxidation in NIDDM patients by stable isotope methodology. U-13C glucose (7mg/kg) was used to asses total glucose oxidation in addition to hepatic glucose output (m+6 mass isotopomer) and glucose recirculation (m+3 mass isotopomer). 3-13C glucose (300 mg) was used in a second experiment to asses more specific the effects of metformin on the pyruvate dehydrogenase complex involved in the oxidative decarboxylation of pyruvate to Acetyl-CoA. Glucose oxidation was determined by indirect calorimetry (VCO_2) and mass spectrometry ($^{13}CO_2$) Patients were admitted to the clinic the night before the first experiment and stayed for three days until the end of the second tracer experiment. After 10 weeks of metformin treatment this experimental setup was repeated. The initial dose of 850 mg of metformin was increased every second week up to a final dose of 2550 mg unless the patient had a fasting plasma glucose (FPG) below 120 mg/dL, which did not happen in this first five patients (age 42 ± 8 years, duration of diabetes 5 ± 1 years). The effect of metformin on metabolic profile is shown in the following table (Chol=cholesterol, TG=triglyceride, FFA=free fatty acids).

	HbA1c [%]	FPG [mg/dL]	Insulin [pmol/L]	Lactate [mmol/L]	BMI [kg/m²]	Chol [mg/dL]	TG [mg/dL]	FFA [mmol/L]
before	8.7±0.6	177±13	213±50	1.22±0.04	42±5	209±7	251±63	1.3±0.3
after	7.5±0.1	155±14	202±33	1.68±0.31	41±4	184±6	195±43	0.5±0.1

The cumulative dose recovery of $^{13}CO_2$ over the six hours of experiment increased by 8% (p=0.03) for U-13C glucose and by 16% (p=0.04) for 3-13C glucose. This increased glucose oxidation with a lower insulin level after metformin treatment suggests improvement of insulin sensitivity in NIDDM patients. Differences in glucose oxidation increase between the two tracers 3-13C and U-13C glucose points to the pyruvate dehydrogenase complex as one possible site of metformin action.

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USEFULNESS OF VOGLIBOSE ADMINISTRATION ON DIABETIC PATIENTS WITH CHRONIC RENAL FAILURE.

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Diabetic nephropathy resulting in chronic renal failure (CRF) often accompanies diabetic retinopathy leading to visual disturbance, and to the difficulty in frequent insulin injection. The purpose of the present study was to evaluate the effect of voglibose on diabetic patients with CRF. Nine such patients with poorly controlled blood glucose (7 treated with hemodialysis and 2 untreated) were selected: seven received voglibose plus insulin concurrently and two voglibose alone. The dose of voglibose was 0.2-0.6 mg/day. HbA1c was measured by HPLC at -1, 0, 1, 3 and 5 months after the administration. The mean HbA1c of the nine diabetic patients before the administration (9.1 ± 2.1%; Mean±SD) began to decrease one month after and significantly ($P<0.05$) decreased to 7.2±1.1% 3 months after the administration of voglibose plus insulin or voglibose alone. The mean HbA1c (7.3 ±1.1) five months after the administration was also significantly ($P<0.01$) lower than that before the administration. No severe adverse effects were noted other than mild abdominal fullness. The administration of insulin plus voglibose or voglibose alone was thus effective in controlling blood glucose. These findings indicate that voglibose is a useful therapy for diabetic patients with end stage renal insufficiency.

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TREATMENT WITH METFORMIN IN NIDDM PATIENTS LOWERS PLASMA AMYLIN LEVEL

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This study was undertaken to investigate if the kind of oral treatment of NIDDM can affect the level of plasma amylin (AMYLIN). AMYLIN (Peninsula Lab.kits), serum C-peptide and insulin in fasting state and 6 min after 1 mg i.v. glucagon injection were measured in 3 study groups: healthy volunteers (CON, n=10), NIDDM patients with glibenclamide treament (GLIB, n=10) and patients with metformin treatment (MET, n=13). AMYLIN (in pM, mean±SEM) was: in CON 1.6±0.24 and 6.5±1.6, in GLIB 3.1±0.4 and 5.5±1.2, in MET 1.8±0.3 and 2.3±0.3, in fasting state after glucagon stimulation, respectively. In another group of 20 patients with newly diagnosed NIDDM the above test and measurements were carried out twice: before (GLIB-0, MET-0) and after 2 weeks of treatment with either metformin or glibenclamide (GLIB-2, MET-2).

The fasting and stimulated amylin levels in plasma were:

	0 min	6 min after glucagon
GLIB-0	2.5±0.2	3.3±0.4
GLIB-2	3.2±0.3	4.6±0.4
MET-0	2.6±0.6	3.5±0.6
MET-2	1.3±0.4*	2.8±0.9*

*There was a marked decrease in baseline and stimulated AMYLIN in metformine group. In the light that increased amylin level can contribute to insulinresistance the present results suggest that metformin therapy can be beneficial in this type of diabetes.

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EFFECT OF ACARBOSE COMBINED WITH SULPHONYLUREA AND BIGUANIDE; A DOUBLE BLIND, CROSSOVER STUDY.

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Acarbose, an alpha-glucosidase inhibitor, is known to improve glycemic control in patients with non-insulin-dependent diabetes mellitus (NIDDM) managed with diet alone, or other antidiabetic agents. However, it is controversial whether this drug is effective when combined in patients treated with sulfonylurea and biguanide (metformin). This study was undertaken to test the efficacy of acarbose in combination with sulfonylurea and metformin. The trial was double-blinded, and consisted of a 4-week run-in period, a 12-week test period, a 4-week wash-out perid and a 12-week crossover-test period. Thirty patients with NIDDM, insufficiently treated with sulfonylurea (n = 10) or sulfonylurea plus metformin (n = 20) were randomized into two groups. During the test or crossover-test period, 100 mg of acarbose or placebo were given three times per day respectively. Before and after 12-week test and crossover-test period, mixed meal glucose tolerance test was performed. Twenty six patients completed the study. Among these, 9 were being treated with sulfonylurea (group A) and 17 with sulfonylurea and metformin (group B). Compared with baseline, acarbose treatment resulted in a significant decrease in the fasting and postprandial 1 and 2 hr serum glucose levels (11.5 $\pm$ 1.9 vs. 9.8 $\pm$ 2.2, 17.5 $\pm$ 2.2 vs. 14.5 $\pm$ 3.2, 16.5 $\pm$ 2.9 vs. 14.0 $\pm$ 3.8 mM, $P < 0.05$ respectively) and Hb A_1 level (11.8 $\pm$ 1.5 vs. 10.6 $\pm$ 2.0 %, $P < 0.05$). This improvement in glycemic control was noted both in group A and in group B. There was no statistically significant change in these values during the placebo period. The incidence of side effects (mainly gastrointestinal symptoms such as flatulance and abdominal pain) was higher in the acarbose treatment period (48 % vs. 11 %, $P < 0.05$) than in the placebo period, but there was no significant difference between group A and B. These results show that acarbose is effective in improving glycemic control in NIDDM patients when used in combination with sulfonylurea, or sulfonylurea and metformin.

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EFFECT OF SULFONYLUREA ON WASHOUT RATE OF ^{201}THALLIUM WITHIN MYOCARDIUM CELLS IN NEWLY DIAGNOSED NIDDM PATIENTS

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Functional K_{ATP} channels existing in cardiovascular system are closed or inactive under physiological circumstences. During hypoxia and/or ischaemia these channels are opened and leads the myocardium to relax in order to protect the myocardium against ischaemia and reperfusion damage. Because the endothelium cell function is disturbed in diabetes mellitus, this relaxation can not be observed as in the healthy subjects which may be attributed to the failure in opening the K_{ATP} channels. In the recent study we performed myocard perfusion scintigraphy with ^{201}Thallium which acts via K_{ATP} channels to 10 newly diagnosed diabetic patients and 10 healthy controls. The mean WORs (washout rate) of 10 diabetic patients before and 3 months after starting sulfonylurea were 43.05 and 27.01 respectively while the mean WOR of normals was 47.09. The washout of ^{201}Thallium in myocardium of diabetic patients was equal to the values of normal subjects ($p>0.05$) whereas after the treatment with sulfonylurea for 3 months the WOR of diabetics decreased to 27.01 that was statistically significant when compared to the pretreatment values ($p<0.05$). This preliminary study suggested us that sulfonylureas act as a K_{ATP} channel closer so the washout of ^{201}Thallium from myocardium decreased after treatment in diabetic patients.

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THE EFFECTS OF ACARBOSE ON INTESTINAL PROTEIN ABSORPTION

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Background It is well known that glucose absorption needs the energy derived from Na^+-K^+ ATPase at the basement membrane, and that sodium ion is mandatory for the glucose transport In these aspects, the absorption of amino acids and dipeptides is similar in nature to that of glucose. Therefore, we investigated the effect of the administration of acrabose, α-glucosidase inhibitor, on protein-metabolism. **Method** Sprague-Dawley rats aged eight weeks were divided into the following four groups: (1) normal control group (group C; n=8), (2) acarbose-treated normal group (group A; n=8), (3) non treated diabetic group (group DM, n=8), (4) acarbose-treated diabetic group (group DMA, n=7). Diabetes was induced by streptozotocine at 60 mg/kg (i.p.) Group A and DMA were fed with the same standard diet as given to group C and DM, except for supplementary acarbose at 200 mg/kg. These groups were observed for 28 days. During the last three days of the observation period, we measured food and intake, urine volume, and fecal volume. After freeze drying, physiological saline was added to a certain amount of each fecal specimen, homogenized, and centrifuged. The resulting supernatant was used as a fecal extraction solution for the quantification of total amino acids, total proteins, and glucose. Data were analaysed by Scheffe's multiple comparison; a *P* value less than 0.01 was considered to be statistically significant. **Result** **1)** Blood glucose levels did not differ between group C (101 $\pm$ 7.8 mg/dl, means $\pm$ SE) and A (67 $\pm$ 7.5) or between group DM (388 $\pm$ 35.4) and DMA (416 $\pm$ 51.3), showing no effect of the administration of acarbose. **2)** Total amino acids in feces significantly increased in groups DM (954 $\pm$ 121 nmol/day) and DMA (1331 $\pm$ 171), compared with group C (397 $\pm$ 104) and A (322 $\pm$ 58.9), but no significant differences were seen between group DM and DMA. **3)** Urine protein significantly increased only in group DM, whereas no significant differences were seen among other groups. **Conclusion** Our data suggested that diabetes may cause impaired protein absorption in the digestive tract, and that the administration of acarbose may influence not only glucose absorption, but protein absorption as well.

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GLIBENCLAMIDE BETA-CYTOPHILOUS EFFECT IN MICE UNDER DIFFERENT TREATMENT SCHEDULES

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In our previous study pancreatic beta-cells alteration with auto-immune insulitis development has been shown in animals after long term glibenclamide (Glib) using. The aim of this study was to investigate whether Glib discontinuous treatment schedule can reduce the deleterious impact of chronic continuous treatment with Glib in C57BL/KsJY-db/m mice. Glib was given once daily *per os* (1 mg/kg) within 3 months (Group 1, n=10) or one-monthly courses three times each separated by four weeks (Group 2, n=8). The study was placebo controlled (n=12). Glib treatment in Group 1 decreased the tolerance to glucose (mean blood glucose levels were 5.8±0.2, 17.9±1.3, 18.3±1.2, 10.8±1.5, 6.7±0.2 mmol/l at 0, 30, 60, 120, 180 min after i.p. glucose challenge, respectively vs 5.2±0.2, 10.3±1.3, 9.1±1.0, 4.6±0.2, 4.3±0.3 mmol/l in controls, $p<0.05$ at 30, 60, 120 min), attenuated the insulinaemic reaction (integral immunoreactive insulin content over i.p. GTT at 0, 30, 60, 120 min was 345±25 vs 598±72 pmol/l in controls, $p<0.05$), increased (3 fold, $p<0.05$) the complement-dependent antibody-mediated serum cytotoxity (CDAMSC) directed to neonatal rats pancreatic islet cells, induced dystrophy in beta-cells of 70% islets and lymphocytic infiltration (mainly IA and IB grades) in the third part of islets. In Group 2 we did not observe a decrease in tolerance to glucose, any insulitis; dystrophic pancreatic islets were recognised in 12% and CDAMSC was increased by 1.5 fold. Thus the discontinuous treatment with Glib attenuated sharply the unfavourable beta-cytophilous impact of Glib chronic using.

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EFFECT OF A NEW α-GLUCOSIDASE INHIBITOR, VOGLIBOSE, IN DIABETIC PATIENTS WITH LIVER CIRRHOSIS

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In patients with liver cirrhosis, there is often postprandial hyperglycemia. The effect of a new α-glucosidase inhibitor (voglibose) on blood glucose levels was evaluated in 8 diabetic patients with liver cirrhosis. The patients received voglibose (0.6mg/day) orally immediately before each meal for 4 weeks. It significantly reduced postprandial blood glucose levels (after breakfast : 203.2 ± 62.2mg/dl to 141.0 ± 70.1mg/dl, $p < 0.02$, after lunch : 179.5 ± 99.8 to 141.5 ± 39.1, $p < 0.05$, after dinnner : 187.8 ± 78.6 to 147.0 ± 44.3, $p < 0.05$), accompanied by a decrease in HbA_{1c} (Before : 6.5 ± 2.8 %, After 4 weeks : 5.6 ± 1.7 %, not significant). Fasting Serum total cholesterol and triglyceride levels which were lower in pretreatment period tended to increase during 4 weeks but the differences were not significant, whereas fasting serum HDL cholesterol levels were unaffected. Fasting serum GOT (AST), GPT (ALT), γ-GTP, ChE and albumin levels tended to improve after voglibose treatment (not significant), but fasting blood ammonia levels were significantly decreased after the treatment (90.4 ± 29.9 μg/dl to 58.8 ± 17.4 μg/dl, $p < 0.01$). Blood ammania levels were increased following a meal in diabetic patients with liver cirrhosis. The peak values were seen at 1 hour after breakfast without voglibose treatment, but at 3 hours after breakfast with the treatment. Voglibose did not cause any side effects except for 2 cases who showed mild hypoglycemic attacks at the early stage of the treatment. These results suggest that a new α-glucoidase inhibitor voglibose has a beneficial effect on postprandial hyperglycemia and liver function tests in diabetic patients with liver cirrhosis.

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SULFONYLUREA EFFECT ON INSULIN RECEPTOR TYROSINE KINASE OF DIABETIC PATIENTS IN DIFFERENT CONDITIONS OF GLUCOSE CONTROL.

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The aim of the present study was to investigate the influence of plasma glucose concentrations on glyburide action on insulin receptor tyrosine kinase activity(IRTK)of patients with diabetes type 2(NIDDM). The patients were divided in four groups according to be or not under treatment, and according to their different plasma glucose concentrations.Group A without treatment, and B, C, and D under Glyburide.Plasma glucose, insulin and glycated haemoglobin(HbA1c) were measured. The insulin binding(Bmax) and(IRTK) toward PolyGlu(4:1)Tyr was evaluated in solubilized receptors from erythrocytes of the patients. Statistical analysis by ANOVA,P <0.05. Glucose(mMol/L) was for A(n=7)15.54 +/-2.49; B(n=12)6.35+/-0.31;C(n=10)9.31+/-0.28;D(n=9)14.16+/-1.00, P=0.001.Insulin(pMol/L), for A=78+/-15; B=68+/-10; C=120+/-38; D=70 +/-15; NS. HbA1c(%) for A=13.4+/-1.8; B=7.4+/-0.4; C=9.8+/-0.7; D=11.5 +/-1.1, P=0.004 .The Bmax(nMol/mg protein)was not different between the groups for A=1.13+/-0.31; B=1.35+/-0.25; C=1.80+/-0.31;D=1.25+/-0.23 ; NS. Kd(nM) for A=9.56+/-2.5; B= 4.23+/-0.94; C=4.80+/-01.10; D=5.11+/-1.20 It was higher in A than in B, P< 0.05. The IRTK activity stimulated by insulin(pMol/min/mg protein)was lower in untreated, than in treated groups: A= 10.5+/-2.5; B=24.0+/-2.7; C=29.8+/-5.3; D=22.4+/-3.6, P= 0.01. The Ka(nM) was lower in the treated group with best glucose control than, in treated and untreated groups with high glucose concentrations, for A=2.3+/-0.3; B=1.7+/-0.2; C=3.6+/-0.8; D=2.4+/-0.4, P=0.04. In conclusion,NIDDM patients treated with glyburide presented higher insulin-stimulated IRTK activity without significant changes in insulin binding. Plasma glucose concentrations did not interfer on insulin-stimulated IRTK activity of patients under glyburide treatment,but the treated patients with best glucose control presented higher sensitivity to insulin for IRTK activation.

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THE EFFECT OF α-GLUCOSIDASE INHIBITOR ON HYPERINSULINEMIA IN DUMPING SYNDROME

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A 55-year-old, skinny woman was referred because of syncope attack. She had received partial gastrectomy 30 years ago. In recent years, she exhibited marked post-prandial hypoglycemia. An abdominal computed tomograph could detect no pancreatic tumor. Her thyroid and adrenal functions were normal. Anti-insulin antibodies were not detectable. 72 hour-fasting test did not induce hypoglycemia. Glucagon and arginine loading tests showed normal pancreatic β-cell functions. 50g-OGTT showed oxyhyperglycemia associated with insulin (IRI) hypersecretion (0'→60'; plasma glucose (PG): 90→237 mg/dl, IRI: 4→300 μU/ml). I.v.-GTT showed normal IRI secretion even though PG levels were as high as those in OGTT. Administration of α-glucosidase inhibitor (α-GI) showed desirable effects on loading of 400 kcal mixed meal (without α-GI; 0'→30'; PG : 79→174, IRI: 3→61, with α-GI; PG : 79→138, IRI: 2→22, respectively). These results suggest that the hypersecretion of IRI during OGTT was induced by glucagon-like peptide-1 (GLP-1), because GLP-1 is considered to play an important role in dumping syndrome. Our results also suggest that α-GI blunted hyperinsulinemia-reactive hypoglycemia, through inhibiting incretin secretion.

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PHARMACOKINETICS OF GLIBENCLAMIDE AND ITS METABOLITES IN DIABETIC SUBJECTS WITH NORMAL AND WITH IMPAIRED RENAL FUNCTION

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The pharmacokinetics of glibenclamide (gb), 4-*trans* -hydroxy-(M1) and 3-*cis* -hydroxy-gb (M2) were compared in 2 x 11 diabetic subjects with impaired (IRF iohexol clearance 7-42 ml/min/1.73 m^2) or normal renal function (NRF; iohexol clearance 75-140 ml/min/1.73 m^2). Serum (48 h) and urine (24 h) samples were obtained after administration of 7 mg gb orally. Concentrations of gb, M1 and M2 were determined by HPLC. All comparisons between groups were analysed by paired non-parametric Wilcoxon test, a two-tailed $p<0.05$ considered significant. Peak serum values of M1 (24-85 vs. 16-57 ng/ml), M2 (7-22 vs. <5-18 ng/ml) and M1 + M2 (32-100 vs. 23-76 ng/ml) were higher in the IRF group. Gb AUC (1153±241 vs. 2086±707 μgh/L) and Cmax (302±88 vs. 463±226 ng/L) were lower and Cl/F (6.31±1.30 vs. 3.70±1.15 L/h) higher in the IRF group. M1 AUC (307±140 vs. 210 ±117 μgh/L and Cmax (36±14 vs. 26±10 ng/L) were higher and Cl/F met (27.5±12.5 vs. 40.7±1.8 L/h) lower in the IRF group. Much lower amounts of the metabolites were excreted in urine in the IRF group than in the NRF group (7.2 vs. 26.4% in 24 h) and there was a strong correlation between metabolite excretion and renal function. No other pharmacokinetic differences were found. The differences in AUC, Cmax and CL/F of gb can be explained by a higher free fraction in the IRF group, which would increase metabolic gb clearance. The inverse findings regarding M1 can be explained by the fact that the metabolites are totally eliminated by the kidneys. After a single dose of gb, neither gb nor its metabolites seemed to accumulate in IRF subjects. As only small amounts of M1 and M2 were excreted in the urine, this indicates one or more complementary non-renal elimination routes (probably biliary excretion).

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THE USEFULNESS OF COMBINATION ON THERAPY WITH INSULIN INJECTION AND α-GLUCOSIDASE INHIBITOR IN NIDDM PATIENTS WITH SECONDARY FAILURE ON SULFONYLUREAS AGENTS

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We investigate the effect of combination thrapy with preprandial rapid-acting insulin injections and α-glucosidase inhibitor(α-GI), which is known to suppress postprandial hyperglycemic, on discontinuation of insulin treatment in non-obese NIDDM patients with secondary failure on oral hypoglycemic agents.

Thirty-six hospitalized patients with NIDDM who showed poor glycemic control during 2 weeks of diet and exercise therapy with almost maximal doses of sulfonylureas were divided into a group treated with rapid-acting insulin injection plus α-GI before each meal(group Ⅰ;n=19), and a group treated with rapid-acting insulin injection alone(group Ⅱ ;n=19). There were no significant differences in daily insulin requirements, FPG, HbA1c or serum fructosamine between groups Ⅰ and Ⅱ. However, both meal-related and premeal glycemia was normalization in group Ⅰ and the piriod of insulin treated was 12.7 days shorter than in group Ⅱ.

These obsevation indicate that combination therapy with preprandial rapid-action insulin injection and α-GI can shorten the period of insulin therapy and facilitate discontinuation of insulin treatment through normalization of postprandial hyperglycemia as well as the resting state of pancreatic beta cells in NIDDM patients with secondary failure on oral hypoglycemic agents.

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HAEMODYNAMIC EFFECTS OF GLIMEPIRIDE AND GLIBENCLAMIDE IN DOGS

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The aim of the study was to compare the haemodynamic effects of glibenclamide (GB; n=6) and those of the latest low daily dose sulphonylurea compound, glimepiride (GM; n=6) in the coronary circulation. Mean arterial blood pressure, heart rate, myocardial tissue flow, myocardial contractile force, and the rate of change of myocardial contraction and relaxation were measured in pentobarbital anaesthesia (Nembutal, CEVA, 133 $\mu mol*kg^{-1}$), during iv. administration (0.4-2-5-8 $\mu mol*kg^{-1}$) of the drugs in dogs. Furthermore, cardiac work was calculated. Both compounds proved to be hypotensive (GM: -21%, $p<0.001$; GB: -38%, $p<0.001$) and reduced the heart rate (GM: -6%, $p<0.05$; GB: -32%, $p<0.05$). Myocardial tissue flow was influenced oppositely by the compounds ($p<0.05$): it was increased by glimepiride (+14%, $p<0.05$), while it was diminished (-39%, $p<0.001$) by glibenclamide. Both compounds was found to be negative inotropic (GM: -52%, $p<0.001$; GB: -49%, $p<0.05$). The rate of change of myocardial contraction was reduced by glimepiride and glibenclamide (GM: -56%, $p<0.05$; GB: -52%, $p<0.05$), but the rate of change of myocardial relaxation was decreased only after glimepiride (-39%, $p<0.001$). Cardiac work was reduced by both drugs (GM: -21%, $p<0.001$; GB: -57%, $p<0.01$). According to the results, glimepiride decreases mean arterial blood pressure less, than glibenclamide does; this newly developed drug practically does not influences heart rate and its cardiac work lowering effect is weaker, than that of glibenclamide, although these differences in the present study were not significant. However, glimepiride exerts an obviously favourable side-effect on the microcirculation of the myocardium. Taking these findings and its very low daily dose into account, glimepiride may also be recommended for the treatment of non-insulin-dependent diabetes mellitus.

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EFFICACY OF MONOTHERAPY WITH ACARBOSE, GLIBENCLAMIDE, GLICLAZIDE, METFORMIN OR PLACEBO IN NIDDM PATIENTS

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This study was planned to compare the different oral antidiabetic agents in NIDDM patients with dietary failure. 43 NIDDM patients (35-65 years of age, BMI<35 kg/m^2, HbA1c=7-9%, duration of diabetes>6 months) were randomized into five groups and treated for 24 weeks with acarbose (A) (n=10), gliclazide (Gc) (n=9), glibenclamide (Gb) (n=8), metformin (M) (n=6) or placebo (P) (n=10) and were evaluated every 6 weeks. No change during 24 weeks of treatment with P ($p>0.05$) could be seen for fasting plasma glucose (FPG), postprandial plasma glucose (PPG) and HbA1c levels (9.3±0.8 vs. 9.0±0.8 mmol/L; 10.6±1.0 vs. 10.3±0.4 mmol/L; 8.1±0.5 vs. 8.0±0.7%, respectively). Significantly lower mean values for FPG, PPG and HbA1c levels were obtained on treatment with A (9.6±1.4 vs. 7.8±1 mmol/L; 13.0±2.1 vs. 9.3±1.2 mmol/L; 8.5±0.8 vs. 7.0±0.7%, $p<0.001$, respectively), Gc (10.4±1.8 vs. 8.1±1.2 mmol/L; 13.4±2.1 vs. 9.7±1.3 mmol/L; 8.4±1.1 vs. 7.4±1.3 %, $p<0.001$, respectively), Gb (10.3±2 vs. 8.1±0.6 mmol/L; 13±2.1 vs. 9.9±2.5 mmol/L; 8.4±1.1 vs. 7.2±0.9 %, $p<0.05$, respectively), M (10.8±1.2 vs. 7.8±0.6 mmol/L; 12.5±1.8 vs. 8.7±0.6 mmol/L; 8.4±0.7 vs. 6.9±0.7%, $p<0.01$, respectively). While the significant increases were observed in the postprandial serum insulin levels following treatment with P, Gc, Gb (19.8±1.2 vs. 22±1.5; 20.5±1.2 vs. 31±1.6; 24.3±1.2 vs. 42±1.5 pmol/L, $p<0.05$, respectively), no significant changes were found following treatment with A and M (20.4±1.4 vs. 16.3±1.3; 21.6±5.5 vs. 16.3±6.2 pmol/L, $p>0.05$, respectively). No marked differences were observed between groups with respect to fasting serum insulin and c-peptide levels after treatment. Our results indicate that A, Gc, Gb and M are effective drugs for the monotherapy of NIDDM patients when diet alone fails. Because postprandial insulin increase has been shown to be associated with increased risk for cardiovascular disease, A and M may be superior to Gc and Gb, which elevates postprandial insulin levels.

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COMPARATIVE INVESTIGATIONS OF THE EFFECTS OF GLIBENCLAMIDE AND ACARBOSE ON CIRCADIAN INSULIN LEVELS IN NIDDM

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Glibenclamide as insulin secretion stimulating drug and acarbose as antihyperglycemic agent represent different mode of actions. Little is known of their effects on circadian levels of insulin and lipids under long term treatment. Therefore we investigated 76 NIDDM patients (average age 58.9 years, average BMI 27.6) before and after a 16 week treatment with acarbose (3x100 mg/d) or glibenclamide (3x1 mg/d) or placebo (3x1 tablet/d) in a double blind fashion. All patients were treated by diet alone, antidiabetic drug therapy was interrupted 4 weeks before the start of the investigation. 24 hours profiles of insulin, blood glucose and lipids (fasting and than of all 3 hours) were carried out under standardized diet and Metabolic Ward conditions. After 16 weeks acarbose therapy the insulin area under the curve decreased slightly (8.8 vs. 8.3 $nmol*l^{-1}*24h$, n.s.), whereas after glibenclamide therapy the insulin area under the curve increased significantly (9.6 vs. 12.1, $p < 0.001$) and under placebo it decreased (8.7 vs. 7.9, $p < 0.01$). Hyperinsulinemia persisted up to 4 a. m. in glibenclamide treated patients. In comparison with the initial values the blood glucose area under the curve decreased (after acarbose 317.8 vs. 289.9 $mmol*l^{-1}*24h$, $p < 0.01$, after glibenclamide 356.9 vs. 236.5, $p < 0.01$), but increased in the placebo group (339.2 vs. 346.3, n.s.), the triglyceride area under the curve decreased slightly under acarbose (66.5 vs. 65.9 $mmol*l^{-1}*24h$) more so under glibenclamide (71.6 vs. 62.5 $mmol*l^{-1}*24h$) and increased under placebo (52.8 vs. 61.8 $mmol*l^{-1}*24h$). Glibenclamide leads to long lasting hyperinsulinemia up to dawn whereas acarbose reduces hyperinsulinemia. Both drugs reduce hypertriglyceridemia obviously due to improved diabetes control.

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ACARBOSE IMPROVES THE METABOLIC ENVIRONMENT OF NIDDM PATIENTS WITH THE PLURIMETABOLIC SYNDROME.

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NIDDM subjects (n=105; age 36-71) were randomised to acarbose (100mg tid) or placebo for 16 weeks, and changes in clinical and metabolic parameters indicative of the insulin resistance syndrome were monitored. Fasting levels of glucose, HbA1c, true insulin, proinsulin, fibrinogen, and lipids were measured 4 weekly, and glucose, insulin and triglyceride responses to a standardised 1.6MJ breakfast were determined at 0, 1 and 2 hours post meal. Analysis of data was by ANOVA with repeated measures on an intention to treat basis. The placebo and acarbose treated groups were similarly matched at baseline for age / sex distribution, duration of diabetes, hip:waist ratio but BMI was higher in acarbose (31±3) than placebo group (29±3) group. Acarbose resulted in significant reductions to fasting levels of glucose ($p<0.0001$), triglycerides ($p=0.03$), and HbA1c ($p<0.003$) over the 16 weeks of treatment. The mean HbA1c level was 0.4% different ($p=0.003$) between groups with respect to baseline change. Fasting insulin or proinsulin levels did not change with Acarbose treatment, but in response to the standard breakfast meal, insulin ($p=0.06$) and glucose ($p<0.0001$) responses were reduced. There were no significant treatment differences for fibrinogen, fasting cholesterol, HDL, BP, weight, waist:hip ratio over time. These data show that Acarbose reduces glucose and triglyceride levels, and limits the insulin response to a mixed meal in NIDDM subjects with the plurimetabolic syndrome. **Acarbose may have potential to limit the rapid progression of atherogenesis associated with insulin resistance and diabetes mellitus.**

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GLIBENCLAMIDE AND DAILY BLOOD PROFILES; COMPARISON OF A SINGLE-DOSE AND DIVIDED-DOSES SCHEDULES

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The aim of this study is to clarify the optimal administration schedule of glibenclamide. Daily blood glucose profiles were measured in 65 Type 2 diabetic patients, on glibenclamide in single-dose (n=39) , on glibenclamide in 2 divided-doses (n=26). Daily blood glucose profiles with before breakfast plasma glucose concentrations ≧ 200mg/dl were excluded. Plasma glucose concentrations were determined at 08.00 (before breakfast), 10.00, 12.00 (before lunch), 14.00, 18.00 (before dinner), 20.00, 24.00, 03.00, 06.00, 08.00 hours. Mean Plasma glucose values at 08.00, 10.00, 20.00, 24.00, 03.00, 06.00 hours were not significantly different between single-dose group and divided-doses group, but those at 12.00, 14.00 , 18.00 hours and mean day (08.00~18.00) blood glucose area under the daily profile were significantly lower in single-dose group than in divided-doses group. The mean total (all day) and night (18.00~08.00) blood glucose areas under the daily profile were not significantly different between single dose-group and divided-doses group, but mean night / total blood glucose area ratio was significantly lower in divided-doses group than in single-dose group . In multiple regression analysis, night/total blood glucose area ratio positively correlated with single-dose administration schedule.

These results suggest that blood glucose levels in the night were lower in the divided-dose schedule than in the single-dose schedule.

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INTERRELATION BETWEEN THE EFFECT OF α-GLUCOSIDASE INHIBITOR AND URINE C-PEPTIDE IN NIDDM.

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α-Glucosidase inhibitors (α-GIs) are useful agents for maintaining good glycemic control by reducing postprandial hyperglycemia. However, it may cause hypoglycemia by the combination with insulin treatment. Whether or not the patients develop hypoglycemia seems to depend on their abilities of insulin secretion. This study was conducted to examine the interrelation between the expression of the effect of α-glucosidase inhibitor and urine C-peptide excretion. 40 patients with NIDDM receiving insulin therapy were hospitalized and administered α-GI after 3 wks when their glycemic control become stable. Patients were divided into two groups: high urine C-peptide (>15μg/day) group (HCP) and low urine C-peptide (<15μg/day) group (LCP). Although α-GI treatment improved circadian variation in blood glucose levels, resulting in a decrease in M value in both group, the effect was significantly more prominent in HCP (M value; HCP: 13.2 ± 0.9 to 3.3 ± 0.4, LCP: 14.7 ± 1.8 to 5.7 ± 0.6). To prevent hypoglycemia, the dosage of insulin was reduced in both group. The rate of the reduction in insulin dosage was significantly higher in LCP than in HCP. These observations suggest that the effect of α-GI can be expressed even in patients with a severe deficit in insulin secretion, and that 15μg/day of urine C-peptide in NIDDM patients treated with insulin can be an indicator of reducing the insulin dosage.

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Pharmacokinetics and bioavailability of repaglinide, a new OHA for patients with NIDDM

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In this open-label, randomised, four-period, crossover Phase I study using a replicate design, key pharmacokinetic parameters (AUC, C_{max}, t_{max}, $t_{1/2}$) and mean residence time (MRT) were determined following repaglinide (REP) 2 mg in tablet form and as an oral solution. Twenty-four healthy male volunteers each received a single dose of REP 2 mg (either tablet or oral solution) on four different occasions, separated by a washout period of ≥ 7 days. All subjects received each formulation twice. For 24 hours after dosing, subjects were monitored and blood samples were collected for a 20-point REP serum concentration-time profile. REP was rapidly absorbed after administration of both formulations (t_{max} solution 32 min (SD 6.47), and t_{max} tablet 50.15 min (SD 31.31)). The extent of absorption was similar in both groups, and there was no significant difference between the tablets and oral solution with regard to the intra-subject variation in AUC (36.03 *vs* 32.79 ng/ml*hours; $p = 0.1127$) and C_{max} (30.96 *vs* 34.46 ng/ml; $p = 0.9217$). The relative bioavailability ($AUC_{tablet}/AUC_{oral\ solution}$) was 1.10 (or 110%) with a 95% confidence interval of 1.03–1.17. Although the total amount of REP absorbed was significantly higher for the tablets than for the oral solution, the difference between the two formulations was negligible. The elimination half-life based on subject means was approximately 32 minutes, ranging from 20–60 minutes. The half-lives determined from the individual REP profiles ranged from 11 to 110 minutes. In conclusion, REP pharmacokinetics demonstrate rapid absorption and elimination, and the total availability of REP was the same for the two formulations, though the rate of absorption was 57% lower for tablets, leading to a 10% lower C_{max} and 35% longer MRT.

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Acarbose improves insulin sensitivity in obese patients with IGT.
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Insulin sensitivity is impaired in obese pat, with IGT and accompanied by hyperinsulinemia, which is supposed to be a risk factor for atherosclerosis and early ß-cell exhaustion.
In a double blind placebo controlled study,12 m. patients(BMI > 28),mean age 52 yrs,with impaired glucose tolerance and fasting hyperinsulinemia (> 27µu/ml)were treated with Acarbose loomg tid or placebo for 6 mo.Before and 24hrs after Acarbose treatment insulin sensitivity was measured by minimal model and euglycemic clamp.Insulin, c-peptide and proinsulin were monitored as well. Following Acarbose,c-peptide was lowered insign. from 3.1 to 3.ong/ml compared to placebo(2.9 vs. 3.2ng/ml).Proinsulin,however,decreased sign.from 2o.3 to 13.6µu/ml(placebo:21.9 vs21.3).Insulin sensitivity rose sign.after 6 mo Acarbose treatment:SI 1.3 to 2.8;glucose infusion rate 3.6 to 5.2 GIR.compared to placebo:SI 1.9 to 1.7 and GIR 4.8 vs 3.8.
Conclusion:Acarbose improves insulin sensitivity and lowers proinsulin in obese pat.with IGT.and may be helpful to slow down further progression from IGT to NIDDM.

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Repaglinide *vs* glibenclamide: a 14-week efficacy and safety comparison

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Repaglinide (REP) is a new short- and fast-acting insulin releaser. This European, multicentre study compared the efficacy and safety of REP with the commonly-used sulphonylurea, glibenclamide (GLIB). A total of 195 non insulin-dependent diabetes mellitus (NIDDM), sulphonylurea-treated patients (94 on REP and 101 on GLIB) participated, of whom 161 completed the study. Prior to the study, all subjects had an HbA_{1c} value of between 6.5 and 12 %, and a fasting blood glucose (FBG) value of between 6.2 and 12 mmol/l. The double-blind, randomised, parallel-group study included an initial screening visit, a 1- to 2-week washout period, a 4-week titration period, and a 10-week maintenance period. Repaglinide (0.5 mg, 1.0 mg, 2.0 mg or 4.0 mg) was administered preprandially with the three main meals, and GLIB (1.75 mg, 3.5 mg, 7.0 mg or 10.5 mg) was administered in the morning, with the exception of the 10.5 mg dose (7.0 mg in the morning and 3.5 mg in the evening). Mean HbA_{1c} values decreased from 7.8 % to 7.5 % in the REP group, and from 8.0 % to 7.6 % in the GLIB group (NS between groups). FBG decreased from 13.1 mmol/l to 10.2 mmol/l in the REP group, and from 13.5 mmol/l to 10.1 mmol/l in the GLIB group (NS between groups). There was a statistically significant difference in mean BG (p = 0.0003) and in the 2-hour post-breakfast BG (p = 0.09) between the treatment groups (mean BG REP = 11.2 ± 0.1 mmol/l *vs* GLIB = 11.7 ± 0.1 mmol/l; post-breakfast BG REP = 12.2 ± 0.2 mmol/l *vs* 13.1 ± 0.2 mmol/l), as shown by the 8-point BG profiles. No significant difference was shown between the two groups in fructosamine levels, lipid profiles, or fasting levels of either C-peptide, insulin or pro-insulin. Both treatments were well tolerated and the frequency of all hypoglycaemic episodes was low (35); 20 in the REP group and 15 in the GLIB group. No significant difference in body weight was detected between the two groups. In conclusion, REP is as well tolerated as GLIB and equally effective in controlling FBG and HbA_{1c} levels, together with a significant improvement in meal-related BG levels in comparison with GLIB.

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An Asian multi-centre clinical trial on acarbose monotherapy in NIDDM patients

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This is a multi-centre study involving 7 centres in Hong Kong, Taiwan, Singapore, Philippines, Malaysia and Korea. The objective of the study is to evaluate the efficacy and tolerability of acarbose in Asian NIDDM patients previously treated with diet. The design is one of randomised, double-blind and placebo-controlled. Eligibility criteria included history of NIDDM of more than 3 months, HbA_{1c} level between 7% and 10%, body mass index ≤35 Kg/m^2, no previous anti-diabetic therapy except diet, and no severe diabetic complication. Patients were randomly assigned to receive acarbose (50 mg tid for four weeks followed by 100 mg tid for twenty weeks) or placebo in a double-blind fashion. After the 24-week treatment period, an intention-to-treat analysis showed a statistically significant difference in the mean decrease in HbA_{1c} between the two treatment groups: 0.74% for the 63 patients in the acarbose group and 0.26% for the 63 patients in the placebo group (P=0.014). Mean fasting plasma glucose concentrations changed from 8.4 mmol/l to 8.0 mmol/l in the acarbose group and from 8.3 mmol/l to 8.8 mmol/l in the placebo group (P=0.017). Mean one-hour postprandial plasma glucose concentration changed from 12.8 mmol/l to 11.7 mmol/l in the acarbose group and from 12.9 mmol/l to 13.7 mmol/l in the placebo group (P=0.005). Incidence of diarrhoea was 14% in the acarbose group and 13% in the placebo group. Eleven patients from the acarbose group prematurely withdrew from the study, six of them because of adverse reactions but none of them was serious and one was due to a lack of efficacy. The results have confirmed the clinical efficacy of acarbose in the management of NIDDM patients of Asian origin.

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REPAGLINIDE DIFFERS STRUCTURALLY FROM THE SULPHONYLUREAS GLIBENCLAMIDE AND GLIMEPIRIDE.

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Repaglinide (repa), (S)(+)-2-ethoxy-4-[2-[[3-methyl-1-[2-(1-piperidinyl) phenyl]butyl]amino]-2-oxoethyl]-benzoic acid, and the sulphonylureas (SU) glibenclamide (glib) and glimepiride (glim) bind to the SU-receptor. Based on structure-activity relationships, we suggested a model for receptor binding involving three pharmacophoric groups ($COOH/SO_2NH$; NHCO/CONH; piperidino/MeO or α-oxo). To gain insight into the structural basis for recently reported differences in binding modi between repa and glib, we examined the X-ray structures and analyzed conformational space, electrostatic and lipophilic potentials. The X-ray structures show an extended conformation for glib, and a more compact shape for repa; in a superposition, the pharmacophoric groups do not significantly overlap. Therefore, we looked for further low energy conformations via molecular mechanic calculations (SYBYL®). Three areas of low energy conformations were identified and further optimized (Gaussian 94®) for repa: I (global minimum; ≡ X-ray), II and III (2.6 and 1.8 kcal/mol higher in energy). For glib two low energy conformations were found: I (≡ X-ray) and II (1.3 kcal/mol). In a superposition of repa-II and glib-II, the pharmacophoric groups fit well; the amidic oxo-groups are located to enable hydrogen bonding to the same binding site of the SU-receptor. To repa's (S)-isobutyl, no counterpart is found in glib. The ethoxy (repa) and cyclohexyl (glib) groups marginally overlap. The hydrophobic potentials calculated for repa-II and glib-II differ significantly. We conclude that conformations II may represent a common binding conformation, and that different binding may be due to lipophilic differences. In addition, we determined the X-ray structure of glim. An analogous comparison repa/glim gave similar results as obtained for repa/glib.

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EFFECTS OF ACARBOSE ON BLOOD GLUCOSE LEVELS IN TYPE II DIABETICS

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Acarbose is an antidiabetic agent that produced a dose dependent reduction in postprandial plasma glucose levels following a starch meal. 100 obese outpatients with type II diabetes (48 men, 52 women, mean age 56, mean body mass index 31.3 kg/m^2, without serious complication) were selected for the study. The average duration of disease was 3 years. They were treated with only diet. They were treated with only diet. They were devided into two groups: the control group (50 patients) were treated with diet (1200 calories/day for women, 1500 calories/day for men) and placebo. The treatment group were treated with diet as the same with the control group and acarbose (300 mg/day) for three months. The mean baseline fasting plasma glucose levels were 198 mg/dl at both groups. At the and of study, acarbose produced significantly decrement in fasting plasma glucose level from 198 to 141 mg/dl in treatment group ($p< 0.001$). In the control group, fasting plasma glucose level decreased from 198 to 180 mg/dl (Insignificant compared with baseline level). In conclusion, we stated that acarbose was effective in controlling the fasting plasma glucose levels in type in type II Diabetes Mellitus.

1266

ADDITIONAL TREATMENT WITH REPAGLINIDE PROVIDES SIGNIFICANT IMPROVEMENT IN GLYCAEMIC CONTROL IN NIDDM PATIENTS POORLY CONTROLLED ON METFORMIN

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This multicentre, randomised trial was designed to compare the effect on glycaemic control of repaglinide (REP) given in combination with metformin (MET) against the effect of either drug given as monotherapy in patients not well controlled on MET alone (mean HbA_{1c}: 8.5) .Eighty three patients were included in this three-armed, double blind, double dummy parallel group study. After a 4-5 week run-in period on their usual dose of MET, patients were randomised to either REP therapy, MET therapy, or REP+MET therapy, and the REP dose was determined through a 4-8 week titration period (initial REP dose: 0.5 mg t.i.d.a.c.; maximum dose: 4 mg t.i.d.a.c.), followed by a 3-month maintenance period. The MET dose was kept constant throughout the study (1-3 g/daily). From the baseline to final visit, MET/REP provided statistically significant ($p< 0.005$) improvement in glycaemic control in comparison with the two monotherapies (mean change in HbA_{1c}: -1.41% (MET/ REP),- 0.38% (REP), -0.33% (MET), mean change in fasting blood glucose (mmol/l): -2.18 (MET/REP), 0.49 (REP), -0.25 (MET). No statistical differences were seen between the two monotherapies and MET/REP with respect to fasting insulin and C-peptide levels, and the lipid profiles. MET and MET/REP treatment caused more gastrointestinal side effects than REP treatment. No severe hypoglycaemic events were observed in any group. There were no major side effects and a total of 9 patients withdrew from the study for various causes unrelated to treatment. In conclusion, REP treatment provided the same glycaemic control as MET with less gastrointestinal side effects. REP/MET therapy induced significant improvements in metabolic control in contrast to either REP or MET bringing HbA_{1c} down into the range of acceptable control . The data also suggest that the combination of REP and MET may have synergistic properties in this type of patients.

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EFFECT OF ACARBOSE ON BODY MASS INDEX IN TYPE II DIABETICS

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In the treatment of obesity, many methods are used either alone or in combination. On this study, the effect of acarbose on body mass index in type II diabetics have been evaluated. 100 obese outpatients (48 men, 52 women, mean age 56) were selected for study. They were classified into two groups as diet plus placebo (the control group) and diet plus acarbose (the treatment group). Diets were 1200 calories/day for women and 1500 calories/day for men. Acarbose was administered 300 mg/day for three months. Their disease duration was three years approximately and they have had no serious complications. Mean baseline body mass index was 31.3 kg/m^2. Acarbose significantly decreased body mass index from 31.3 to 28.0 kg/m^2 in the treatment group compared with control group. In controls, weight reduction was only minimal and insignificant compared with baseline value (The control group's body mass index decreased 30.3 kg/m^2). This study has shown that acarbose is usefull in the treatment of obesity with mild diabetes and impaired glucose tolerant.

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EFFECT OF ACUTE ADMINISTRATION OF GLICLAZIDE ON INSULIN RELEASE IN NIDDM.

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Sulphonylureas (SU) show variations in stimulating insulin release. We studied the effect of acute administration of 160 mg gliclazide on insulin release during a hyperglycemic clamp in 12 NIDDM patients, (mean$\pm$sem) age 50$\pm$2.6 yr, NIDDM duration 5.5$\pm$1.4 yr, BMI 24.1$\pm$0.6 kg/m^2. Home SU were stopped for 3 days. Baseline blood glucose levels were 9.6$\pm$0.6 mmol/l. After a 3.5 hr hyperinsulinemic euglycemic clamp (blood glucose 4.6$\pm$0.04 mmol/l) and a 60 min wash out period, a hyperglycemic clamp at 8 mmol/l was started. One hour before the start of the hyperglycemic clamp, gliclazide or placebo (randomized, double blind, cross-over) was administered. The insulin- and C-peptide levels did not differ from 0-10 min (first phase) (both $P>0.5$). So, it is likely that this moderate hyperglycemia does not provoke a first phase in these NIDDM patients. However, second phase insulin release (30-240 min) was significantly enhanced by gliclazide and very poor on placebo. Plasma insulin levels (log-transformation) were significantly higher from 1 hr onwards (all $P<0.05$); geometric mean (95% CI) insulin increased from 13(10-18) vs 10 (7-15) mU/l at the start of the clamp to 17(13-22) vs 11(9-13) at 1 hr, and 20(15-27) vs 13(11-18) mU/l at 4 hr (gliclazide vs placebo). Mean (95% CI) C-peptide levels were also increased from 0.42(0.30-0.60) vs 0.28(0.24-0.33) nmol/l to 0.94(0.65-1.35) vs 0.75(0.59-0.96) nmol/l at 4 hr (gliclazide vs placebo). In conclusion, in long-standing NIDDM gliclazide markedly enhances second phase insulin release at a moderately elevated blood glucose level.

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EFFECTS OF ACARBOSE ON LIPID PROFILE IN TYPE II DIABETES MELLITUS

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It has been demonstrated that acarbose leads to an improvement in insulin sensitivity by reducing postprandial plasma glucose levels, hyperinsulinemia and hypertriglyceridaemia. Efficiency of acarbose on lipid metabolism was evaluated in 100 type II diabetic patients (48 men, 52 women, mean age 56). They were classified into two groups as diet plus placebo (the control group) and diet plus acarbose (the treatment group). Diets were 1200 calories/day for women and 1500 calories/day for men. Acarbose was administered 300 mg/day for three months. Their disease duration was three years approximately, and they have had no serious complications. They don't have any antilipemic treament. Acarbose decreased total cholesterol and LDL cholesterol level significantly compared with pretreatment and control value (pretreatment total cholesterol: 239 mg/dl, control total cholesterol: 227 mg /dl, treatment group total cholesterol :219 mg/dl, $p< 0.01$; pretreatment LDL cholesterol: 151 mg/dl, control: 142 mg/dl, treatment group: 132 mg/dl, $p< 0,001$). HDL cholesterol level increased significantly compared with pretreatment and control value (pretreatment 41.14 mg/dl, control: 41.46 mg/dl, treatment: 43.56 mg/dl, $p< 0.001$), Triglycerid level slightly decreased in this study, probably the treatment duration is not enough for this effect. As a result, acarbose leads to a reduction in total cholesterol and LDL cholesterol levels and to an increasement HDL cholesterol level. Thus the results suggested that acarbose might be a favorable effect for the treatment of diabetes mellitus.

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CLINICAL CHARACTERISTICS AND OUTCOME OF NIDDM PATIENTS WITH ORAL HYPOGLYCEMIC AGENT FAILURE.

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To investigate oral hypoglycemic agent (OHA) failure in NIDDM and the relationship between β-cell function and the outcome of short-term insulin therapy, we studied 44 NIDDM patients treated with OHA. Of these, 25 failed to response OHA while the other 19 responded well. The patients with OHA failure had lower body mass index (BMI), longer duration of diabetes as compared with those with OHA success. The β-cell function between 2 groups was not statistically significant. Patients with OHA failure were further divided into poor β-cell function (Gr. A, n= 9/25) or fair β-cell function (Gr. B, n=16/25) according to their C-peptide responses to intravenous glucagon stimulation. Age at diagnosis, duration of diabetes, fasting plasma glucose (FPG) and glycohemoglobin (HbA1c) were similar in both groups. In contrast, Gr. A had lower BMI ($P< 0.03$). Five patients in Gr. A and 8 patients in Gr. B underwent glucagon stimulation test 1-24 months after insulin therapy. The β-cell function was still poor in 4/5 patients in Gr. A, however, it was stationary in all patients in Gr. B. Successfully switching to OHA therapy was observed in 1 patient in each group and sustained for 10 and 4 months, respectively. In OHA failure patients who had poor β-cell function, 3/5 and 1/5 patients had positive antimicrosomal antibody and antithyroglobin antibody, respectively. However, 2/2 patients had negative insulin antibody and only 1/2 patient had HLA DR3. In conclusion, (1) most OHA failure patients need long-term insulin treatment; (2) patient's β-cell function can't predict the success of conversion to OHA after short-term insulin therapy.

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EFFECT OF ACARBOSE ON HbA_1C LEVELS IN TYPEII DIABETES MELLITUS

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HbA_1c level is one of the parameters of optimal diabetic control. We studied the effect of acarbose on HbA_1c level in type II Diabetes Mellitus. The patients were selected from the outpatients whom didn't receive any insulin or sulfanilurea treatment and doesn't have any serious diabetic complication. 100 obese outpatients with type II diabetes (48 men and 52 women, mean age 56) were taken in the study . They were classified into two groups as diet plus placebo (the control group) and diet plus acarbose (the treatment group). Diets were 1200 calories/day for women and 1500 calories/day for men. Acarbose was administered 300 mg/ day for three months. Their disease duration was three years approximately. Initially, their average HbA_1c levels was 9.13 %. In the treatment group , HbA_1c level decreased to 7,08 %. This result were statistically significant compared with baseline and the control value ($p< 0,001$, control group's HbA_1c level is 9,02 %). We concluded that acarbose was effective in controlling glycaemic levels and also protective from complication in type II Diabetes Mellitus.

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BLOOD GLUCOSE LOWERING EFFECTS OF REPAGLINIDE IN RATS AND DOGS.

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Repaglinide is a novel non-sulphonylurea compound for the treatment of NIDDM. The glucose lowering effects of repaglinide were investigated in fasted Wistar rats and fasted beagle dogs. Furthermore, effects of repaglinide in rats were compared to those of glibenclamide and glimepiride. Compounds were administered orally via gavage (rats) or capsules (dogs) and blood glucose and insulin were determined at several time points p.a.. In rats repaglinide was administered in a dose range of 0.003 mg/kg to 0.3 mg/kg, and both glibenclamide and glimepiride were tested in a dose range of 0.03 mg/kg to 10.0 mg/kg. ED_{50} values were calculated for the effects after 120 min, the time where the maximal glucose lowering effects were obtained with all three substances. Repaglinide was the most potent antihyperglycaemic compound in rats (ED_{50} 9.9 µg/kg). Its potency was 18 times higher than that of glimepiride (ED_{50} 182.1 µg/kg) and 25 times higher than that of glibenclamide (ED_{50} 254.9 µg/kg). In dogs repaglinide (doses tested 0.01, 0.03, 0.1 mg/kg) caused a pronounced antihyperglycaemic effect (ED_{50} 28.3 µg/kg). Insulin levels were only transiently elevated. Maximal increase (about 3 fold increase versus control) was seen at 60 and 90 min after administration with control levels being reached after 4 and 6 hours p.a.. The activity profile of this novel, very potent, antihyperglycaemic compound makes repaglinide a promising new treatment for NIDDM.

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THIS ABSTRACT HAS BEEN WITHDRAWN BY THE AUTHOR.

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REPAGLINIDE, A NEW RAPID AND SHORT ACTING NON-SULPHONYLUREA INSULIN SECRETAGOGUE INHIBITS ATP-SENSITIVE POTASSIUM CHANNELS (I_{KATP}) IN ISOLATED HEART MUSCLE CELLS

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The effect of the non-sulphonylurea insulin secretagogue repaglinide (repa) on I_{KATP} was investigated in single isolated guinea pig ventricular myocytes and compared with the sulphonylurea insulin secretagogues glibenclamide (glib) and glimepiride (glim). Currents were measured using the whole cell voltage clamp technique and a negative voltage ramp from +60 to -100 mV (-20 mV/s). I_{KATP} was induced by superfusing the myocytes with 30 µmol/l cromakalim in the presence of 3, 10, 30, and 100 nmol/l repa, glib, and glim. Baseline currents present at the beginning of the experiment were substracted to separate I_{KATP} from other currents. At maximum drug effect, concentration response curves were constructed using peak I_{KATP} and IC_{50} values (the concentrations of half maximal inhibition of the I_{KATP}) were calculated. The slope of the concentration response curves did not significantly differ among the tested drugs. IC_{50} values (95 % confidence interval) were: repa 10.4 (8.3-13.0) nmol/l, glib 9.2 (6.8-12.6) nmol/l, glim 25.4 (17.8-36.4) nmol/l. The IC_{50} of repa and did not significantly differ from the IC_{50} of glib, however, both IC_{50} were significantly ($p<0.01$) lower than the IC_{50} of glim (about 2.5 times). In conclusion: There were no qualitative differences between the non-sulphonylurea insulin secretagogue repa and the sulphonylurea insulin secretagogues glib and glim in their ability to inhibit I_{KATP}. The relative potency of the tested drugs to block I_{KATP} in cardiac cells corresponds to that in pancreatic β-cells, however, the absolute potency was about 100 to 200 fold less.

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COMPARISON OF THE CLINICAL EFFECT OF DIFFERENT INITIAL DOSES OF ACARBOSE IN DIABETES WITH RESISTANCE TO SULFONYLUREA THERAPY

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Improvement of glycemic control by suppression of postprandial hyperglycemia using acarbose (Glucobay®: GB) has been well documented, but a problem lies in the occurrence of gastrointestinal side effects, especially abdominal distention and flatulence. In this study, the effect on glycemic control and the incidence rate of adverse reactions were compared for initial GB doses of 300 mg/ day and 150 mg/ day. **[Methods]** GB was given at 300 mg/ day (study A) or 150 mg/ day (study B) as the initial dose together with a sulfonylurea (SU) in NIDDM patients with SU resistance. Study A (20 patients) was continued for a mean period of 6.3±1.7 months, and was followed by study B (8.3±1.7 months) including 19 patients different from those in study A. **[Results]** In study A, 3 patients stopped treatment due to severe side effects and the dose was decreased to 200 or 150 mg/ day in 9. In study B, all patients could tolerate the initial dose and the dose was increased to 300 mg/ day in 7 cases. The incidence of initial side effects was 76.5% in study A and 78.5% in study B. Side effects were far less severe in study B as compared with study A. FPG was lowered by 15.6% in study A and by 18% in study B, while HbA1c fell by 13% and 16%. Another study is now in progress using an initial dose of 100 mg/ day. **[Conclusion]** Acarbose was administered in combination with SU therapy to diabetics with SU resistance. When acarbose was started at a low dose and increased after an appropriate period, side effects decreased but the improvement of glycemic control was similar to that achieved at a high initial dose if the treatment period prolong.

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GLUCOSE-DEPENDENCY OF THE GLUCOSE LOWERING EFFECTS OF REPAGLINIDE.

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Repaglinide is a novel antihyperglycaemic compound which is currently evaluated in phase III clinical trials. The aim of the present study was to investigate the glucose lowering activities of repaglinide under normoglycaemic as well as hyperglycaemic conditions. Therefore, fasted Wistar rats were orally dosed with repaglinide together with 0, 0.5, 1.0, 2.0 and 3.0 g/kg glucose as a bolus. Blood glucose levels were determined at several time points after administration. Glucose levels were significantly decreased under all experimental settings with doses of $\geq$ 0.01 mg/kg repaglinide p.o.. With dosages of $\geq$ 0.1 mg/kg repaglinide, the initial increase in blood glucose which was seen after the glucose boli was totally blunted, and glucose levels even fell below starting values. The ED_{50} values calculated for the effects after 120 min were: 12.3 (0.5 g glucose/kg), 9.9 (1.0 g glucose/kg), 14.5 (2.0 g glucose/kg) and 12.8 µg/kg p.o. (3.0 g glucose/kg), respectively. The amount of repaglinide needed to achieve a decrease in blood glucose of 1 mmol/L was calculated to be 10.3, 9.3, 7.0, 8.4, and 7.2 µg/kg p.o. after glucose loads of 0.0, 0.5, 1.0, 2.0, and 3.0 g/kg, respectively. This means that less repaglinide is needed in the hyperglycaemic state to achieve the same reduction in blood glucose levels. As also supported by published in vitro data, repaglinide turns out to be more effective in lowering blood glucose levels in the hyperglycaemic state.

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BLOOD GLUCOSE CONTROL WITH ACARBOSE (COMPARISON WITH SULFONYLUREAS OR INSULIN THERAPY)

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Acarbose is an α-glucosidase inhibitor expected to possess many advantages as a novel means of controlling blood glucose levels, quite distinct from sulfonylureas (SUs) and insulin preparations. To investigate the advantages of acarbose, patients well-controlled (mean HbA1c over three months < 7.5%) on either diet alone, relatively low doses of SUs (≦glibenclamide 2.5 mg/day) or insulin (≦10 U/day), were subsequently treated for one year with acarbose (300 mg/day) and results compared to prior treatment. Subjects consisted of 77 NIDDM outpatients with 24, 23 and 30 cases, respectively, who had previously been controlled on SUs (Group S), insulin (Group I) or diet therapy (Group D). Mean age was 59.5 years, while the mean duration of NIDDM was 8.2 years. Improved HbA1c levels were noted in the D group, but remained unchanged in the S or I group. Total cholesterol (TC) and HDL (HDL-c), had increased from 187.7±28.4 and 49.8±45.4 mg/dl to 200.1±33.3 and 57.3±18.2 mg/dl, respectively, while triglycerides (TG) decreased from 96.3±45.4 mg/dl to 80.4 ±30.5 mg/dl. In Group I, both TC and TG remained unchanged, while HDL-c increased from 49.8±12.5 to 54.1±14.3 mg/dl. Baseline TC and HDL-c in the D group increased from 200.7±35.0 and 53.4±11.8 mg/dl to 217.7±34.6 and 57.7±12.2 mg/dl, respectively, while TGs decreased from 113.3±43.7 to 84.3 ±33.8 mg/dl. Apoprotein-AI in group S increased from 108.4±19.7 to 115.1± 20.8 mg/dl and apoprotein-B in groups S and I decreased from 91.9±20.6 and 85.8 ± 22.1 mg/dl to 82.2 ± 19.0 and 80.2 ± 19.5 mg/dl, respectively. Improvement was also noted in the 19 patients with abnormal urinary protein excretion (trace albumin ≧30 mg/g.Cr); urinary albumin levels falling from 51.5±37.6 to 30.7±22.8 mg/g.Cr. Acarbose (300 mg/day) is as effective as 2.5 mg/day of glibenclamide or 10 U/day of insulin for controlling the blood glucose level. Acarbose also showed a potential to improve serum lipid profiles and reduce urinary excretion of proteins.

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REPAGLINIDE, A POTENT AND ORALLY ACTIVE HYPOGLYCAEMIC BENZOIC ACID DERIVATIVE.

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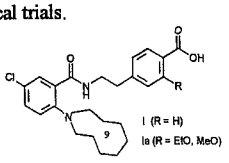
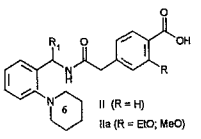
Aiming to improve hypoglycaemic activity and pharmacokinetic properties of benzoic acid derivatives I and II, we have investigated the effect of an additional ethoxy or methoxy substituent (compounds Ia, IIa). The compounds, synthesized by acylation of the corresponding amines, were orally administered to fasted female rats via gavage. Blood glucose was determined at several time points post administration (p.a.). Compounds Ia were found to be less active than I, whereas compounds IIa (R_1 = alkyl, phenyl) showed higher activity and longer duration of action than II. Structure activity relationship studies revealed that hypoglycaemic activity of IIa is optimal when R = ethoxy and R_1 = lower alkyl, ω-alkenyl, or (cycloalkyl)methyl. Racemic AG-EE 388 ZW (R = OEt; R_1 = isobutyl) represents one of the most active compounds IIa; maximum hypoglycaemic activity was observed 2 hours p.a. (ED_{50} = 22 µg/ kg). Its R-enantiomer, AG-EE 624 ZW, lowered blood sugar only marginally after 1 mg/kg, whereas the S-enantiomer, AG-EE 623 ZW (repaglinide), was found to be highly active (ED_{50} = 9.9 µg/kg). Being more potent than the sulphonylureas glibenclamide (ED_{50} = 255 µg/kg) and glimepiride (ED_{50} = 182 µg/kg), repaglinide was selected for development and is currently undergoing phase III clinical trials.





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THE BLOOD GLUCOSE LOWERING EFFECT OF ACARBOSE IS NOT INFLUENCED BY A SIMULTANEOUS THERAPY WITH MAALOX®70

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Aim of this single-centre 4-fold double-blind crossover study was to investigate the influence of 10ml Maalox®70 (antacid) on the pharmacodynamics of the α-glucosidase inhibitor acarbose after administration of 75g sucrose. 24 healthy male volunteers were randomized in four treatment groups and received 100mg acarbose, or a placebo tablet, or the combination of 100mg acarbose plus 10ml Maalox®70, or a placebo tablet plus 10ml Maalox®70. Wash out phases of 6-10 days separated the various successive treatments. Venous blood glucose concentration was determined using the hexokinase method and serum insulin was determined by radioimmunoassay. Standard laboratory investigations for the judgement of safety were done before and after the study. To assess a possible interaction of 10ml Maalox® 70 and acarbose, postprandial blood glucose and serum insulin levels were compared as maximal concentrations and „area under the curve" (AUC) over 4 hours. Integrated blood glucose and serum insulin response were calculated using the log linear trapezoidal approximation. Decision of bioequivalence (= no pharmacodynamic interaction) was based upon two one-sided t-tests. Adverse events (flatulence, diarrhoea) were reported from 14 resp. 15 out of 24 volunteers in the acarbose treatment groups. Only 1 resp. 3 volunteers in the placebo groups (headache, flatulence) reported side effects. No trends in the other laboratory parameters were detectable. No significant difference in blood glucose and insulin between acarbose or acarbose plus Maalox®70 or placebo and placebo plus Maalox®70 could be found. Testgroups receiving acarbose showed a significant decrease in postprandial blood glucose and serum insulin increment ($p<0.01$). AUC- and Cmax values of acarbose plus Maalox®70 and acarbose were almost identical. No influence of Maalox®70 in addition to acarbose or placebo was seen. Conclusion: Maalox®70 need not to be classified as contraindication when medicated together with acarbose.

1280

ORAL ANTIDIABETIC DRUGS ARE GIVEN BOTH INAPPROPRIATELY AND CARELESSLY.

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A survey was made to assess the quality of initial prescribing of oral antidiabetic drugs, with particular regard to the firm recommendation in the British National Formulary that they should not be given until patients have been shown not to respond adequately to at least three months' dietary restriction. The clinical histories of 100 consecutive patients with diabetes of more than one year's duration attending a hospital diabetic clinic were analysed. Patients taking insulin were excluded. Most had received their initial advice elsewhere, either from their general practitioner or from another hospital department: 14 were being treated with diet alone, 46 had taken a proper period of diet followed by tablets and 40 had started tablets immediately at the time of diagnosis. There was no instance of inappropriate early prescribing of tablets by members of the Diabetic Clinic team. Sulphonylurea-induced hypoglycaemia can be an important problem. Therefore the 81 patients in this survey who were taking sulphonylureas were asked if they had been warned about this when they started taking the drug: 24 (30%) remembered receiving advice, 57 (70%) did not. Sixteen (20%) had suffered from symptoms which probably had been due to hypoglycaemia. There was no significant difference between the incidence of these symptoms in the groups which could or could not remember receiving advice about hypoglycaemia. It appears that when doctors who do not specialise in diabetes mellitus initiate treatment in a newly diagnosed patient too many prescribe oral antidiabetic drugs too early and give advice about hypoglycaemia too late.

1281

CHOLESTYRAMINE DOES NOT IMPAIR THE EFFECTS OF VOGLIBOSE (AO-128) ON GLUCOSE AND INSULIN

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Voglibose(V) is a new α-glucosidase inhibitor for treatment of non-insulin-dependent diabetes mellitus. This randomised, parallel group study was aimed at investigating whether cholestyramine(CH), which is known to bind drugs, interacts with voglibose and thereby reduces its effects on the postprandial rise in blood glucose(G) and insulin(I). 24 healthy subjects received 5 mg V three times daily from day 1 through the morning of day 21. 12 of the volunteers additionally received 4 g CH once daily on day 7 and 4 g CH twice daily from day 8 through the morning of day 21. On days 7 and 21 the volunteers received a carbohydrate rich standard breakfast. Blood samples of 10 ml were taken at -0.25, 0.25, 0.5, 1, 1.5, 2, 3 and 4 h relative to dose intake for the determination of G and I. The effect of CH on the postprandial G and I control of V was investigated by means of the $AUC_{0\text{-}4h}$ (linear trapezoid rule) and C_{max} of G and I by means of an ANCOVA (SAS 6.11) with baseline G and I as a covariate. There was no statistically significant difference in G and I on day 7 and day 21 with respect to $AUC_{0\text{-}4h}$ (V/V+C day 7; day 21: 370/366; 347/356 mg*h/dl and 65/47, 73/52μU*h/ml) and C_{max} (V/V+C day 7; day 21: 103/105; 104/103 mg/dl and 25/20; 30/23 μU/ml). The adverse event spectrum was similar in both dosing groups. It can be concluded, that the combination of 5 mg V three times daily and 4 g CH twice daily is safe and CH does not impair the effects of V on the postprandial rise in G and I.

1282

BIOTRANSFORMATION OF [14C]REPAGLINIDE IN HUMAN, CYNOMOLGUS MONKEY, DOG, RABBIT, RAT AND MOUSE

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Repaglinide is presently under development as a new oral antidiabetic insulin secretagogue. After oral or IV administration of [14C]repaglinide (AG-EE 623 ZW) or the racemate [14C]AG-EE 388 ZW, the radioactive metabolite profiles in plasma, urine and bile or feces were analyzed using radio-HPLC and MS. The compound was very well absorbed after oral administration in the rat, dog and human (80 - 100 %). In the rabbit, absorption was 50 - 100 %, in the mouse about 45 %. In all species, total radioactivity was excreted mainly with bile, and the excretion in urine was comparatively low: rat and dog 1 % of the dose, mouse 3 %, monkey 6 %, human 8 % and rabbit 30 %. Repaglinide was metabolized to various degrees according to the following pathways: conjugation to yield the acyl-glucuronide (M7) or tauride (M6), oxidative opening of the piperidine ring (M2), oxidative N-dealkylation of M2 with formation of the aromatic amine (M1), hydroxylation of the piperidine ring (M4) and N-oxidation (M12). In plasma of all species the parent compound represented 56 - 98 % of the radioactivity. The other metabolites mentioned above comprised up to 0.5 - 7 %. Repaglinide was excreted almost exclusively metabolically in all species. Biliary or fecal clearance of the metabolites was the most dominant route. M2 formation was the major pathway in human (68 % of the dose), monkey (82 %) and rabbit (46 %). The further formation of M1 was the second pathway in human (14 %) followed by hydroxylation of the piperidine ring (M4). The major pathway in the dog (70 %) and in the rat (53 %) was the conjugation with glucuronic acid (M7). M2 represented the second important metabolite fraction (dog 20 %, rat 32 %).
The metabolic clearance of repaglinide and the excretion of the metabolites with bile may represent an important safety aspect in patients with impaired renal function.

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Insulin Therapy in IDDM

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EFFECT OF DAILY DOSE OF INSULIN ON METABOLIC CONTROL AND DEVELOPMENT OF LATE DIABETIC COMPLICATIONS IN TYPE I DIABETES.

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Introduction: Type I diabetic patients with low daily dose of insulin have preserved ß cell function or an enhancement in insulin sensitivity. The effect of low daily dose of insulin on metabolic control and late diabetic complications have not been well established in type I diabetic patients. **Objective:** The aim of the study was to determine whether daily dose of insulin in patients with type I diabetes is related with metabolic control, chronic complications, and preserved ß cell function. **Patients, material, and methods:** Patients with type I diabetes (n=380) were identified from the out-patients' clinics of the Cruces Hospital. To be included, patients had to have diabetes onset before age 32, were ketosis prone, and were treated continuously with insulin. Two groups of patients with more than 10 yr. of diabetes evolution were selected according to their daily dose of insulin: group 1 (n=16) with high dose of insulin (> 0.7 UI/Kg) and group 2 (n=15) with a low dose (< 0.5 UI/Kg). Clinical data (including the presence of chronic complications), metabolic control (HbA1c) and preserved ß cell function were studied in the two groups. Basal and after glucagon C peptide test was done to measure ß cell function. **Results:** No differences were found between the two groups of patients in the sex, body mass index, years of evolution of type I diabetes (22±9 vs. 22±8 yr. respectively), and diabetic late complications with exception of no proliferative diabetic retinopathy (NDPR).

Group	age (yr.) at diagnosis	Ketoacidosis (100 patients/yr.)	daily dose of insulin (UI/Kg)	C-peptide test (nmol/l) 0'	6'	HbA1c (%)	NDPR (%)
1	13±10	9.1	0.97±0.22	0.13±0.19	0.16±0.19	9±1	25
2	21±8	3.2	0.38±0.06	0.13±0.16	0.16±0.23	8±1.3	60
	p<0.05	**p<0.001**	**p<0.001**	**p=ns**	**p=ns**	**p<0.05**	**p<0.01**

Conclusions: 1. Differences in the daily dose of insulin have been found in type I diabetic patients. **2.** Patients with a low daily dose of insulin were older at disease onset than those with a high dose. **3.** The degree of metabolic control was better in patients with a low dairy dose of insulin than in those with a high dose. Severe ketoacidosis was more frequent in the later group. **4.** There were no differences in the residual ß cell cell function after 10 yr. of diabetes evolution between patients with low and high daily dose of insulin . **5.** Suprinsingly, non proliferative diabetic retinophaty was more frequent in type I diabetic patients with a low daily dose of insulin than in those with a high dose.

1284

SEPARATE VERSUS SINGLE GLUCOSE-INSULIN-POTASSIUM INFUSION REGIMENS IN DIABETICS UNDERGOING HEART SURGERY

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Diabetic patients undergoing heart surgery, particularly coronary artery by-pass grafting, have an increased morbidity and mortality as compared with nondiabetic patients. Despite the existence of a great variety of perioperative regimens devised for the treatment of diabetics, we aimed to compare the efficiency of 2 different perioperative insulin regimens in diabetic patients undergoing heart surgery. Seventeen diabetic patients (group 1) (G1) received a single intravenous (IV) 5% glucose, regular insulin and potassium infusion plus regular insulin intramuscularly every 4h according to the value of the capillary blood glucose. Sixteen diabetic patients (group 2) (G2) received a separate IV infusion of 30% glucose (20 mL/h) and 0,9% saline containing regular insulin according to the hourly value of the capillary glycemia (0,5-12U/h), delivered by independent pumps. The results are expressed as mean ± SD. To compare the results we used the Student's *t* test and the Fisher's exact test. There were lower mean glycemic levels in G2 (201.81 ± 59.84 mg/dl) than in G1 (228.31 ± 100.75 mg/dl); however, these differences did not reach statistically significant values ($p > 0.05$). The frequency of ketosis was significantly higher in G1 than G2 (7 episodes/16 patients vs. 1 episode/16 patients; $p = 0.04$). Regarding the hypoglycemia, there was no significant difference between the two groups (G1 - 2 episodes; G2 - 0 episodes; p = NS). The doses of insulin given in the first 12h post-operatively were significantly higher in G2 than in G1 (49.40 ± 15.44 U vs. 22.76 ± 8.87 U; $p < 0.0001$). We conclude that despite demanding technical equipment and specialised personnel, the separate glucose and insulin infusions regimen makes possible to give the adequate insulin needs in insulinresistant states, like heart surgery and to obtain a better glycemic control with less complications.

1285

INCIDENCE OF IATROGENIC HYPOGLYCAEMIA IN VARIOUS TYPES OF INSULIN -TREATED DIABETES

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Hypoglycaemia is a common and potentially dangerous side effect of treatment with insulin in diabetic patients. The present study was designed to evaluate retrospectively the incidence of mild and severe hypoglycaemic episodes in various types of insulin-treated diabetes. A total of 165 patients'medical records were analysed. The patients were cathegorized into 3 groups as follows: 1) juvenile-onset IDDM (age at onset <30 yrs, n=33), 2) adult-onset IDDM (age at onset ≥30 yrs, n=18), 3) NIDDM (n=114). All patients were taking conventional (mixture of short-intermediate acting insulins twice daily) therapy for at least one year. Incidence of mild hypoglycaemic episodes were found to be significantly lower in NIDDM group (1.02 episodes.patient^{-1}.year^{-1}) compared with juvenile (3.45 episodes.patient^{-1}.year^{-1}) and adult (1.78 episodes.patient^{-1}.year^{-1}) onset IDDM groups ($p<0.0001$ and $p<0.001$, respectively). However, the incidences are similar when compared between the two IDDM groups. In contrast, incidence of severe hypoglycaemic episodes were comparable among juvenil/adult-onset IDDM and NIDDM groups (0.20 episodes.patient^{-1}.year^{-1} vs. 0.10 episodes.patient^{-1}.year^{-1} vs. 0.15 episodes.patient^{-1}.year^{-1}, respectively; p=NS). These data suggest that while mild iatrogenic hypoglycaemia due to conventional insulin therapy is a less frequent problem in NIDDM compared with juvenile/adult-onset IDDM, severe iatrogenic hypoglycaemic episodes are encountered as frequently as in juvenile/adult-onset IDDM.

1286

CHARACTERISTICS AND OUTCOME OF KETOSIS-ONSET DIABETICS IN TAIWAN.

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The occurrence of diabetic ketoacidosis (DKA) has generally been considered as significant β-cell damage and patients with ketosis-onset are usually classified as insulin-dependent diabetes mellitus. To known the characteristics and outcome of these patients, we studies 36 cases who had DKA at diagnosis. There were 19 males and 17 females, with age between 13 ~ 73 years old, body mass index (BMI) 12.4 ~ 33.7 kg/m^2, HbA1c 5.2 ~ 15 % and fasting C-peptide 0.10 ~ 4.58 ng/ml. At 1 ~ 2 weeks after DKA, these patients received glucagon stimulation test and were treated with or without insulin according to their β-cell function. Eleven patients (Gr. A) whose incremental C-peptide (Δ CP) > 0.7 ng/ml were treated with diet (n=1) or oral hypoglycemic agents (OHA, n=10).Twenty-five patients with Δ CP < 0.7 ng/ml were treated with insulin (Gr. B). During 0.5 to 5 year follow-up, only 1 patient in Gr. A needed insulin treatment. At initial, Gr A had high BMI (23.8 ± 1.5 vs. 19.8 ± 0.8 kg/m^2, P <0.02) as compared with Gr. B. The age and HbA1c were not significantly different between 2 groups. Our data indicate that not all ketosis-onset diabetic patients need permanent insulin treatment. Those with higher BMI and better β-cell function may control their diabetes with diet and/or OHA.

1287

CAPILLARY GLYCATED HAEMOGLOBIN ASSAY AND IMPROVED METABOLIC CONTROL IN DIABETIC PATIENTS.

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Because the results are available at the time of the visit, capillary assays of glycated haemoglobin could help physicians in the ambulatory management of diabetic patients. In order to test the outcome on insulin prescription and metabolic control, a prospective study was conducted in 50 insulin-treated diabetic patients with $Hb_{A1c} < 8\%$, randomised into two groups. In the first group, patients and physicians were informed of the result of the capillary assay (group I). In the control group (C), the assay was performed but the result was kept unknown until the end of the study. Hb_{A1c} (HPLC, with a delay in result delivery), and capillary monitoring of blood glucose were performed and used by physicians. Both groups were followed up over 4 months. Results were compared with the chi-square test, and with the analysis of variance. At inclusion, clinical and biological characteristics of patients did not differ significantly between groups. After 4 months of follow-up, Hb_{A1c} (HPLC) improved in (I) (mean±SE: -0,14±0,13%), whereas it was worse in (C) (+0,30±0,14%; $p<0,05$). Insulin doses were most frequently changed in (I) during visits ($p<0,05$). The number of hyperglycaemia decreased in (I) (-4,5±2,5 vs. +1,6±2,1; $p<0,05$). The number of hypoglycaemia did not change significantly. These results suggest that capillary assay of glycated haemoglobin with immediate result could help for managing insulin treatment in ambulatory follow-up of diabetic patients. An improvement of metabolic control could be obtained by reducing the frequency of hyperglycaemia. The improved understanding of the results of Hb_{A1c} by patients could also be involved.

1288

INTENSIFIED THERAPY AFTER DCCT.

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The main interest of our study was based on the fact, that division of insulin application from two to three or four daily injections, can improve metabolic compensation of diabetes, without any other changes in therapeutic approaches. The aim of the study was also to clarify notions as intensified therapy (IT) and multiple insulin injections (MII).

In our study group was 51 children and adolescents with type I diabetes, treated and followed-up for 5 years in Children Diabetes Center of the Slovak Republic. Treatment of patients was started conventionally by two injections per day, after that, patients were switched for MII (3 or 4 daily injections). We compared markers of metabolic compensation, such as glycated hemoglobin (HbA1c) and fructosamine (FAM), levels of C-peptide (CP) and insulin autoantibodies (IAA) at the time of the start of the study and later on, every sixth month period. We didn't find any significant difference.

Our study group was divided into subgroups, according to certain criterias, which can influence metabolic control, as family history of diabetes, ketoacidosis and age of the onset of diabetes, keeping diabetic diet and physical activity. We found the fact, that the worth metabolic control was in the subgroup of patients, who have diabetes in parents or siblings. We prove importance of keeping diabetic diet and physical activity.

1289

FAILURE OF HIGH-DOSE INSULIN TREATMENT TO IMPROVE B-CELL FUNCTION IN DIABETIC NOD MICE.

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High-dose insulin treatment in the first period after clinical onset of insulin-dependent diabetes mellitus (IDDM) has been found to improve beta-cell function in humans. The aim of the present study was to examine whether this finding could be reproduced in an IDDM animal model, the spontaneously diabetic NOD mice, since, if so, the possibility of investigating the underlying mechanism would be provided.

Newly diagnosed diabetic female NOD mice were randomized into three groups composed of a conventionally insulin-treated group (n=10) injected subcutaneously with 15 IU/kg per day of NPH for 14 days followed by five days without insulin, a high-dose insulin treated group (n=8) injected subcutaneously with 150 IU/kg per day of Actrapid for 14 days followed by five days without insulin and an untreated group (n=11). A reference group of age matched non-diabetic untreated female NOD mice (n=11) was included in the study.

The amount of insulin extracted from the total pancreas, presumed to give an indirect measure of beta-cell function, was found to be not significantly different between the three diabetic groups, but at a level of about 100 fold less than in the non-diabetic group. No significant differences between the groups of diabetic mice were found in blood glucose values at the end of the study.

We conclude that no improvement of beta-cell function could be demonstrated in newly diagnosed diabetic NOD mice after early high-dose insulin treatment.

1290

SAFETY AND METABOLIC CONSEQUENCES OF THE TRANSFER OF INSULIN REQUIRING DIABETICS (IDDM AND NIDDM) FROM RAPITARD MC 40 IU/ML TO INSULIN MIXTARD HM 40 IU/ML

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The study was performed in order to assess the safety and metabolic consequences of the transfer of insulin requiring diabetics from the mixed animal insulin preparation to the human insulin preparation. The study was an open, uncontrolled, multicenter trial with a duration of 16 weeks. In the 4 week run-in period, the patients were treated with Rapitard MC and in the 12 week human insulin.The study was performed at 6 different centers. Totally, 86 patients were included in the trial Of these, 83 patients completed the study. Three patients dropped out the study.

The patients included in the study were IDDM (61 patients) or NIDDM (25 patients) with an average duration of treatment of 2.86 years, SD 2.19 years. Metabolic control was generally stable with HbA1c levels less than 25 % above the normal ranges for the laboratory of the center in question.During the study, the number and severity of hypoglycaemic episodes were recorded in patient's visiting booklets. At visits to the clinic, the following parameters were recorded : Adverse events, blood glucose profiles (fasting blood glucose and preprandial blood glucose in the evening taken the day before the visit), insulin dose, HbA1c, and weight. During both study periods, the number of episodes reported per patient was generally low. Very few severe hypoglycaemic episodes were reported and there were no reports of hospitalisations due to hypoglycaemic episodes. There were no statistically or clinically significant differences between the number of episodes reported in treatment periods. There also seemed to be no difference in the severity of the episodes reported. For the IDDM patients and preprandial blood glucose at the end the human insulin period was on average approximately 20 mg/dl lower as compared with the end animal treatment period. The decrease was apparently not related to a simultaneous increase in insulin dose. This decrease in blood glucose level is, however, not reflected in a decrease in HbA1c level, which did not differ significantly at the end of the 2 study periods. For the NIDDM patients the change did not result in any changes in metabolic control as evaluated. In conclusion, transfer of patients (IDDM and NIDDM) from the animal source insulin preparation to the human insulin, is not associated with any change or increase in the frequency or severity of hypoglycaemic episodes nor in metabolic control. Therefore, patients can safely be changed from Rapitard MC to Mixtard 30 HM whilst maintaining the same total daily insulin dose.

1291

Is Sweating a Useful Indicator of Nocturnal Hypoglycaemia?

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Many IDDM patients eschew tight glycaemic control because of a justified fear of severe nocturnal hypoglycaemia. As sweating is frequently considered a "classical" warning symptom, we have evaluated the potential of a nocturnal alarm system which measures small amounts of perspiration equivalent to a resistance of 75 kohms/cm^2.

12 IDDM patients (3 male, aged 22 - 52 yrs, duration of diabetes 23 [11 to 25] yrs; median [IQ]) of whom 4 had a history of hypoglycaemia unawareness, used the alarm each night over 4 weeks. Patients also recorded blood glucose levels on retiring, on waking and during any symptomatic episodes of hypoglycaemia during the night. The alarm sounded **correctly** for only 50% of the 14 symptomatic episodes (blood glucose <3.5mmol/l). The "missed" episodes were recorded by 5 individuals of whom 3 already had hypoglycaemia unawareness. The alarm sounded **incorrectly** 17 times (blood glucose ranging between 4 - 19mmol/l). We were concerned to note that in patients with unawareness, 50% of retiring blood glucose values were <7 mmol/l and 20% of waking values were <4mmol/l, suggesting a high rate of nocturnal hypoglycaemia.

In conclusion, detection of sweating is not a particularly helpful aid to the prevention of nocturnal hypoglycaemia. More effort should be directed at manipulation of diet and insulin dose adjustment to prevent hypoglycaemia, on the basis of recorded blood glucose levels, in patients with hypoglycaemia unawareness.

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NIGHT INJECTIONS OF REGULAR INSULIN IMPROVE DIABETES CONTROL IN C-PEPTIDE NEGATIVE PATIENTS.

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The main aim of the present randomized over-cross study was to compare the short-term effects of two intensive insulin regimens differing just in overnight therapy: 1.(R) = regular insulin at 10 p.m. and at 2.30 a.m.; 2.(L) = long acting (Ultratard HM) at 5.30 p.m. During day, preprandial boluses were adapted according to selfmonitoring in both regimens. Doses of Ultratard HM were calculated individually. Thirty five type 1 (insulin-dependent) diabetic men (aged 19-41 years, diabetes duration 3-26 years) were randomized (odd numbers started with regimen R) and treated with each regimen for 2 weeks. At the end of each test-period blood glucose (BG) and free insulin (FIRI) profiles (16 estimations/d) and insulin dose/kg body mass/d (INS) were registered. In the whole group the mean BG (MBG) and the INS was lower with the R than with the L (7.78±0.323 vs. 9.05±0.375 mmol/l and 673±27 vs. 763±29 mU/kg body mass/d). There was no difference in FIRI-medians between both regimens (0.189±0.011 vs 0.212±0.013 nmol/l, p>0.05), however, the FIRI at 19.00 h, 20.00 h and 22.00 h was higher with the L and at 5.00 h with the R. In 25 patients the regimen R resulted in lower MBG than the L (6.88±0,413 vs 9.85±0.381 mmol/l, p<0.01) even though the INS here was also lower (0.67±0.033 vs 0.800±0.052 u/kg/d, p<0.01). In 10 patients the R resulted in higher MBG than the L (9.19±0.704 vs 6.99±0.489 mmol/l, p<0.05), however, under the R less insulin (0.667±0.052 vs 0.744±0.055 u/kg/d) was injected in this group. Conclusion: night injections of regular insulin result in better diabetes control than Ultratard HM in 71 percent of estimations. The lower insulin dose in the R shows better efficacy of this regimen.

1293

The pharmacokinetics of sc Semitard MC, human NPH ge, Rapitard MC and human NPH 30:70 ge insulin in normals.

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The insulin kinetics and blood glucose responses to Semitard MC, Rapitard MC, human NPH ge and human NPH 30:70 ge were compared in healthy, non-diabetic male subjects (n=12). Mean (SD) age was 33.9±11.1 years and BMI of 24.9±2.9 kg/m^2. All were involved on four study days each one week apart. After basal (fasting) samples were taken 0.2U/kg of the allocated insulin was injected subcutaneously as a bolus into the anterior abdominal wall and blood samples were taken at frequent intervals over the next 24 hours.

Exogenous Insulin	Semitard	NPH	Rapitard	30:70
T_{max} (h)	6.17	8.02	1.88	3.15
C_{max} (mU/l)	13.05	5.76	10.62	13.81
AUC_{0-24} (h.mU/l)	149.60	89.96	69.49	121.40
Glucose				
T_{min} (h)	11.60	21.00	9.58	6.00
ΔC_{min} (mmol/l)	-1.85	-1.55	-1.59	-1.85
AUC_{0-24} (h.mmol/l)	-31.03	-22.73	-22.50	-29.96

The C_{max} for exogenous insulin concentration was significantly higher ($p<0.05$) with Semitard vs NPH and NPH 30:70 vs Rapitard, the T_{max} being different only between NPH 30:70 and Rapitard ($p<0.05$). The transfer of patients from Rapitard to NPH 30:70 may require a dose reduction, whereas switching from Semitard to NPH a dose increase may be needed.

1294

EEG CHANGES IN NORMOGLYCEMIC IDDM PATIENTS TREATED WITH HUMAN COMPARED TO PORCINE INSULIN.

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Eight IDDM subjects (mean age 39.4 ± 2.1 y. [SEM], mean duration of diabetes 21 ± 3.6 y., mean HbA1c 7.7 ± 0.5 %, C-peptide < 90 pmol/l and no longterm complications) with history of serious neuroglycopenic episodes on treatment with human insulin (1986/87) participated in a crossover trial with human (HI) vs porcine (PI) insulin to investigate different effects on brain functions under normoglycaemia. Sleep and sleep-EEG was ascertained in three consecutive sessions on PI, followed by HI and again on PI. Mean blood-glucose values before (HI: 8.8 ± 1.1; PI: 7.0 ± 0.7), during (HI: 8.0 ± 1.9; PI: 6.6 ± 0.6) and after sleep (HI: 9.8 ± 1.6; PI: 7.9 ± 1.0) (mmol/l ± SEM) were not different. *Results:* The insulin effects consisted in changes of nonREM sleep EEG in the 14-Hz bin spindle freqency range. PI and HI may produce different effects on spindle generating mechanisms in the thalamocortical system and in sleep.

	PI_1	PI_2
mean EEG power density as % change PI vs HI	17.1	10.9
standard error mean [SEM]	3.15	4.50
p-value (compared to HI)	< 0.001	0.047

Statistics: Analyses of variance for repeated measures was done and post-hoc testing by paired student's t-test with Bonferroni adjustment. *Conclusions:* PI and HI have different effects on the 14-Hz spindle frequency component of the sleep EEG and therefore affect differently specific functional aspects of the brain under normoglycemic conditions. It is not unreasonable to conclude that also other brain functions could be differently affected by the two types of insulin.

1295

TWICE DAILY VS THRICE DAILY INSULIN REGIME IN POST RENAL TRANSPLANT NIDDM PATIENTS.

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Glycaemic control in Post Renal Transplant patients has always been a challenge. A sharp raise of Blood Glucose towards evenings (4–7 pm) & normal to low Fasting Blood Glucose values are being observed consistently. Use of high doses of T.Prednisolone during day time is thought to be the cause. The Aim of the study was to see the effectiveness of Twice Daily Vs Thrice daily regime of Insulin in Glycemic control. 16 cases of post renal transplant are being followed up since July 1990. 10 patients are on thrice daily regime (3 times short acting with addition of intermediate acting Insulin added to the night dose) and 6 are on twice daily regime (split mix of short and intermediate acting Insulin). Tab. Glipizide 5 mg tid is also being continued. FBS & PPBS are being done every month and HbA1c once in 3 months in all the patients. Dosage adjustments based on SMBG has been taught to all the patients. Though the FBS and PPBS values appear to be well within the target (FBS < 120 mg/dl and PPBS < 180 mg/dl) in both the groups, mean HbA1c values are significantly higher in the twice daily regime group (Mean HbA1c 8.2) compared to the thrice daily regime (Mean HbA1c 7.6). To conclude HbA1c values predict Glycemic control better than FBS and PPBS values and thrice daily Insulin regime is better than twice daily regime.

1296

Somatostatin and Insulin Therapy for the Treatment of Diabetic Ketoacidosis

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The presence of increased concentrations of growth hormone and glucagon accentuate diabetic ketoacidosis(DKA), so suppression of those hormone by somatostatin may be beneficial in patients with DKA.

The effect of somatostatin analogue, octreotide, injection plus low-dose insulin infusion in diabetic ketoacidosis (DKA) was compared to that of low-dose insulin infusion.

In fifteen patients of DKA, randomly we treated 5 patients while they received octreotide, 50 μg every 6 hour, subcutaneous injection and low-dose insulin infusion(4U/h). Ten patients were treated with low-dose insulin infusion only in addition to conventional fluid therapy. 24 hour serum glucose, urinary ketones and blood pH were monitored as parameters of successful treatment.

All patients were recovered without episode of late hypoglycemia or severe complications. The median time that serum glucose levels dropped to less than 7.8 mmol/L in octreotide and insulin group(group1) was 20 hour and in insulin group(group2) was 72 hour ($p<0.01$). Ketoacidosis disappeared in octreotide and insulin group in 19.6 ± 7.5 h and in insulin group in 79.5 ± 19.5 h ($p<0.01$). The exponential course of glycemic decline between two groups were evaluated and the logarithmic slopes were respectively -0.77 in group1 and -0.52 in group2.

Addition of octreotide to treatment with low-dose insulin infusion reduced and resolved acidosis in a shorter time.

1297

MULTIPLE INSULIN INJECTION DURING FASTING RAMADAN IN IDDM PATIENTS

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Fasting the Holy Month of Ramadan represents one of the five pillars of Islam. People with insulin-dependent diabetes mellitus (IDDM) are usually advised against fast, to avoid potential complications of fasting and insulin therapy, such as hypoglycemia or diabetic ketoacidosis (DKA). Despite this advise some patients insist on fasting thus, exposing themselves to these complication. This trial was conducted in a group of IDDM patients, who insisted to fast, to assess the outcome of fasting Ramadan regarding the acute complications and glycaemic control. A group of 15 patients (male:9 - famale:6), 11 IDDM and 4 insulin treated non-insulin-dependent diabetes (NIDDM), with a mean duration of diabetes of 8.5 years (5-12 years) were studied. All had a full clinical, and laboratory assessment for body mass index (BMI), HbA1 and Lipid profiles before and after Ramadan. They were started on three insulin injections a day, 2 before meals (sunset and dawn) of short acting and one in the late evening of intermediate acting insulins. They performed daily capillary blood glucose monitoring (4-6 times/day) and frequent daily urine testing for ketones. They had free access to the team members throughout the study. All the 15 patients completed the month uneventfully, with no serious acute complications (no hypoglycemia or DKA). Although some patients showed some improvement in HbA1, BMI and Cholesterol values, it did not however reach statistical significance. We therefore conclude that, Multiple insulin injection therapy can safely be used, with proper self-monitoring and close professional supervision in IDDM patients insisting to fast Ramadan, without deteriorating the glycaemic control. Larger controlled studies are needed to further support these findings.

1298

DIABETIC KETOACIDOSIS
A STUDY OF 75 EPISODES

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Abstract: Seventy five episodes of diabetic ketoacidosis (DKA) were admitted to New Children Hospital, Cairo University from January 1993 to January 1995 and managed on two protocol oriented systems were analyzed. Fourty five episodes (60%) occurred in newly diagnosed diabetics and 30 episodes (40%) in known diabetics. The most common precipitating factors in known diabetics were omission of insulin (86%), infection (36%) and stress (4%). Blood sugar levels at admission was significantly correlated to the degree of dehydration ($P<0.05$). The mean pH was 6.97 = 0.16 and was significantly correlated to the respiratory rate. The serum potassium was positively correlated to the blood glucose and negatively correlated to the pH value. Although the serum osmolality was higher in comatosed patients compared to conscious patients, the difference was not significant ($P> 0.05$). On the other hand a significant correlation was noticed between consciousness level and both blood glucose concentration and degree of acidosis ($P<0.05$). At admission, no significant correlation was observed between hyperglycemia and either hyperketonemia or bicarbonate level. Therefore, alleviation of hyperglycemia should not be interpreted as an indication of reduction in insulin infusion rate. This was also confirmed by the shorter duration of reversal of ketoacidotic state when insulin infusion was not reduced and dextrose 10% added if acidosis still existed (protocol II), compared to reducing the insulin dose and adding dextrose 5% whenever pyperglcemia is corrected even in the presence of acidosis. (Protocol I). Four patients succumbed giving a mortality rate of 5.3% and one patient developed severe neurologic sequelae giving a morbidity rate of 1.2%.

1299

A TRIAL OF INSULIN-LIKE GROWTH FACTOR-I AS AN ADJUNCT TO MULTIPLE INJECTION THERAPY IN INSULIN DEPENDENT DIABETES

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53 adolescents with IDDM (26M, median age(range) 16.1yr (10.8 to 20.6)) were randomised in a multicentre, double-blind, placebo-controlled trial of subcutaneous rhIGF-I (Pharmacia) at two doses (40µg $kg^{-1}day^{-1}$, n18 ; 20µg $kg^{-1}day^{-1}$, n18), compared with placebo (n17) for 6 months. All were on multiple injection insulin regimens. Baseline mean(sem) HbA1c (40µg kg^{-1} 7.9%(±0.4); 20µg kg^{-1} 7.7%(±0.5); placebo 7.7%(±0.3)), insulin dose (40µg kg^{-1} 1.11(±0.08); 20µg kg^{-1} 1.02(±0.06); placebo 1.04(±0.07) units $kg^{-1}day^{-1}$), and duration of diabetes (40µg kg^{-1} 7.0yr(±2.2); 20µg kg^{-1} 8.0yr(±2.0); placebo 9.3yr(±2.5)) were comparable. Nine subjects withdrew (40µg kg^{-1} 1; 20µg kg^{-1} 4; placebo 4). IGF-I levels were significantly increased at 6 months following rhIGF-I: 40µg kg^{-1} 362(±22)ng/ml, 20µg kg^{-1} 263(±22)ng/ml vs. placebo 187(±30)ng/ml ANOVA p=0.001. At 3 months HbA1c values were lower following rhIGF-I (40µg kg^{-1} 7.4%(±0.4), 20µg kg^{-1} 7.8%(±0.5) vs. placebo 8.0% (±0.3)), reflecting dose-related reductions from baseline : ΔHbA1c median(range) 40µg kg^{-1} -0.5%(-2.8 to +0.5), 20µg kg^{-1} +0.1%(-1.4 to +1.6) vs. placebo +0.3%(-1.0 to +1.5) ANOVA p=<0.05. Sustained improvements were less evident at 6 months (ΔHbA1c 40µg kg^{-1} 0.2%(-2.8 to +1.5), 20µg kg^{-1} 0.7%(-1.1 to +3.1), placebo 0.8%(-0.7 to +2.9), p=ns). No differences in insulin requirements, BMI, GFR or Ua/Uc were seen (ANOVA p>0.05). Retinal appearances and hypoglycaemia frequency were unaffected by treatment allocation. In conclusion rhIGF-I is well tolerated and at 40µg $kg^{-1}day^{-1}$ can improve glycaemic control even in well controlled adolescents on multiple injection therapy.

1300

ALLERGY TO HUMAN BIOSYNTHETIC INSULIN; Morales Pérez F.M., Gonzalo M.A, Barquero Romero J, Alvarez Barreiro J.A and Díaz-P. de Madrid J. Hospital Regional Universitario "Infanta Cristina", Badajoz., Spain.

Introduction: Immunologic and allergic reactions used to be common with the first preparations of animal insulin. Nowadays, however, are very unusual due to the use of biosynthetic insulin. Immune reactions and allergy to insulin could be under genetic control. In addition, its appearance may be related to intermittent administration of the insulin. **Case report:** A 32-year's old woman was diagnosed of Type I Diabetes Mellitus in November 1995. She was put on treatment with human biosynthetic insulin (Actrapid® at breakfast and lunch time, and Mixtard 30® before dinner). Six weeks later, she began to present, five minutes after insulin administration, an oedematous reddish itchy lesion in the injection site that reached 8-10 cm of diameter in hours. This lesion disappeared progressively, persisting after 48 hours an indurated zone. Patient had no previous history of atopic syndrome or allergy to drugs. She showed positive Prick cutaneous tests for all preparations of human insulin, while Prick protamine latex tests were negatives. Patient had normal serum levels of total IgE, but high levels of IgE specific for human insulin (8.11 kU/L), and also for porcine (6.78 kU/L) and bovine (7.78 kU/L) insulin. A cutaneous biopsy showed findings compatibles with angiodema (perivascular mononuclear, non eosinofilic, infiltration and oedema in deep dermis). Treatment with antihistaminics (cetirizine 10mg/day) was started with marked improvement of symptoms, but therapy could not be discontinued to date. **Summary:** We present an extremely unusual case of local allergy to human biosynthetic insulin in an IDDM patient not previously sensitization and that had been treated continuously. Specific IgE levels and cutaneous tests demonstrate IgE mediated sensitization to insulin. In this case permanent treatment has partially improve symptoms.

1301

THE INFLUENCE OF INTENSIFIED DIABETES MANAGEMENT ON COMPLICATIONS INCIDENCE IN THECOURSE OF PREGNANCIES IN WOMEN WITH INSULIN DEPENDENT DIABETES MELLITUS.

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OBJECTIVE: The aim of this paper was to evaluate the influence of Staged Diabetes Management (SDM) on the complications incidence in pregnant women with type I diabetes mellitus (IDDM).

STUDY DESIGN: 176 pregnant women with IDDM took part in the study, divided into two groups: SDM group: 88 women with well controlled diabetes (average blood glucose level (BG) in the 24 hours glucose profile BG<95 mg/dl) and non SDM (NSDM) group: 89 pregnant women before Staged Diabetes Management program application (BG>95 mg/dl). The average age and duration of diabetes between the groups was comparable..

RESULTS: The most frequent complications in the groups were urinary tract infections (UTI): i.e. asymptomatic bacteriuria and pyelonephritis. The incidence of UTI was: 21.6 % in SDM group and 36 % in NSDM group ($p<0.05$). The SDM group patients had a lower risk of infection primarily in the third trimester. The NSDM group had elevated infection risk during all trimesters of pregnancy. Neither infant weight nor the time of delivery was affected by the course of infection. NSDM group patients had a higher rate of poorly controlled diabetes with a high predisposition to hyperglycaemia ($p<0.05$). Preeclampsia was diagnosed in 6.9% of SDM patients, and 18% of NSDM patients ($p<0.05$). Ketoacidosis and polyhydramnion occurred over two times more commonly in NSDM women. The SDM group had longer mean pregnancy duration (37,28±2,8 weeks) compared with NSDM group women (36.3±3.36 weeks), ($p<0.05$). The caesarean section rate decreased from 50% (NSDM group) to 29.9% (SDM group), ($p<0.05$). Apgar scores differed (SDM group: 8.01±1.51 points; NSDM group: 7.49±1.69 points).

CONCLUSIONS: Good metabolic control in pregnant type I diabetes using SDM increases the duration of pregnancy and decreases the incidence of serious complications, i.e. urinary tract infections and preeclampsia. It has also a positive influence on pregnancy duration and newborn health.

1302

NOVOPEN® 1.5 IN INDIAN WOMEN WITH GESTATIONAL DIABETES MELLITUS.

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The acceptance and clinical results of insulin treatment with NovoPen® 1.5 and syringe were compared in 52 Indian women with GDM. Gestational age was 20-30 weeks at recruitment, and GDM was defined according to WHO criteria. Insulin (Actrapid® HM or Mixtard® 30HM) was instituted at fasting plasma glucose > 90 mg/dl and 2 h - post 50g glucose level > 160 mg/dl). Insulin was administered using NovoPen® 1.5 with G28 needle, or syringe. The patients were followed every 2 weeks until delivery.

Results:

	NovoPen® 1.5	Syringe	P value
Insulin dose	25 U/day	22 U/day	0.47
HbA_{1c}- change	-0.8%	-0.7%	0.75
weight - change	+4.7kg	+4.7kg	0.99

There was no difference in frequency of hypoglycemia. The overall operation and convenience of using NovoPen® 1.5 was rated high by the majority of the patients. 32%of respondents reported that NovoPen® 1.5 caused "less pain than expected", and 68 reported that it caused "no pain".

Conclusion: NovoPen® 1.5 - and syringe administration of insulin caused similar improvement in glycemic control in Indian GDM patients. NovoPen® 1.5 was well accepted, and considered either painless or to cause less pain than expected by the patients.

1303

THE INFLUENCE OF INTENSIFIED DIABETES MANAGEMENT ON NEONATAL COMPLICATIONS INCIDENCE IN INFANTS OF WOMEN WITH INSULIN DEPENDENT DIABETES MELLITUS.

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OBJECTIVE: The aim of this study was to investigate the influence of intensified diabetes management according to Staged Diabetes Management (SDM) on the complications incidence in newborns of mothers with type I diabetes mellitus (IDDM).

STUDY DESIGN: 155 infants took part in the study, divided into two groups. SDM group (n=81) was composed of infants whose mothers had average blood glucose level (BG) in the 24 hours glucose profile of BG<95 mg/dl. NSDM group (n=74): children which were born before Staged Diabetes Management program application (24 hours glucose profile average BG > 95 mg/dl). The ages and diabetes duration in mothers of infants included into different groups were comparable.

RESULTS: We found significant differences between the two groups for duration of pregnancy (SDM group: 37.28±1.98 weeks; NSDM group. 36,3±3,36 weeks). Neonates from SDM group had significantly higher umbilical cord blood pH (pH=7.297±0.088) than those from NSDM (pH=7.268±0.105). Postpartum hypoglycaemia and hyperbilirubinaemia were two times more common in NSDM group. RDS was seen more frequent in NSDM group children (19.8%) than in SDM group (11.1%). Congenital foetal malformations occurred almost two times less frequently in the SDM group than in the NSDM (5.4% versus 9.8% respectively). Early newborn death was seen only in NSDM group (incidence rate: 3.8%).

CONCLUSIONS: Good metabolic control archieved after Staged Diabetes Management program application in the IDDM pregnant women decreased the incidence of serious neonatal complications: newborn death, foetal congenital malformations, hypoglycaemia and RDS .

1304

CLINICAL ASPECTS IN THE TREATMENT OF IDDM

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We studied the clinical characteristics concerning treatment of 207 IDDM patients with a mean age of 49 yrs and a mean duration of 15 yrs. These patients were categorized into the abrupt-onset type (A), 131 cases, and the slowly -progressive type (S), 76 cases. The mean HbA1c for the latest 2 yrs was 7.8 ±1.5%. The distribution of the patients with mean HbA1c value of <7, 7~, 8~, 9% ≦ was 27, 40, 19 and 14%, respectively. The insulin dose (M±SD) was 34 ±14 U/day, and 70% of the subjects used more than 4 times injections daily. The amount of insulin in the poor glycemic control group [HbA1c ≧9%, 30 cases, mean 10.4%] was significantly larger than that in the good glycemic control group [HbA1c <7%, 55 cases, mean 6.5%] (39 vs 30U, $p<0.01$). BMI was also significantly larger in the poor glycemic control group (22.7 vs 20.1, $p<0.01$), as was the frequency of BMI larger than 23 (37 vs 9%). The amount of insulin injected daily in the A and S groups was 35 vs 31U, $p<0.05$. The distribution of patients with more than 4 insulin injections a day was 76 vs 58% (ns) in the A and S groups, respectively. Among the subjects with duration of diabetes more than 10 yrs, the prevalence of retinopathy, nephropathy and neuropathy was higher in the poor glycemic control group (n=17) than in the good glycemic control group (n=37); 77 vs 65%, 53 vs 24 and 59 vs 33, respectively. However, these differences were not significant. These diabetic complications were not significantly different between A and S. In summary, in our IDDM patients, 27% were under good control and when the fair control cases were added, the rate was 70%. Compared with the good control group, the poor control group used a larger insulin dose and revealed a higher mean value of BMI. Thus, overeating was speculated in the poor control group.

1305

ORAL REHYDRATION IN THE DIABETIC CRISIS

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The DM is one of the main causes of medical consultation and hospital admition in Costa Rica. Only the cost of admition are over $500.000 per year. This study show our experience with the oral rehydration (OR) applied to diabetic patients with glycemias over 250 mg/dl, and no cetosis. 18 pts were treated with 250 ml of water every 15 min and 5 to 10 uds of cristaline insulin/hour.

	Average	St D	p
Age	49.8	19.6	
IMC	28.1	5.7	
Initial Glyc	442	90.4	
Glyc 1 hr.	364	90.6 (-17%)	NS
Glyc 2 hrs.	342	103 (-22%)	NS
Glyc 3 hrs.	266	102 (-39%)	0.03
Glyc 4 hrs.	233	101 (-47.2%)	0.003
Glyc 24 hrs.	230	91 (-47.9%)	0.003
Glyc 48 hrs.	246	105 (-44%)	0.003
Insulin	8.3 UI		
Water	5333 ml		

It is showed the lowest levels of glycemia reached during the 4 hours procedure are still present 48 hours later. Without this program all the patients, otherwise would be addmited.

1306

HOW DO PATIENTS WITH DIABETES MELLITUS TYPE I HANDLE THE INJECTION-MEAL INTERVAL IN DAILY LIFE?

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In order to compensate for the delayed onset of action of regular insulin an injection meal interval (IMI) is often recommended for insulin treated diabetic patients. Nevertheless, how and if diabetic patients practice the IMI in daily life was evaluated only in a limited number of studies with selected patient groups. Within a population-based public health study with 684 adult patients with diabetes mellitus type 1 a random sample of 202 patients (41 % women, age 35±10 years (mean±SD)), duration of diabetes 18±11 years) was asked about their handling of the IMI. 10 patients who practiced only long-acting insulin were excluded from the analysis. 12 patients adapted their IMI so frequently, that they could not give a mean value for it. Of the remaining 180 patients 64 % reported to practice an IMI of 0-15 min, 13 % used an IMI of 20 or 25 min and only 23 % used an IMI of 30 min or longer. A flexible IMI was practiced by 134 patients (70 %), whereas 58 patients (30 %) used a fixed IMI. 122 (91 %) of the 134 patients using a flexible IMI stated that the usual interval was 15 (0, 60) min [median (range)]. 62 % of these patients practiced an interval of 0-15 min, 16 % an IMI of 20 to 25 min and 21 % an IMI of 30 min and longer. From the patients using a fixed IMI, 67 % used an interval of 0-15 min, 7 % one of 20 or 25 min and 26 % one of 30 min and longer. Their median IMI was 8 (0, 60) min. The patients who used a flexible IMI made the choice of the IMI according to the following criteria: preprandial glycaemia 73 %, time of day 37 %, physical exercise 34 %, carbohydrate content of meal 25 %. 69 % of the patients selected the IMI according to the actual situation. Patients using a flexible/fixed IMI receive information about the choice of the IMI from: treatment and teaching programmes 62/47 %, own experience 55/38 %, physician 26/34 %. Up to now this is the largest questionnaire for the use of IMI and the first in a randomly selected group of patients. It shows, that more than 60 % patients with type I diabetes in daily life only practice a short injection meal interval between 0 and 15 min.

1307

TEMPORAL RELATIONS OF GLYCOSYLATED HAEMOGLOBIN HbA1c WITH BLOOD GLUCOSE LEVELS AND FLUCTUATIONS

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Glycosylated Haemoglobin (HbA1c) is widely used as index of diabetic control but its exact temporal relations with the daily glycaemic excursions are not fully elucidated. In order to further investigate the above relations we studied 78 diabetics who did self-monitoring of their blood glucose (BG) 2-5 times/daily, at 08:00, 11:00, 17:00, 20:00 and 23:00, alternatively, for two months, while HbA1c was measured by HPLC at the end of this period. HbA1c showed a good correlation with the mean BG of the 2-month period, r =.5953, p<.001 and also with the mean BG at each time point, r =.5256, r =.4474, r =.3717, r =.4797 and r =.6020, respectively, p<.001. Thus an HbA1c value of 5.3% reflects mean 2-month BG of 5.8 mmol/L, a value of 6.0% mean BG of 7.3 mmol/L, HbA1c value of 7.0% mean BG 9.6 mmol/L and HbA1c value of 8.4% reflects mean BG of 12.5 mmol/L. Dividing the 2-month period in four 2-weeks periods, HbA1c showed good correlation with the mean BG of each of them, r =.4890, r =.5321, r =.5595 and r =.5498, from the first to the fourth respectively, p<.001. Dividing the patients in those with great daily glycaemic excursions i.e. 5.0 mmol/L (Group A) and those with small, 1.1 mmol/L, (group B), we found that the correlation coefficient between HbA1c and mean BG over the two months was r =.5750 for group A and much stronger, r =.7142, for B, p<.001. The other correlations between HbA1c and glucose values within each group showed similar differences, beeing always stronger for group B, which had the smallest BG diurnal variations. **Conclusion:** In Type 1 diabetics **a)** HbA1c is associated with the mean glucose over 2 months and also with the mean BG at different time points during the day **b)** The association is much stronger in patients with stable control than in those with great daily glucose excursions, a fact which must be considered when interpreting the HbA1c results.

1308

GLUCOSE EFFECTIVENESS ON CSII THERAPY IN NEWLY DISCOVERED IDDM PATIENTS : MINIMAL MODEL ANALYSIS

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It was shown that hallmark of states of reduced glucose effectiveness appears to be the insulopenic state. The aim of our study was to evaluate the effect of CSII treatment (Novo Nordisk Infuser MKII using Velosulin) in a group of 22 newly discovered IDDM patients (age: 22.23 ± 1.19 years; BMI: 21.19 ± 0.53 kg/m^2) on glucose effectiveness. Glucose effectiveness (Sg; 10^{-1} x min^{-1}) was calculated using MINMOD program (copyright R.N.Bergman) at 0, 2 and 12 weeks. Obtained results were presented as mean ± SE. Student t-test was used for statistical analysis. Patients were divided into two groups: group A (patients entering into clinical remission; n = 6; age: 23.83 ± 2.68 years; BMI= 22.6 ± 1.52 kg/m^2) and group B (without remission; n = 16; age: 21.63 ± 1.33 years; BMI= 20.66 ± 0.42 kg/m^2). Comparing Sg in group A and B following results were obtained: 0 week (A vs. B; 0.102±0.038 vs. 0.232±0.091, p > 0. 05); 2 weeks (A vs. B; 0.270±0.052 vs. 0.158±0.023, p < 0.05); 12 weeks (A vs. B; 0.133±0.029 vs. 0.206±137, p > 0.05). Statistically significant increase (p<0.05) for Sg in patients entering remission was found only after 2 weeks of CSII in comparison with patients without remission. We did not obtained increase for Sg in 2 and 12 weeks on CSII in comparison with the beginning of treatment in groups A and B. In conclusion, our results showed that CSII therapy had some effects on glucose effectiveness, but this could be only one among the relevant factors that determined the outcome of the disease in IDDM patients.

1309

INTENSIVE INSULIN THERAPY DOES NOT INCREASE THE INCIDENCE OF HYPOGLYCAEMIA

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We aimed to analize the episodes of hypoglycaemia in a group of well educated patients with diabetes, who adjust doses of rapid acting insulin before meals. 60 patients with type I diabetes, 26 female and 34 male, aged 16-52 years, mean diabetes duration 10.74±8.67 years, treated with intensive insulin therapy for 1.8±1.6 years, HbA_{1c} 7.4±0.6 % were entered into the study (group A). 60 patients with type I diabetes treated conventionally, with HbA_{1c} 9.6±1.2 %, were used as controls (group B). We evaluated the number of hypoglycaemic episodes per week distinguishing between mild (no third person's help required) and severe hypoglycaemia (first aid given by family/doctor). We noticed significantly fewer hypoglycaemic events, both mild and severe, in group A in comparison with group B (mild hypoglycaemia 1.16±0.68 vs 3.20±1.86 episodes/week, $p<0.05$; severe hypoglycaemia 0.00 vs 0.03±0.01 episodes/week, $p<0.05$). The results of our study suggest that intensive insulin therapy in educated patients who adjust the doses of rapid acting insulin before meals not only allows for more free life style and better metabolic control of diabetes, but also decreases the incidence of hypoglycaemia.

1310

BETA CELL SECRETION DURING CSII THERAPY IN NEWLY DISCOVERED IDDM PATIENTS ENTERING REMISSION

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The aim of our study was to evaluate long effects of CSII treatment (Novo Nordisk Infuser MKII using Velosulin) in a group of 22 newly discovered IDDM patients (age: 22.23 ± 1.19 years; BMI: 21.19 ± 0.53 kg/m^2) on basal and stimulated beta cell secretion. Glucagon stimulation test (Glucagon, 1 mg iv, Novo Nordisk) was performed at 0, 2, 12, 24 and 52 weeks and C-peptide (RIA Inep, Zemun; nmol/l) determined at 0 and 6 min. Results were presented as mean ± SE. Student t-test was used for statistical analysis. Patients were divided into two groups: group A (patients entering into clinical remission; n = 6; age: 23.83 ± 2.68 years; BMI= 22.6 ± 1.52 kg/m^2) and group B (without remission; n = 16; age: 21.63 ± 1.33 years; BMI= 20.66 ± 0.42 kg/m^2). Comparing basal beta cell secretion in group A and B following results were obtained: 0 week-0min (A vs. B: 0.186 ± 0.047 vs. 0.178± 0.029, $p>0.05$); 0 week-6min (A vs. B: 0.252 ± 0.052 vs. 0.276 ± 0.0469, $p>0.05$), 2 weeks-0min (A vs. B; 0.110 ± 0.037 vs. 0.184 ± 0.033, $p>0.05$); 2 weeks-6min (A vs. B; 0.174 ± 0.063 vs. 0.270 ± 0.043, $p>0.05$); 12 weeks-0min (A vs. B: 0.220 ± 0.066 vs. 0.171 ± 0.057, $p>0.05$); 12 weeks-6min (A vs. B: 0.602 ± 0.204 vs. 0.282 ± 0.0295, $p>0.05$). There was no statistically increase in C-peptide levels (basal or stimulated) between groups A and B after 2 and 12 weeks on CSII therapy in comparison with 0 week ($p>0.05$). In conclusion, patients entering into clinical remission during CSII therapy increased stimulated beta cell secretion over course of three months. This increase was not statistically significant comparing with stimulated C-peptide levels at 0 week as well as comparing with beta cell secretion in patients without clinical remission.

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THE *GETREM* STUDY: NEW PRELIMINARY FINDINGS FOR THE CLINICAL REMISSION IN IDDM USING LOGISTIC REGRESSION

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Strict metabolic control within the first year of insulin dependent diabetes (IDDM) is thought to be a key factor for achieving clinical remission (i.e. suspension of insulin therapy according to the parameters issued by the International Diabetes Immunotherapy Group). The aims of the GETREM project are two fold: (i) to evaluate the frequency and duration of spontaneous clinical remission in an European population of newly diagnosed IDDM patients (aged 5-35 years), who are followed up for a period of 36 months, with a common protocol and without any adjunctive immuno-intervention; and (ii) to identify the predictive factors for remission of IDDM patients diagnosed between 5 and 35 years of age. A total of 189 consecutive patients with newly diagnosed IDDM according to WHO criteria were recruited in a participating centres (Belgium, Czech Republic, Estonia, France, Germany, Hungary, Italy, Poland, Romania, Sweden and Turkey) over an extended period of 36 months. Various clinical characteristics (age, gender, etc), history (severity, diabetic siblings, etc) and integrated parameters of metabolic control (HbA1c, blood glucose, total home insulin dose adjusted for body weight, etc) were obtained. So far, 33 patients (17.5%) experienced full clinical remission; mean duration of remission was 7.4±5.8 SD months, but there was some variation between centres. Contingency table analysis showed statistically significant associations ($P<0.05$) between remitted patients and all the above measurements, except age. The 'best' logistic regression fit for remission included predictive factors such as study centre, total home insulin dose adjusted for body weight, and gender, in that order of importance, which predicted 90 % of the patient outcomes correctly. We conclude, that logistic regression analysis provides useful results for the identification of factors, that are predictive of clinical remission in recent-onset IDDM.

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DEFINING INSULIN NEEDS BY MEANS OF CONTINUOUS SUBCUTANEOUS INSULIN INFUSION (CSII) IN TYPE I DIABETES MELLITUS.

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In the treatment of Type I diabetic outpatient, even in multi-injectional therapy, we often encounter difficulties in defining specific insulin requirements. For the purpose of re-defining insulin doses and launching an optimized insulin treatment program according to the DCCT model, 20 C-peptide deficient Type I diabetic patients with poor diabetes control (age 40.7±16, duration of diabetes 18±10yrs, BMI 23.2±3, HbA1c 9.0±1.2%) who were undergoing a therapy of four injections a day (with intermediate insulin at bed-time) were treated by our division. Upon entry, subcutaneous insulin infusion (CSII) (Microjet Quark U100, Bayer) was initiated using the following parameters: initial basal rate correspondent to 40% of the total daily dose administered, and the remaining 60% divided into boluses given at meal-time and based on carbohydrate intake and the foreseen insulin requirements. No modification to previous dietary habits which consisted of three meals a day were made. The resulting carbohydrate amounts were: breakfast 38.5±19gr, lunch 95.5±33gr, supper 90.5±30gr. During treatment the capillary-glucose levels were verified daily (One-Touch H, Lifescan). The basal insulin requirements, which resulted as 0.25±0.09 U/Kg/die, was defined by researching the dose which - in the absence of nocturnal glycemia <90 mg/dl or episodes of hypoglycemia - allowed us to bring the glycemia to a level between 100 and 130 mg/dl at 7am, bringing daily changes which are not superior to 10% of previous dose. The insulin requirements obtained were therefore divided into four injections a day with 60% of the daily dose given as intermediate insulin at bed-time and the remaining 40% divided and administred as an adjunct to the three prandial boluses of regular insulin.

The method was simple and easy. It allowed for brief periods of recovery (8.5±2.8 days) and helped us to establish that the total dose of insulin/kg/die upon release from the hospital (POST) was significantly lower that the one at time of entry (PRE)(PRE 0.74±0.26 vs POST 0.65±0.19U $p<.005$). In particular, this was more relevant for the bed-time dose (PRE 12.4±5.0 vs POST 9.5±2.9U $p<.005$), but also significant to the dose given at supper time (PRE 12.4±4.9 vs POST 10.4±3.4U $p<.025$). The ratio between carbohydrates and insulin (breakfast 5.0±2.3, lunch 7.9±2.4, supper 9.2±2.8 gr/U confirmed that the major need of insulin was during breakfast ($p<.005$).

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A NOVEL APPROACH FOR COMMENCING PATIENTS ON INSULIN THERAPY

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We recently conducted an Australia-wide survey showing that inpatient or 1:1 outpatient education are the predominant strategies used when commencing NIDDM patients on insulin. However, in Newcastle, NIDDM patients with Secondary Oral Failure (SOF) are converted to insulin in an innovative group education program if SOF is confirmed following a month-long pre-course assessment period. The uniqueness of this course has prompted us to assess the epidemiological characteristics of patients seen during the first 4 years of the course. A follow-up was conducted at 1-5 years after initial attendance to see if the course met its educational and therapeutic objectives. Patients' satisfaction with the course and costs to the patients and the health system were also measured. During the 4 year study period the Diabetes Education Centre converted 251 patients (129 male and 122 female; age 63±5.5yrs; duration of NIDDM 9.4±3.7 years) with SOF to insulin. Pre-course 82% of the patients were symptomatic, with an average HbA1c of 9.2±1.6% and a mean fasting glucose of 12.4±5.1 mmol/L. Twenty six patients died in the interim and 8 were lost to follow-up. Of the 217 patients available for follow-up 172 completed questionnaires on different aspects of diabetes care and had their HbA1c measured. The number of symptomatic patients, HbA1c and fasting glucose levels decreased significantly during the follow-up period (39%, 8.1%±1.3; 10.5±4.3 mmol/L; respectively, p<0.001). Only six patients were admitted to hospital for hypoglycaemia, but all were aware of the precipitating cause. All but one patient could manage injections at home without help. The patients stated that the course met their expectations and patient satisfaction levels were high. As expected, weight gain 1-5 years after conversion to insulin was significant (10.2±3.5kg). The course proved to be cheaper than other commonly utilised education approaches. In conclusion, the objectives of the course were met while providing a satisfying and inexpensive alternative to either inpatient or 1:1 outpatient conversion to insulin.

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LONG TERM EFFECT OF CSII THERAPY ON INSULIN SENSITIVITY IN NEWLY DISCOVERED IDDM PATIENTS : MINIMAL MODEL ANALYSIS

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It was postulated that strict metabolic control at the beginning of IDDM may have effect on the subsequent outcome of the illness. The aim of our study was to evaluate the effect of CSII treatment (Novo Nordisk Infuser MKII using Velosulin) in a group of 22 newly discovered IDDM patients (age: 22.23 ± 1.19 years; BMI: 21.19 ± 0.53 kg/m^2) on insulin sensitivity. Insulin sensitivity (Si; 10^{-4} x min/mU/l) was calculated using modified MINMOD program (copyright R.N.Bergman) at 0; 2; 12; 24 and 52 weeks. Data are presented as mean ± SE while t-test was used for statistical analysis. Patients were divided into two groups: group A (patients entering into clinical remission; n = 6; age:23.83 ± 2.68 years; BMI = 22.6 ± 1.52 kg/m^2) and group B (without remission; n = 16; age: 21.63 ± 1.33 years; BMI = 20.66 ± 0.42 kg/m^2). Comparing insulin sensitivity between group A and B following results were obtained: 0 week (A: 0.383 ± 0.078 vs. B: 1.85 ± 0.506; p > 0. 05); 2 weeks (A: 1.592 ± 0.715 vs. B: 2.651 ± 0.396; p > 0.05); 12 weeks (A: 2.783 ± 0.814 vs. B: 4.501 ± 0.984; p > 0.05); 24 weeks (A: 3.280 ± 0.741. vs. B: 2.451 ± 0.563; p > 0.05) and 52 weeks (A: 5.445 ± 0.944 vs. B: 4.865 ± 0.667; p > 0.05). Statistically increased Si in comparison with 0 week was achieved in group A after 12 weeks (0.383 ± 0.078 vs. 2.783 ± 0.814; p < 0.05) as well as in group B after 12 weeks (1.85 ± 0.506 vs. 4.501 ± 0.984; p < 0.05). In conclusion, insulin sensitivity was significantly increased in newly discovered IDDM patients after 3 months of CSII therapy. There was no significant difference in insulin sensitivity between patients entering remission and patients without remission after long term (52 weeks) follow up.

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AN EVALUATION OF 158 DIABETICS WITH INTENSIFIED TREATMENT ACCORDING TO THE BASIS BOLUS INSULIN THERAPY REGIMEN

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A total of 158 diabetics (D) on basis bolus insulin therapy regimen (BBIT) could be evaluated in our Diabetes Center (DC). Of these 81 had been already admitted with the BBIT regimen (group A=gA), 77 were transfered from several less intensified treatment strategies to the BBIT regimen during their stay at the DC (group B=gB). The underlaying diagnoses (gA+gB) were: in 117 cases diabetes mellitus (dm) type 1, in 17 dm type 2a, in 12 dm after pancreatitis, in 6 dm type 2b, in 3 gestational dm, in 3 cases no allocation to a certain type of diabetes was possible. Mean duration of dm was in gA 13,9±3ys (range:1-42ys), in gB 11±3ys (range:1-41ys). During a period of 20 days patients underwent a structured educational program counting 22 units of appr. 1 hour. Seven units were necessary to explain basic algorhythemes, 4 for the handling of monitoring and injecting devices, during the rest of the units general aspects of diabetes were discussed. The mean dayly insulin dose at admission was 52,9±7 IU in gA and 40,9±6 IU in gB. A dose reduction could be achieved to 32,2±5 (-39,2%) IU by means of improved algorhythmes in gA and to 30,6±5 (-25%) IU by transfere to the BBIT regimen in gB. The mean number of blood glucose tests per day (both groups) counted 7,2 (range:5-10). As expected the mean HbA1c levels at admission were lower in gA (8,2±2) than in gB (9,1±2). At discharge this value did not differe significantly in the two groups (gA:7,6±2 gB:7,9±2). The results of this analysis prove that a stay at the DC is a good means to transfer insulin treated D to the BBIT regimen and to improve glycemic control in patients already running on BBIT.

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7 YEARS EXPERIENCES OF INTRAPERITONEAL INSULIN INFUSION

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116 patients (69 female,47 male) have been treated by the CIPII using a Titanium-Portsystem.The Portsystem was implantated intraoperative.Insulin-infusion is regulated by an insulinpump which is connected with the system at the Titan-button and an i.p.double score catheter.The insulin effect is directly. **Reasons for CIPII:** Brittle-Diabetes, severe hypoglycaemia,skin reactions under CSII-therapy, late complications. **Basic data:** Typ 1 n = 100 (86,2%), Type 2 n = 16 (13,8%), average of age 36,1±11,7 years, diabetes duration 16±9,6 years, CIPII treatment time 4,2±2,0 years. **Results:** Laboratory data: In comparison to CSII we didn`t find any significant difference for creatinine,GOT, GPT, Cholesterol, HDL, Apolip A and B until 36 month follow up.g-GT (start 13,4 ± 12,4,follow up 4 years17,7±17,4 - p <,0001) and Triglyceride (start 106±64,follow up 4 years 130±97, p ,0006) shown statistical differences.Using CSII HbA1c <8% in 59,5%, using CIPII < 8% in 73,7%. 48 cases had to be explantated during treatment time of 2,85 years. Reasons:catheter occlusion, fibrin coat around the catheter (10%) or other catheter problems (18%), skin infection (30%) ,intraabd.infection (15%), penetration of titan button through the skin(7%),intraabd.adhaesion (18%),briden(2%).Skin infection might be based on mechanical irritation.Predominant Staph.aureus was found (detail data will be shown). In less than 15% the infection is manifestated within the first 30 days after the implantation, in nearly 50% within one year and more. The titaninum doesn`t seem to be a good material for a long time i.p. insulin delivery via port system.To avoid problems with the intraperitoneal catheter it is usely to change the catheter ambulatory (data will be shown).The Port-System-acceptance is very high. Patients are content because of direct effect of insulin,get more flexibility and spontaneous lifestyle without severe hypoglycaemia.**Conclusion:** For now this Titanium-Port seems to be limited because of the complication events which are discribed.Nevertheless we are convinced that the CIPII-therapy has to become a fixed part in diabetes treatment.

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POSITIVE ATTITUDES AMONGST PATIENTS AND NURSES TOWARDS 'DIAS'- A TOOL FOR PROVIDING INSULIN DOSE ADVICE

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DIAS is a computer model of glucose metabolism which is designed to provide insulin dose advice for IDDM patients based upon data collected from home (dietary intake, insulin dosage and blood glucose measurements). To assess the attitudes of patients and specialist nursing staff towards the use of DIAS as a clinical tool, 35 Diabetes Specialist Nurses (DSNs) and 20 IDDM patients (10 males and 10 females aged 36.3 ± 3.7 yr) were given a demonstration of the system and asked to complete a structured response form. Responses to written statements were recorded using a five-point Likert scale; where '1' = 'strongly disagree' and '5' = 'strongly agree'. The attitude of DSNs was positive. Most felt that by using DIAS they would feel more confident about altering insulin doses (Mean Likert Score (MLS) = 3.2) and would examine blood glucose levels more specifically (MLS= 3.5). They also felt that DIAS was 'a good idea' (MLS = 4.03) which they would want to use in their patient group (MLS = 3.6). Compared to DSNs, patient attitudes were more strongly positive, agreeing that DIAS would improve their glycaemic control (4.0 +/- 0.13 (patients) vs. 3.27 ± 0.12, (DSNs), $p < 0.01$), enable them to cope more with changes in their normal routine, (4.0 ± 0.15 vs. 3.20 ± 0.16, $p < 0.01$) and make them more likely to monitor blood glucose levels (3.6 ± 0.22 vs. 2.91 ± 0.13, $p<0.01$). Patients also agreed that by using DIAS it would be easier to monitor their diabetes (MLS = 4.2) and they would feel more confident about adjusting their insulin dosages (MLS = 4.1). The previous use of computer systems to assist in providing insulin dose advice has proved relatively unsuccessful. The benefits of analysing attitudes towards DIAS prior to implementation are twofold; firstly by providing an indication of its likely acceptance by potential users and secondly by highlighting important concerns which can be addressed before the technology becomes generally available

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EXPERIENCE OVER THE LAST 11 YEARS WITH CONTINUOUS SUBCUTANEOUS INSULIN INFUSION (CSII)

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Since 1985 1033 patients got the CSII.All patients get an each hour preprogramable pump (H-TronV®) and surface stabilized insulin (H-Tronin®). **Basic data:** male n= 496 (48%), female n= 537 (52%),diabetes mellitus typ 1, n=875 (85%), diabetes typ 2, n=158 (15%),age in years, (std.,range) 34,4 (± 13,4, 9 - 78),diabetes duration in years,(std., range)12,6 (± 9,4, 0-50),CSII treatment time in years,(std., range)3,5(±3,1, 0,3 -11). 383 patients (41,2%) are treated longer than 4 years. Average duration of hospitalisation in the Nightclinic (mix from in-and outpatient management) is in nights (std, range)10,7 (±3,9, 1-30). Indications for treatment in the Nightclinic are find out the basalrate configuration and praeprandial insulin dosage,train pump handling and further education.**Results:**890 patients (86,2%) accept CSII and stay at our center.36 patients (3,5%) stopped the therapy, 84 patients (8,1 %) leave center and 23 patients (2,2 %) deceased. Reduced arrise of biochemical hypoglycaemic blood sugar levels compared with the intensive conventional therapie regime (ICT) are observed.The mean HbA1 value before start of CSII is 10,2% (std, range), (± 2,2, 6 -19). After 5 years of CSII treatment mean HbA1 value is 8,7% (std., range),(± 0,9, 6,2-12,1).Each basalrate configuration ist strictly individual.Target is blood sugar (fasting conditions / 24 hours). We observed no correlation between basalrate (BR) and Body Mass Index (r-squared o,51), BR and summary IU before CSII (r-squared 0,18). **Conclusions:**CSII with H-Tron® is well accepted by more than 85 %.The risk of severe hypoglycaemia is lower than under ICT.The mean HbA1 value shows a reduction of more than 1,5% in comparison to further therapy. Pump treatment is the best subcutaneous insulin strategy for insulin treated diabetic patients but it is not indicated in case of non compliance, drug abusus, handicaped persons (mental/physical) and good metabolic control by other insulin therapies.

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EFFICACY AND SAFETY OF HUMAN INSULIN COMPARED TO LOCALLY-PRODUCED CHINESE INSULIN

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Human insulin from Novo Nordisk is currently being introduced in China. A number of diabetes clinics participated in a prospective observational cohort-study, with the aim to collect data on patients who were transferred from Chinese produced animal insulin to Novo Nordisk highly purified insulin. The Chinese insulins were from "Xuzhou Biochemical" and "Shanghai Biochemical". The patients were evaluated before transfer to human insulin and 3 months after. 43 clinics submitted data on 630 patients.

Results	Before Human Insulin (HI)	After 3 months HI	Difference	Unit
Insulin dose	37	30	-22%	median u/day
HbA_{1c}	9.1	7.2	-18%	median %
Mild hypo.	0.40	0.26	N.S.	events/pt/mth
Severe hypo.	0.53	0.19	N.S.	events/pt/yr
Local injection reaction	110	5	-95%	total no. of cases

Conclusion: The data should be interpreted with care; the study was an open observational study with many possibilities for bias, and there was no quality control of source data. However, taken as a whole, the data shows a marked improvement in glycemic control, despite a reduction of the insulin dose. Concurrently, the frequency of local adverse reactions dropped markedly. The recommendation to reduce the insulin dosage by 10-30% after transfer to human insulin is supported by the study, confirming the higher bioavailability of highly purified human insulin compared to animal source insulin of lower purity. The benefits in efficacy and safety of transferring patients from locally-produced animal insulin to highly purified Novo Nordisk human insulin were clearly demonstrated.

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INSULIN LISPRO IMPROVES GLYCEMIC CONTROL IN IDDM PATIENTS UNDER CONTINUOUS SUBCUTANEOUS INSULIN INFUSION (CSII)

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The application of insulin lispro (LP) on CSII in IDDM patients seems to be an ideal method for simulating physiological insulin secretion. The rapid onset of action accounts for lower postprandial glucose values, the shorter duration of action reduces the frequency of hypoglycemic episodes. The aim of this study was to investigate the effect of LP with CSII by contiuous tissue glucose monitoring under daily life condition. We included 10 IDDM patients under CSII treatment with regular insulin (RI) in the study. 24 hours continuous tissue glucose monitoring has been performed twice on each patient, the first day with RI and the second with LP. For changing from RI to LP the patients have been advised to keep the basal rate of the insulin pump as before and to reduce bolus infusion to about 80% of their usual dosage. Continuous tissue glucose measurement has been performed via microdialysis using the "Ulmer Zuckeruhr" system. One tissue glucose value per minute has been measured. All patients measured capillary blood glucose with their own glucose meters and decided independently about insulin dosage and food intake. Glucose profiles have been evaluated by following parameters: mean blood glucose (MBG), Schlichtkrull M-value (M) and number of hypoglycemic episodes. All investigated metabolic parameters have been improved under lispro insulin therapy. MBG was reduced from MBG_{RI}=138+/-13mg/dl to MBG_{LP}=132+/-6mg/dl, M-value was significantly ($p<0.05$) improved from M_{RI}=24.1+/-4.3 to M_{LP}=16.8+/-1.9, indicating a more stable metabolic control. The frequency of hypoglycemic episodes decreased not significantly from 1.1 hypo/day in the RI group to 0.33 hypo/day in the LI group. We conclude that lispro insulin improves metabolic control in IDDM patients under CSII.

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CIRCULATING INSULIN ANTIBODIES IN THREE TYPES OF YOUNG DIABETICS ON BOVINE INSULIN THERAPY.

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The WHO classifies diabetes mellitus of the young in developing countries into IDDM and malnutrition related diabetes(comprising of protein deficient pancreatic diabetes(PDPD) and fibrocalculous pancreatic diabetes(FCPD). 42% of the young diabetic (age < 30 years) we see are IDDM, while 28% and 13% of them are PDPD and FCPD respectively. Immune modulation is reported in malnutrition. However, there is no information on insulin antibody (IA) response in different categories of young diabetics treated with bovine insulin. We studied 52 consecutive young diabetics(IDDM,n=26; PDPD,n=17; FCPD, n=9) on bovine insulin therapy. IA was measured using radioligand assay and expressed in standard deviation scores (SDS) above healthy controls. On participation in the 5th IA proficiency program (University of Florida, U.S.A.), our assay had 100% specificity and 90% sensitivity. The three groups of diabetics were comparable in age. The mean BMI in IDDM, PDPD and FCPD were 16.7 ± 2.5, 16.0 ± 3, 17.1 ± 2.3 Kg/m2 respectively. The IA SDS in the three groups were 70 ± 23; 65 ± 28; 72 ± 19 respectively. The differences observed were statistically not significant. However, the IA SDS of the entire group correlated inversely with their BMI ($P < 0.05$). Total insulin dose (IU/day) required for acceptable glycemic control was significantly higher ($P < 0.05$) in the patients with IDDM (35 ± 7) when compared to patients with PDPD and FCPD (26 ± 12 and 21 ± 4). There was no significant relationship between IA positivity and the presence or absence of microvascular complications such as retinopathy and nephropathy. To conclude, antibody response to bovine insulin therapy is comparable in three groups of young diabetics and there was no relationship between IA SDS and microvascular complications in them.

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EFFICACY OF CONTINUOUS INSULIN INFUSION: WHOM AND WHEN?

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This study was done to test efficiacy of continuous subcutaneous insulin infusion therapy (CSII) in 19 diabetic patients (13 chronic resistant type 1 and 6 newly diagnosed type 1 diabetics with ketoacidotic episode) whom we could not be able to achieve metabolic regulation with either conventional or multiple insulin injections regimen. CSII was performed in chronic resistant type 1 diabetic patients (mean chr. age 22±9 years, mean duration of diabetes 7.4±8.1 years) for 9 to 60 days (mean 38±16 days), in newly diagnosed type 1 diabetics (mean chr. age 21±8 years, mean duration of diabetes 1.5±1.4 months) for 14-40 days (mean 24±9 days). Before CSII therapy, daily insulin requirement was 0.88±0.24 IU/kg/day in chronic resistant type 1, 0.71±0.21 IU/kg/day in newly diagnosed type 1 diabetics. Insulin requirements of both group to obtain normoglycemia is reduced by 31% following CSII therapy and became 0.61±0.18 IU/kg/day; 0.49±0.13 IU/kg/day respectively. Although there were no significant side-effects, we observed local skin inflammation on catheter implantation area in one patient, hypoglycemic attack in another and a mild ketoacidosis in another one during CSII therapy. This study shows that CSII therapy is useful and effective in newly diagnosed type 1 diabetics with ketoacidosis, on the other hand in chronic resistant type 1 patients CSII therapy may lead to rebound phenomena at the end of therapy. However, we believe development of new techniques is needed to eliminate the inconvenience during the switch over to conventional insulin therapy from CSII therapy.

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RELIABILITY OF THE BLOOD GLUCOSE DATA AND ITS INFLUENCE ON GLYCAEMIC CONTROL DURING TREATMENT OF DIABETIC PREGNANT WOMEN

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Pregnant diabetic women have been classified as the most motivated group of patients for realization of the intensive insulin treatment. In order to check reliability of the data collected by this group of patients at home and those reported to physician during routine clinical assessment of the current metabolic control, a study of 20 pregnant diabetic women has been carried out.. Study group was treated by multi-injection technique with minimum 4 blood glucose (BG) measurements per day and clinical assessment every 3 weeks. All data were recorded automatically into memory of the Bayer glucometer M+ without knowledge of the patient. Comparison of the data reported by the patients and recorded by glucometer M+ led to following results: as an average for whole study group 181 values of BG per patient were recorded by glucometers and 239 BG values per patient were reported by patients. Generally only 53.9% of BG measurements recorded automatically by glucometers were reported by patients. Remaining values were changed (26.7%) or not recorded (19.4%) in the registration form. Influence of reliability of the data on realization of the intensive insulin treatment was assessed based on comparison of the glycaemic control described by "J" and "MBG" indices for both group of the data. Mean values of both indices were respectively: 35.0 and 137 mg/dl for recorded values and 26.2 and 127.0 mg/dl for reported values by patients. Much higher and statistically significant difference was calculated for an universal "J" index (related in part to BG variations). In conclusion obtained results indicated, that for highest motivated group of patients in diabetes treatment reliability of data reported to physician is low and could led to wrong realization of the treatment regarding to diet and insulin doses. An application of the direct transmission of the BG data from patient home to the control unit in the hospital could be in this case beneficial. Project related to evaluation of the efficiency of the stated above telematic transmission of the data has been started in 1996 and will be terminated in 1998.

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WHAT ABOUT ANTI INSULIN IMMUNOGENICITY ON A LONG TERM IN DIABETIC PATIENTS TREATED WITH IMPLANTABLE INSULIN PUMP?

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Treatment with implantable pump for peritoneal insulin delivery is associated in some cases to an important increase of anti insulin antibodies (AIA). This increase does not seem to have a deleterious metabolic effect but it has to be carefully evaluated on a long term. We examined the evolution of AIA level and possible influence on metabolic and clinical parameters over a long implantation duration. In 20 type I IDD patients (m age ± SD=37y ± 13, m duration of diabetes = 19y ± 14) we studied AIA level before and until 54 months after implantation of a programmable pump (Minimed* M 2001) delivering insulin peritoneally (U 400 21 PH Hoechst*). We examined influence of discontinuous pump use on AIA level in 10 patients who temporarily stopped insulin pump treatment owing technical problems. AIA level (measured by RIA) observed 42 months after pump implantation was correlated with HbA1c, fasting plasma free insulin, incidence of hypoglycemia (n of glycemia < 65 mg/dl/month) and daily insulin requirements. AIA level significantly increased as soon as the 3rd month after implantation and this increase was sustained throughout the study period (M0=20 ± 17% (m ± SD) M3= 28±24%*,M12= 32 ± 29%* M24= 33 ± 29%* M36= 37± 25%* (n=12) M48= 42±19%* (n=8) M54=43±17%* (n=6)*p<0.05 vs M0). In patients who temporarily discontinued implanted pump use, AIA level tended to decrease 3 months after switching from IP to SC insulin infusion (35±25 % vs 30±19 % (NS)). But recovery of IP insulin infusion was associated with a significant rebound of AIA level (45± 25 % $p < 0.05$). In 10 patients, under IP treatment we did not observe any correlation between AIA level measured 42 months after pump implantation and concordant clinical and metabolic parameters. Despite what was suggested in some previous studies, AIA level do not tend to decrease over long term IP insulin.Moreover, discontinuation of IP insulin infusion has a stimulating effect. This rise of AIA level does not induce significant metabolic consequences.

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GLYCAEMIC CONTROL IN DIABETES MELLITUS WITH OPEN LOOP INSULIN INFUSION PUMP

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Open Loop insulin infusion therapy for earlier and smooth control in uncontrolled diabetes was employed in patients with ketoacidosis, infections, pregnancy or going for surgery. All the patients underwent detailed physical examination & routine investigations for diabetic complications before they were put on infusion therapy. Out of 101 patients included in study 42 patients had ketoacidosis and infections or both. 7 were pregnant diabetics and rest were requiring earlier control for surgical intervention. Insulin infusion was started with Nikisso Myfuser Pump and 50 patients received intravenous while 51 received subcutaneous insulin infusion. Initial glycemic control was achived in 3 to 7 hours with minor side effects as headache, flushing, giddiness, sweating, leg cramps, etc. This was followed by intensive conventional therapy which made surgical intervention or discharge possible in 2 to 5 days. When subjected to statistical analysis, significant increase in insulin requirement was observed with longer duration of diabetes, poor previous control of diabetes, ketoacidosis and infections. In this study employing short term use of open loop infusion followed by intensified conventional therapy not only the initial glycemic control was smooth but it also facilitated earlier surgical intervention or control of infection and ketoacidosis. Application of correlation coefficient provided us with mathematical equations to predict dose of conventional insulin therapy from the initial insulin requirement.

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FACTORS PREDICTING CLINICAL REMISSION IN AUTOIMMUNE DIABETES MELLITUS

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Objective: The aim of the study was to describe the course of clinical remission in adult patients with autoimmune diabetes mellitus treated with multiple insulin injection therapy and to identify factors predictive for the occurrence and the length of remission periods.
Material and methods: Sixtytwo consecutive adult patients, 43 men and 19 women, with a mean age of 26.5 years, were analyzed retrospectively. The patients, who had their diagnosis in 1986-1991, received treatment with multiple insulin injections. Remission was defined as maintenance of HbA_{1c} <6.5% with an exogenous insulin requirement of <0.4 U/kg/24 h for a minimum of one month.
Results: Thirty-eight (61%) of the patients entered remission between 1-18 months following diagnosis, with a peak prevalence at 5 months. The duration of remission was longer in males than females (10±12 vs. 2±3 months; p<0.01). Long duration of symptoms prior to diagnosis was associated with shorter remissions (p=0.05) and sub-normal serum bicarbonate levels at onset were predictive for both shorter (p<0.01) and less frequent remission periods (p<0.01). Age, BMI, HbA_{1c} and blood glucose at onset did not significantly influence the frequency or the duration of remission.
Conclusions: Multiple insulin injection therapy induces remission in a majority of adult patients with autoimmune diabetes mellitus. Gender as well as the length and extent of beta-cell demand prior to diagnosis influence the duration of the remission periods.

PS 30
Insulin Therapy in NIDDM

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INSULIN THERAPY IN NIDDM: EFECTS ON THE ARTERIAL BLOOD PRESURE AND THE ENDOTHELIN-1 PLASMA LEVELS

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Clinical and experimental evidences have suggested that insulin has hypertensive effect. Stimulation of vasoconstrictor endothelin-1(ET-1) secretion may be the involved mechanism. The aim of this study was to evaluate the possible effect of insulin treatment on arterial blood presssure (BP) and the ET-1 plasma levels in NIDDM. Patients: Seven NIDDM patients(5 males, and 2 females, mean age 64±7 years, diabetic duration 7±5years) admitted to hospital to initiate insulinization due to secondary failure of oral hypoglucemic drugs (SFOH) were studied. 24-hour arterial blood pressure monitorization (MAPA, SpaceLabs 90207) and body mass index(BMI) were measured before (time a), after three days (time b) and 1year(time c) after insulinization. Moreover ET-1 plasma levels were determined in times a and b. Results: Insulin treatment didn't produce any variation on systolic (124±11/120±7/127±13 mmHg in the times a, b and c, respectively) and diastolic (72±5/71±3/71±5 mmHg in the times a, b and c, respectively) 24-H BP. We found no difference in the systolic day/night differences, 8±11/7±7/11±13 mmHg, nor in diastolic day/night differences, 8±9/8±6/10±7mmHg in the times a, b and c, respectively. There were no significant differences among ET-1 plasma levels in the times a and b and no differences in the BMI were found. Conclusions: In our patients with SFOH 1) Insulinization did not induce an increase in BP in the short or medium term. and 2) Short term evaluation of insulinization did not result in any variation of plasma ET-1 levels. Therefore, our data do not support the hypothesis of exogenous insulin as a risk factor for hypertension.

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EFFECTS OF COMPLEMENTARY INSULIN THERAPY ON SERUM LIPID, LIPOPROTEINS AND APOPROTEINS

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The aim of this prospective study was to evaluate the effects of complementary insulin therapy, consisting of a bolus of 1 to 8 units of short-acting insulin before each meal (4-6x daily) and sometimes at 2.30 a.m., on concentrations of serum lipids, lipoproteins and apoproteins in type 2 (non-insulin-dependent) diabetic patients, unsatisfactorily controlled with longacting insulin 1-2x daily (INS 1-2x/d). A group of 34 type 2 diabetic patients (age 61,8±0,97 years, duration of diabetes 13.35±8.07 years, C-peptid 2.5±0,15 ng/ml) completed the study. After 8-10 weeks of complementary insulin therapy, the dose of insulin per day was reduced (49.6±22.5 U/d vs. 36.6±13.3 U/d, p<0.001) without significant change of the glycemic control (HbA1c 9,2±0,37 vs. 8,9±0,35, p>0,05). However, the number of proatherogenic and antiatherogenic lipoprotein particles was improved (decreased apo B: 1.7±0.52 g/l vs. 1.5±0.94 g/l, p<0.01, apo AI/Lp AI: 2.9±1.01 vs. 2.3±0.98, p<0.01 and increased Lp AI particles: 0.6±0.10 g/l vs. 0.7±0.12 g/l, p<0.0001) and BMI also decreased (29.4±4.28kg/m^2 vs.28.9±4.24 kg/m^2, p<0.05). Compared means±SD. These results demonstrate that complementary insulin therapy probably induces antiatherogenic lipoprotein changes in type 2 diabetic patients previously treated by INS 1-2x/d. Thus, this type of therapy should be used more often and start earlier, and should be preferred to longacting insulins. Supported by grants MZ ČR IGA 1617-3 and 0715-3.

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HYPOGLYCEMIC EFFECTS OF ORAL ADMINISTRATION OF REGULAR INSULIN BY SONICATED LIPOSOMES IN TYPE II DIABETES.W.de la Torre,R.Narváez,A. Guerrón,L.Ocampo,M.Arroyo,P.Pérez, and N. Bernal.Hospital de la Policía and SOLCA,Av.Mariana de Jesús, Quito-Ecuador.

We studied the hypoglycemic effects of Oral administration of Regular Insulin (1Qu/ml) incorporated in to Sonicated Liposomes (ORISL) to type II diabetic patients (n=14;age of 59+/-8 years);seven newly diagnosed and seven with 5+/-4 years of disease (Basal C peptide of 2,3+/-0.7 ng/ml;Hb A1 9,8/-1% and fasting blood glucose of 231+/-32 mg/dl)in a double blind placebo controlled fashion. Preprandial glycemia (n=8/48h) and glycosuria (n=4/48h) were determined at stage 1 (washing),stage 2 (administration of ORISL or placebo),and stage 3 (washing). Patients were hospitalizated and isocaloric diet (30 Kcal/Kg of weight:1825+/-155 Kcal/day) was given.
Body weight ramain stable in both groups throughout the entire study period. The glycosurias did fall at stage 2 and 3 (p=-0.05),and specially in stage 2 of the ORISL group (351+269 mg/l) that placebo group (566+/-318 mg/l)p=-0.05. Capilar glycemia fell in the same groups and same stage 2 (-16% vs -7%, Chi^2: 3,9;p=0.04).
In summary,hypoglycemic effects were observed associated to ORISL administration, however,its clinical significance remain preliminary. Miscellaneous circunstances such as preparation,storage,administration,biodisponibility,etc should be taken in to account when interpreting results of ORISL. It has been demostrated that selective hepatic insulinization occurs associated to use of liposomes as drug carriers. On this basis results are encouraging for continuing the search of an alternative way to subcutaneous administration of insulin.

1330

LONG TERM CONTROL AND WEIGTH GAIN AFTER INSULIN TREATMENT IN ORAL HYPOGLYCEMIC AGENTS FAILURE IN TYPE 2 DIABETES.
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Insulin therapy after oral hypoglycemic agents (OHA) failure in type 2 diabetes is controversial because a good metabolic control is difficult to achieve and side effects,as macrovascular risk factors can be worsened.The aim of the study was to measure the long term metabolic control achieved by insulin treatment in a routine management and the evolution of lipid,weigth and blood pressure. A hundred and three type 2 diabetic patients (mean age 63,6+/- 9,6 years,14+/- 8 years of mean diabetes evolution) were trasfered to insulin treatment because unaceptable glycemic control (74 patients) or metabolic symptons of hyperglycemia (28patients).After a year of insulin treatment ,Hba1c changed from 9,8+/-1,6% to 8,08+/-1,4% (p<0,001) and to 8,38+/-1,4% after 2 years (p<0,001).Mean weigth increment after one year was 5+/-3,8 kg (p<0,001) and 6,8+/-4,4 kg after 2 years (p<0,001).Weigth gain was related to Hba1c decrement after one (r=0,49 IC 95% 0,28-0,66) and two years (r=0,46 IC 0,31-0,73)(p<0,05) but not with insulin daily dosage.Hba1c reduction after one year was related to Hba1c at insulinization (r=0,70 IC 0,54-0,81)and inversely to insulin dosage (r=-0,36 IC-0,56-0,12). Plasma triglycerides were lowered by insulin treatment (p<0,05) but not plasma cholesterol or blood pressure. At the moment of insulin introduction,patients with metabolic symptons have higher Hba1c (p<0,01)and weigth loss (p<0,05) than patients without, getting a similar weigth and Hba1c after a year.After two, a new derangement in metabolic control (p<0,05)was found.Nor diabetic education,previous or after insulin therapy,nor glycemic autoanalysis has effect on postinsulin Hba1c.In conclusion ,a better but modest metabolic control can be reached with insulin treatment in OHA failure without other risk factors derangements.

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Comparative study of night time vs day time single dose Insulin in NIDDM.
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OBJECTIVE: To compare the efficacy of a single premixed Human insulin (Neutral: Isophane=30:70) before breakfast or dinner in Non Insulin Dependent diabetes mellitus(NIDDM) with secondary failure to oral hypoglycemic agents. DESIGN & METHODS: Fourty four NIDDMpatients were randomised to treatment with either pre-breakfast(Group A) or pre-dinner(group B) single dose Insulin alongwith Gliclazide 80mg daily. Metabolic profiles were done at 0,8,16 and 24 weeks. RESULTS: Glycemic control was improved significantly in both the groups after 6 months. Fasting blood glucose was significantly lowered compared with the baseline in both grouyps. The mean change ± SD in Group A was -60.5± 12.5mg% and in Group B -42.5± 9.5mg%, the reduction being more prominent in Group A than Group B($P < 0.001$). Glycosylated haemoglobin was lowered from 9.6± 1.6mg% to 7.2±1.4mg% in Group A($p < 0.002$) and from 9.5±1.5mg% to 7.8±1.6mg% in Group B(p 0001).Total serum cholesterol and triglycerides were also lowered significantly from the baseline value in both groups. The weight gain was more pronounced in the Group B(3.5±0.4Kg) than Group A(1.8±1.6Kg; A vs B, $p<0.001$) and the change was inversely correlated with initial weight. CONCLUSION: The night dose administration of premixed Human Insulin has better control on glucose level and serum lipids in NIDDM with combination therapy. Weight gain was more prominent in patients given Insulin at day time compared to the patients using Insulin at night time.

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TRANSIONS TO INSULIN THERAPY DURING A 25 YEAR PERIOD, IN IKEDA HOSPITAL
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Ikeda Hospital has contributed to the care and management of diabetes for the past 25 years. Insulin therapy has made rapid advances in the fields of diabetes treatment and insulin delivery systems. Transitions to insulin therapy in Ikeda Hospital were investigated during this 25 year span. The number of diabetic patients that visit Ikeda Hospital for the first time, shows a marked yearly increase,119 in 1972 and 1889 in 1996, respectively. The ratio of insulin-treated diabetic patients to all diabetic patients was 16% in 1972, and 48% in 1996, respectively. The number of insulin-treated diabetic patients has increased markedly since 1990. In the majority of cases, insulin was the sole source of drug treatment until 1985. Thereafter, combination therapy of insulin and sulfonylurea increased. However, recently combination therapy of insulin with α-glucosidase inhibitors, Basen® and Glucobay®, has increased. With regard to types and daily insulin injections,intermediate insulin was injected once a day before 1980, in the majority of cases. Later, the frequency of pre-mixed and rapid-acting insulin usage, (twice a day) gradually increased. In 1990, the pen-type insulin delivery system, the Novopen®, was introduced in Japan, resulting in an increase in the frequency of intensive insulin therapy. Nowadays, 95% of insulin-treated diabetic patients use the pen-type system, and 70% of them inject insulin two or more times a day. The mean dosage of insulin has changed from 29U/day in 1972 to the present value of 18~20U/day. The mean age of insulin introduction has changed from 40 years in 1972 to 58 years in 1996. Mean fasting plasma glucose levels have changed from 170~180mg/dl in 1972 to 140~150mg/dl in 1996. The rate of progress in insulin therapy has been especially noteworthy these past ten years. It is imperative to manage diabetic patients correctly.

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Intensified insulin therapy improved metabolic control without changes of hypoglycemia in 64 insulin treated patients with non insulin dependent diabetes mellitus (NIDDM)

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Introduction: As a result of the successful treatment of patients with insulin dependent diabetes mellitus (IDDM) with intensified insulin therapy (IIT), many diabetes care centres started to apply IIT also on younger patients with NIDDM. However, hardly anything is known about the efficacy of such a insulin treatment. The aim of this study was to evaluate the long-term success of the treatment of 64 NIDDM patients with IIT. Patients and Methods: 64 insulin treated patients with NIDDM participated consecutively in a structured diabetes treatment and teaching programme for IIT [Scholz et al. 1992]. 83 % of them could be examined 16,7 ± 6,2 month later. Data before participation: age 52,8 ± 6,2 years, time since diagnosos of NIDDM 13,8 ± 5,6 years, HbA1c 9,95 ± 2,13 % (HPLC Diamat, normal range 4,5 - 6,3 %), body mass index (BMI) 27,57 ± 4,02 kg/m^2, number of blood glucose self controls (BGSC) 3,9 ± 8,7 per week, insulin dose (ID) 0,53 ± 0,17 IU/kg, injections 3,4 ± 1,3 per day, ID self adoption (IDSA) 0,5 ± 1,8 per week. Results: HbA1c levels decreased significantly (8,53 ± 1,63 %, p = 0,0001). BMI (28,5 ± 3,52 kg/m^2), BGSC (17,5 ± 10,5 per week), ID (0,67 ± 0,28 IU/kg), injections (4,5 ± 1 per day) and IDSA (4,6 ± 4,3 per week) increased significantly (p = 0,0001). The incidence of severe hypoglycemia remained unchanged (0,0077 per patient and year). Patients with good metabolic control (HbA1c < median minus one SD) changed significantly more often the ID and had significantly lower BMI than patients with bad metabolic control (HbA1c > median plus one SD). Conclusion: Younger insulin treated NIDDM patients were able to adopt the strategies of IIT and succeeded in improving their metabolic control. Patients changing more often their ID and heaving lower BMI achieved better merabolic control.

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EFFECTS OF INSULIN TREATMENT IN TYPE-2 DIABETIC PATIENTS: THE AUSTRIAN INSULIN INTERVENTION STUDY.

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Aim: In a prospective multicenter intervention trial the effects of 4 different therapeutic strategies in Type-2 diabetic patients insufficiently treated with sulfonylureas alone were evaluated after one year.
Methods: 134 patients (age [mean±SD] 63±5 years, diabetes duration 12±4 years) with an HbA1c between 8-11% received randomly sulfonylureas + metformin (S+M), bedtime NPH insulin + sulfonylureas (I+S), premixed insulin twice daily (I) or premixed insulin twice daily + metformin (I+M).
Results: At present 93 patients reached 1 year, data of 86 subjects were evaluated.

Group	S+M (n=23)	I+S (n=21)	I (n=23)	I+M (n=19)
HbA1c Baseline	9.6 ± 1.0	9.6 ± 0.8	9.5 ± 1.0	10.0 ± 0.8
HbA1c after 1 year	8.9 ± 1.5	8.7 ± 1.6	8.3 ± 1.9**	8.2 ± 1.4**
Δ HbA1c	- 0.7	- 0.9	- 1.2	- 1.3
p value vs. Baseline	<0.001	<0.001	<0.001	<0.001

Mean ± SD, **p<0.01 vs. S+M

Metabolic control improved in all 4 groups, however, insulin given twice daily showed the most favourable outcome. 6 patients in the S+M group were switched to insulin after a mean of 8,2 months because of insufficient metabolic control. BMI increased in all groups (S+M: +0.5, I+S: + 1.1; I: +1.3; I+M + 1.1).
Conclusion: Insulin therapy has a better metabolic effect compared to metformin in type-2 diabetic patients insufficiently treated with sulfonylureas, however, this is achievement is hampered by an relevant increase in body weight in this group.

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COMPARISON OF THE EFFECTS OF TWO PREPARATIONS OF INSULIN IN NIDDM PATIENTS WITH SECONDARY SULPHONYLUREA FAILURE

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The aim of the study was to investigate the effects of Actropid HM (Novo Nordick A/S) and Regular Insulin (Qing Dao Biochemical Pharmaceutical Company). 16 cases of NIDDM patients with secondary sulphonylurea failure were divided into two groups which had no differences in age, sex, height, body weight, duration and severity (with homogenous Fasting Blood Glucose, 2-hour postprandial Blood Glucose and 24 hour urinary glucose) of the disease. During the clinical trial, all patients were administrated with stable diet calories and activity and were prevented from hypoglycemic agents such as Metformin and Glucobay etc. Patients of group A were injected Regular Insulin 12, 8, 8 unites, while group B were injected Actropid HM 12, 8, 8 unites, both subcutaneosly and 30 minutes before 3 meals each day. 3-5 days after, blood glucose control parameters (as we described in Chinese Medicine Journal in 1989;69:108) were evaluated. Blood glucose were assessed by One Touch II (Life Scan Company). The following data of the two groups:1.Fasting Blood Glucose(FBG); 2. 2 hour Postprandial Blood Glucose(PBG);3.Mean Blood Glucose (MBG); 4.Mean Amplitude Glycemic Excursions (MAGE);5.M-value (Modified Schlichtkrull's); 6.Mean Indices of Meal Excursion(MIME); 7.24 hour urinary glucose all had significant differences (P<0.05 or P<0.01 respectively). It was found that Actropid HM had better effects than domestic Regular Insulin in NIDDM patients with secondary sulphonlurea failure.

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OBESE NIDDM PATIENTS SHOW A PROGRESSIVE INCREASE OF INSULIN RESISTANCE THAT CAUSES SECONDARY FAILURE OF ORAL AGENTS. AE. Pontiroli, PM. Piatti, M.Pacchioni and R. Camisasca, Istituto San Raffaele, Università degli Studi di Milano, Milan, Italy

The pathogenesis of secondary failure of oral agents (SF) is still a matter for debate; a few studies have shown that SF is due to a progressive red-uction of insulin release in lean NIDDM, but not in obese NIDDM, sug-gesting that, in the latter, SF is due to a progressive increase of insulin resistance, likely mediated by obesity, ageing, duration of NIDDM, and chronic hyperglycemia, hyperlipidemia, hyperinsulinemia. To test this hypothesis, 46 obese (BMI 26-46 kg/m^2) NIDDM patients aged 35-65 yrs (15 on sulphonylureas, and 31 on insulin after being treated by diet and by oral agents) were evaluated for insulin resistance by the HOMA index (insulin/22.5$^{-\ln \text{glucose}}$). Patients on sulphonylureas were younger (49.9±2.8 vs 55.8±1.0 yrs, p<0.05) had shorter duration of NIDDM (5.7±1.5 vs 16.8±1.6 yrs, p<0.01), and showed lower fasting BG (6.3±0.4 vs 9.6±0.4 mmol, p<0.01), and lower HOMA (4.6 ±0.7 vs 9.8±1.4, p<0.01) than patients on insulin; BMI, fasting insulin and trig-lycerides were similar; HOMA and duration of NIDDM were the only significant risk factors for SF at multiple regression analysis. In 6 patients with a significant reduction of body weight, withdrawal of insulin and reduction of HOMA occurred, with no change of insulin levels. To evaluate the role of obesity per se, HOMA was calculated in 65 obese subjects with normal glucose tolerance, similar for age and for BMI, with obesity lasting <10 to >30 yrs; in these subjects increasing dur-ations of obesity were not accompanied by an increase of HOMA (ave-rage 3.2±0.3, p<0.01 vs NIDDM). These data suggest that a progres-sive increase of insulin resistance, linked to duration of NIDDM, is the main cause of SF in obese NIDDM patients, reversible after weight loss.

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CSII IN POORLY CONTROLLED NON-INSULIN-DEPENDENT DIABETIC PATIENTS

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It is evident that tight diabetic control can reduce cardiovascular morbidity and mortality in NIDDM patients. Insulin is often needed to achieve acceptable balance. The most efficient way to deliver insulin conventionally is multiple insulin injection therapy. Sometimes even this therapy seems to be ineffective. We treated 8 such resistant patients with continuous subcutaneous insulin infusion (CSII) using insulin pumps (Nordisk Infuser MII, Hoechst Infusor V100). Mean HBA1c decreased from 10.8 % to 8.4 % in 24 months. Mean body weight increased from 95.5 kg to 97.0 kg (BMI 34.7 – 35.6). Mean insulin dose increased from 86.5 U/d to 93.5 U/d. Only minor technical problems were met. The patients were very pleased with pump treatment. No ketoacidosis or hypoglycaemia was seen. One old patient died of myocardial infarct. One very obese patient was resistant to even this kind of intensified therapy.
Conclusion: CSII with insulin pump is an effective, reliable and handy way to treat the most insulin resistant NIDDM patients.

PS 31
Childhood and Adolescence

1338

Coincidence of insulin-dependent diabetes mellitus and coeliac disease.

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The authors performed prospective assessment concerning IgAEmA serum presence in 201 children with IDDM chosen randomly among 600 patients from Diabetic Outpatient Clinic.In 7,5% of children serum IgAEmA were found.In 3,5% IgAEmA and villous atrophy were found.In these children the only clinical was growth deficit or/and body mass deficit.In children with IgAEmA and villous atrophy silent coeliac disease was diagnosed. These children were put on modified diabetic diet based on gluten-free products.Children with IgAEmA and normal intestinal mucosa will need observation and further genetic tests (a latent coeliac disease was diagnosed in them).

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ARTERIAL HYPERTENSION IN CHILDREN AND YOUTHS WITH TYPE I DIABETES MELLITUS

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Arterial hypertension contributes significantly to the progression of diabetic sequelae such as retinopathy and nephropathy. Early registration and therapy are thus extremely important for prevention. The frequency of arterial hypertension (1-3% in non-selected children) in diabetic children and youths, as well as its relationship to clinical parameters in this group as one at risk of cardiovascular diseases, should be examined. 110 children and youths (aged 7-18 years) with type 1 diabetes mellitus (diabetes duration 0-16 years) underwent ambulatory blood pressure monitoring (ABPM) under clinical conditions, with the cuff size especially adjusted. Measuring intervals of 20 minutes by day and 30 minutes by night were selected. The activity protocol and the actual times of falling asleep were taken as a basis for calculating the mean values. Patients with sleeping disorders were excluded from the evaluation. The results from the workgroup "pediatric hypertension" served as reference values. From 107 patients who could be evaluated (45 boys and 62 girls), 11 (10%) showed pathological mean RR values (mean value by day increased: n=11; mean value by night increased: n=11). The diastolic mean values by night correlated with the diabetes duration ($r=0.32$, $p<0.001$). A diabetic retinopathy or nephropathy existed in two of the patients in each case. In diabetic children and youths, increased blood pressure mean values occur during ABPM more often than in a non-selected group (10% vs. 1-3%). Due to the cardiovascular complications to be expected, prospective investigations must confirm these results and consistent therapy guidelines be drawn up.

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COELIAC DISEASE IN CHILDREN WITH IDDM.

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We aimed to assess the prevalence and characteristics of children with coeliac disease (CD) in a major paediatric and adolescent diabetes clinic. Children with IDDM and biopsy-proven CD, aged < 17.0 years were included and compared with control IDDM subjects without coexisting illness, matched for sex, age and duration of diabetes. 30 patients (22 females, 8 males, age range 7.4-16.9 yrs) with CD were identified from a total clinic population of 1148 (prevalence 2.6%). Two CD subjects had coexisting autoimmune hypothyroidism which was adequately treated. Age at IDDM diagnosis (overall mean ± SD 4.9 ± 3.4 yrs) and IDDM duration (7.2 ± 3.2 years) were similar in both groups. Age at diagnosis of CD was 8.7 ± 3.1 years (range 2.8 - 13.9 years), with the diagnosis being made 3.8 ± 3.1 years (range 0 - 10.8 years) after onset of IDDM. Adequate HbA1c and growth data were available in 26 CD subjects and their controls. Current HbA1c was 8.9 ± 1.2 % in CD subjects and 8.7 ± 0.8 % in controls (p=NS), with no differences between groups when analysed by age categories (< 13 and 13-17 years). HbA1c measured 8.6 ± 0.9% (range 6.8 - 10.3, n=19) in CD subjects on the determination closest and prior to coeliac diagnosis. These values were not different from overall total clinic HbA1c means of 8.6 ± 1.3 % (age 5 - 17), 8.8 ± 1.4 % (age 13-17) and 8.5 ± 1.2 % (age 5-12.9). Current height standard deviation score (SDS) was -0.15 ± 1.10 in CD subjects vs 0.12 ± 0.87 in controls, weight SDS was 0.26 ± 0.85 vs 0.51 ± 0.67 and BMI SD was 0.10 ± 0.73 vs 0.27 ± 0.63 (all p=NS). HbA1c and growth parameters were not influenced by either duration of IDDM or CD. Thus, in a large paediatric and adolescent diabetes clinic the prevalence of coeliac disease was 2.6 %. HbA1c and growth parameters were not different to controls; trends toward higher HbA1c, and lower SDS for weight, height and BMI in coeliac subjects were not significant.

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EARLY TUBULAR INVOLVEMENT IN CHILDREN WITH DIABETES MELLITUS TYPE 1.

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Early tubular alterations were studied in a cross sectional study of 44 children with diabetes mellitus type 1 (IDDM) and normoalbuminuria. The patients were divided into 4 groups according to the duration (5-10 respectively 10-15 years) and to the metabolic compensation (mean level of HbA_{1C} for the last 5 years < 9% respectively ≥9%). 32 aparently healthy children served as normal controls. From the overnight urine collections, the urinary excretion of epidermal growth factor (EGF), retinol binding protein (RBP) and N-acetyl-β-D glucosaminidase (NAG) were used as an index of renal tubular function. The ratio of EGF/creatinine in urine was significantly lower in all children with IDDM when compared to controls (p<0,0001). The excretion of EGF was lower in children with poor control of metabolic compensation, independently from the duration of the disease however the difference was not statistically significant (p<0,06). We did not find a significant difference between the groups when comparing the excretion of albumin, RBP and NAG. No significant correlation was found between glomerular and tubular functions. Under conditions of maintained glomerular integrity the decrease in urinary excretion of EGF could indicate a functional tubular damage caused by diabetes itself or be a sign of early renal involvement. Furthermore, the present study suggests that the predictive value of the other markers of tubular function is relatively low for evolution of diabetic nephropathy.

1342

HYPEROSMOLAR NON-KETOTIC DIABETES AFTER HEART LUNG TRANSPLANT IN A CYSTIC FIBROSIS CHILD.

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A 13 year old boy with cystic fibrosis, hepato-splenomegaly, and end-stage respiratory dysfunction had an orthotopic heart-lung transplant. Pre-transplant fasting glucose was 4.6 mmol/L, weight 32.9 Kg, height 1.35 m. Post-operative broncho-pneumonia was treated with antibiotics and pulsed methyl prednisolone. At discharge random glucose was 7.5 mmol/L. He was taking acyclovir, nystatin, taurine, ursodeoxycholic acid, cotrimo-xazole, colistin, pancrease, vitamins, frusemide, azathioprine and cyclosporin. Six weeks later he was re-admitted too weak to move, with severe malaise, nausea, thirst (drinking sugary fluids) and polyuria. Pulse was 132 bpm, blood pressure 107/61 mmHg. The transplanted organs appeared to be functioning well. Urinalysis showed a trace of ketones, pH was 7.3, venous plasma glucose 86.8 mmol/L, sodium 136 mmol/L, potassium 6.3 - mmol/L, urea 26.4 mmol/L. He was treated with continuous intravenous Actrapid insulin, 0.9% then 0.45% saline infusion, and eventually 5% dextrose infusion. Potassium was added to the infusions. Recovery was complicated by hyper-natraemia (162 mmol/L) and abdominal pain due to severe constipation. He remains well on 21 units of Mixtard 30 pen insulin twice daily. He weighs 35.2 Kg. Hyperosmolar non-ketotic diabetic states are rare in children.

1343

PREVALENCE OF DYSLIPIDAEMIA IN DIABETIC CHILDREN AND YOUNGS. EFFECT OF IMPROVED GLYCAEMIC CONTROL.

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We studied the prevalence of dyslipidaemia in 264 children with insulin-dependent diabetes (IDDM) (53% males, mean age 13.8 ± 3.3 years [range 3 to 18], mean diabetes duration 4.0±3.8 years; mean HbA_{1C} 8.3 ± 2.1%) and in 321 control subjects (53% males, mean age 13.8 ± 3.4 years). In addition, we examined the effect of 3 months glycaemic control optimization on dyslipidaemia in a subgroup of 77 poorly controlled IDDM subjects. Cut-off for hypercholesterolaemia, hipoalfalipoproteinaemia and desirable LDL-cholesterol were the proposed by the National Cholesterol Education Program. Hypertriglyceridaemia was defined as the mean + 2 SD of control population

	IDDM patients	Control group
Cholesterol >5.2 mmol/L	20.3% *	6.5%
Triglycerides >1.5 mmol/L	5.0%	2.5%
HDL-cholesterol <0.9 mmol/L	7.4%	3.7%
LDL-cholesterol >2.9 mmol/L	44.5% *	15.3%

(* p<0.00001. χ^2)

Compared to control subjects, diabetic boys, but not girls, showed a higher prevalence of hypoalfalipoproteinaemia (12.9% vs 4.5% ; p<0.01; χ^2). After glycaemic control optimization (HbA_{1C} decreased from 9.3 ± 2.4 to 6.1 ± 1%. p<0.0001; paired t-test), the prevalence of dyslipidaemia was similar to that in healthy children. In conclusion, hypercholesterolaemia is common in children and young subjects with IDDM, but disappears after glycaemic control optimization.

1344

IS SENSITIZATION TO LATEX THROUGH INSULIN INJECTIONS A PROBLEM FOR CHILDREN WITH DIABETES ?

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The potential induction of allergic sensitization to latex from insulin vial tops stimulated an investigation of the prevalence of specific IgE to latex in serum and the relationship to atopic disease in children with diabetes.

In a cross-sectional study, serum samples of 112 children with type I diabetes (age: 15 (5 -18 years); diabetes duration: 6 (1 - 14) years; median (range)) were investigated for total IgE, IgE screening for inhalational and nutritional allergens, and specific IgE to latex.

Specific IgE for inhalational and/or nutritional allergens was found in 42 (38%) children (atopic group). 7 children (6%) exhibited specific IgE (0.61 (0.40-3.84) kU/L) to latex in serum although none reported clinical symptoms of latex allergy. All latex sensitized children were found in the atopic group. This prevalence of latex sensitization of 17% (7/42) in atopic children with diabetes is comparable to the frequency described in atopic children without diabetes. These seven patients had higher serum total IgE levels (328 (113-1000) kU/l) than atopic patients without latex sensitization (n=35; 124 (24-857) kU/L; $p<0.05$) or patients without atopy (n=70; 33 (2-339) kU/l; $p<0.001$). No differences in age or diabetes duration were observed between either group.

Sensitization to latex is found exclusively in children with atopic sensitization and appears to be related to atopic disease and not to frequent contact to latex through insulin injections. However, atopic patients may be at risk for reactions secondary to latex from insulin vials and syringes.

1345

CHILDHOOD INSULIN-DEPENDENT DIABETES AND ST VINCENT DECLARATION

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Aim: Based on the goals of the St Vincent declaration, a multidisciplinar team was created in our department in 1992, to provide treatment and follow-up for diabetic patients previously followed by our non specialised consultations. The goal of this work is to evaluate the results of this approach on the follow-up of this patients. Materials and Methods: We selected a population of 69 patients (38 M and 31 F) with a mean age of 13.8 ± 6.6 years upon diagnosis. In 42 cases the diagnosis was done before the creation of this team and after in the remaining 27. We evaluated and compare clinical and laboratorial parameters before and after the creation of this unit. Results:

	Diagnosis before 1992			Diagnosis after 1992
	<1992	1992-95	p	
Age at diagnosis (y)	12.0+7.4	___	___	16.6+3.9
Duration of diabetes(y)	5.5±5.4	9.4±5.4	<0.05	1.9±1.0
BMI (kg/m2)	20.0±2.0	23.6±2.1	<0.05	21.4±3.0
BP systolic (mmHg)	116.7±12.9	117.6±20.7	ns	115.0±10.9
BP dyastolic (mmHg	72.4±10.9	77.3±7.3	<0.05	71±15.2
HbA1c (%)	8.7±2.2	8.2±1.6	ns	7.6±1.5*
Retinopathy (%)	11.9	26.1	ns	0
Nephropathy (%)	2.3	23.3	<0.05	0
Hypoglycemia/pt/y		0.012		0.018+

*vs HbA1c before 1991, $p<0.05$; +vs hypoglycemia 1992-95, ns

Conclusions: After the creation of the multidisciplinary team an improvement in HbA1c values was observed in patients whose diabetes was identified before 1992, although not statistically significant. The lowest value of HbA1c was attained by diabetic patients diagnosed after 1992 (the difference vs the other group was significant). This multidisciplinar approach had ensured a better care of these patients and a more complete achievement of the goals of St Vincent declaration.

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INTENSIVE INSULIN THERAPY AS A METHOD OF CHOICE FOR TYPE I DIABETIC TEENAGERS ACTIVELY PRACTISING SPORT.

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The aim of the study was to evaluate the effectiveness of intensive insulin treatment in a group of type I diabetic teenagers with an active sport practice. For the study, we recruited 31 patients with type I diabetes, aged 11 - 19 years, with mean diabetes duration 5.8±4.0 years. 13 patients (group A) were treated with intensive insulin therapy, where doses of rapid acting insulin were adjusted according to planned meal and physical activity. 18 patients treated conventionally were used as control subjects (group B). We evaluated: fasting plasma glucose (FPG), mean blood glucose (MBG) and HbA_{1c}. Moreover, we estimated daily insulin demand and incidents of hypo- and hyperglycaemia (>15 mmol/l). We observed that patients in group A in comparison with group B presented better metabolic control of diabetes (HbA_{1c} 6.76±0.68 vs 8.70±1.71 %, $p<0.05$, FPG 6.56±0.90 vs 8.96±2.89 mmol/l, $p<0.05$, MBG 8.00±1.56 vs 11.13±3.68 mmol/l, $p<0.05$), significantly fewer incidents of hypoglycaemia (0.58±0.61 vs 1.63±1.44 incidents/week, $p<0.05$) and hyperglycaemia (0.38±0.05 vs 2.34±2.32 incidents/week, $p<0.05$). Our results suggest, that in the group of young patients with type I diabetes, who actively practise sport, intensive insulin therapy seems to be the method of choice.

1347

METABOLIC CONTROL AND SOCIAL SECURITY IN CHILDHOOD

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At the Nutrition and Diabetes Unit of the Elizalde Hospital , infant-juvenile diabetic patients are cared for. They come in 85% of the cases from the Urban Cone of Buenos Aires; many of them with serious economic difficulties. We part from the supposition that belonging to a Social Security means work for the father or person that is the economic support of the home, the possibility to go to the appointments and the better accessibility to the necessary expenses for the control and treatment. This would lead to a better metabolic control. With the purpose of evaluating this hypothesis, we took 186 infant-juvenile diabetic patients treated in our Unit and considered their dependence to some social cover , relating it with the metabolic control submitted in 1995(X of 2 or more HbA1c measured by ion capture, IMX Abbot).We classified the metabolic control as : very good(X under 7.2), good (X of 7.2 to 7.99),fair(X 8 to 8.99), bad (X over 9).70% of the patients did not have social cover. When classified, the patients of very good control, in half of the cases (47.83%) did not have social cover. This proportion increases in the measure that the metabolic control worsens (81.41% in patients with HbA1c equal or over 9%, p 0.01).The fulfilment to the programmed appointments was considered, and it was greater in patients with social cover (ns), and it was verified that the insufficient number of appointments (3 or less) is two times greater in patients without social cover. Of the 59 patient with Social Security, half (50.83%) had very good or good control and the rest of them (49.17%) had fair or bad control. The 127 patients without Social Security presented fair or bad control in 74% of the cases. Conclusion: The metabolic control in children and young diabetic is a variate related to situations of familiar risk, educational, emotional and economic. The accessibility to medication and to control elements should be a right for all persons carrying diabetes mellitus.

1348

EFFICACY OF TWO TIMES BASAL INSULIN INJECTION IN INTENSIVE INSULIN THERAPY OF IDDM CHILDREN

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In Japan, the incidence of IDDM is very low, therefore insulin therapy is not developed well. Many pediatricians still treat IDDM children by twice daily injection instead of multiple daily injection (MDI). Recently, MDI (four times injection is common in Japan) is recommended for prevention of diabetic complications. However, starting and continuing MDI (RRR/N) instead of twice injection is very difficult for IDDM children, because they must be taken to hospital for starting intensive therapy and they sometimes forget bed time injection when they feel sleepy. We tried three times injection for nine IDDM children ; mixture type insulin or regular plus NPH insulin before breakfast and supper, regular insulin before lunch. Two of nine children can not control their glucose level by four times injection. They can easily accept new treatment at out patient clinic, and their HbA1c level significantly decreased. It is likely that basal insulin level is more stable at twice basal insulin injection comparing single bed time insulin . The division basal insulin into two (three times injection treatment) is very useful for glucose control in IDDM children.

1349

MICROALBUMINURIA/CREATININURIA RATIO IN A POPULATION OF INFANT-JUVENILE DIABETICS.

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At our Nutrition and Diabetes Unit the detection of microalbuminuria is done in a 12-hours urine nocturnal collection. Taking into account the uncertainty of the gathering in infant-juvenile patients with results no very reliable our **objective** is to validate the use of the microalbuminuria/ creatininuria ratio in a random urine sample in this population. First we determinate the microalbuminuria/creatininuria ratio in a normal population of 50 healthy children and adolescents with normal urine. The mean value found was 0.019 ± 0.003 (range 0.07 to 0.029). In 1995-96 , 354 microalbuminurias were done with the Randox inmuno-turbidimetic method; 120 samples were detected with pathologic values over or equal to 15 microg/min, on the other hand all these samples had a microalbuminuria/creatininuria ratio also pathologic over or equal to 0.03. From these, 23 patients registered 2 or more pathologic values (persistent microalbuminuria). All of them were postpuberal and had a duration of diabetes of over 4 years, (X age 17.46 , range 14.05 and 21.55 years; X duration 9.02, range 4.2 and 15.83 years). We **conclude** that the microalbuminuria/creatininuria ratio is useful in the infant juvenile diabetic patient. In our experience we value as pathologic a ratio that is distant more than 3 standard deviation from the mean of the normal population. Persistent microalbuminuria was not found previous to puberty or before 4 years of desease.

1350

PATIENT PREFERENCE FOR INSULIN LISPRO VERSUS HUMULIN R IN ADOLESCENTS WITH TYPE I DIABETES.

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Insulin lispro (LP) (Humalog®), an analogue of human insulin, is faster absorbed and has shorter duration of action than regular human insulin (HR) (Humulin R®). At the end of a randomized, crossover, open-label, phase III study of LP versus HR involving 481 adolescents with diabetes, patients and their parents completed a questionnaire to explore their impressions of LP and HR. Pubertal males and females ages 9 to 18 years were studied. Treatment periods were 4 months, and consisted of either LP or HR before meals, in addition to NPH insulin given 1,2 or 3 times/day. More than 82% of patients and their parents indicated that LP made the patient's activities easier. Similarly, more than 85% of patients indicated their preference to use LP in the future ($p<0.001$).

	% of Patients Responding			
Patients' Responses to "Which rapid-acting insulin.."	**HR**	**LP**	**No Preference**	**p value**
made the patient feel better?	11.21	65.2	23.6	<0.001
made the patient's activities easier?	6.5	82	11.4	<0.001
did the patient like the best?	9.6	85.8	4.7	<0.001
is the patient likely to use in the future?	7.7	85	4.2	<0.001
Parents' Responses to "Which rapid-acting insulin.."				
better "controlled" the patient?	10.2	69.3	20.5	<0.001
made the patient's activities easier?	4.4	85	10.4	<0.001
is the patient likely to use in the future?	6.3	87.4	3.2	<0.001
made life easier?	3.2	85.3	11.6	

In conclusion, adolescent patients with diabetes prefer LP over HR for their pre-meal insulin, and their parents agree.

1351

OXIDATIVE STRESS AT ONSET AND IN THE EARLY STAGES OF DIABETES MELLITUS TYPE I IN CHILDREN AND ADOLESCENTS.

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Oxidative cell damage (OxCd) was evaluated in 45 diabetic type I patients. Twenty-four were prepubertal 2-12 yr. old (PrpD), and 30 were adolescents and young adults 13-24 yr. old (AdD) without clinical symptoms of neuropathy, retinopathy and nephropathy. OxCd was evaluated one week after the onset of clinical symptoms in PrpD and 2-22 years after the onset of diabetes in AdD. The results were compared with those of 60 healthy age- and sex-matched controls.

Plasma values of malondialdehyde (HPLC evaluation), protein carbonyl groups and superoxide dismutase erythrocyte activity were higher than in controls at the onset of diabetes ($p<0.001$) and in the AdD group ($p<0.001$). In contrast, plasma values of alpha-tocopherol, ß-carotene, erythrocyte glutathione peroxide activity and glutathione erythrocyte content were lower than controls at the onset of diabetes ($P<0.002$) and in the AdD group ($p<0.002$).

In summary, our results show that an imbalance between oxidant-antioxidant status is already present at the clinical onset of diabetes in children and that this imbalance continues thereafter resulting in increased oxidative stress in diabetic patients . The clinical significance of this higher oxidative damage in the development of vascular complications in type I diabetic patients remains to be elucidated.

1352

COMPARATIVE STUDY OF INSULIN LISPRO AND REGULAR INSULIN IN 481 ADOLESCENTS WITH TYPE I DIABETES.

J. Holcombe, S. Zalani, and V. Arora. Eli Lilly and Company, Indianapolis, Indiana, USA.

Insulin lispro (LP) (Humalog®), an analogue of human insulin, is more rapidly absorbed and has a short duration of action compared with regular insulin. The safety and efficacy of LP and regular human insulin (HR) (Humulin R®) were compared in 481 pubertal children (mean age 14.9, range 9.1 - 18.9 years) with Type I diabetes in a multinational, crossover, randomized, open-label study. Males and females who had achieved at least Tanner Stage II puberty were studied. Treatment periods were 4 months, and consisted of either LP or HR before meals, in addition to NPH insulin given 1,2 or 3 times/day. Eight-point glucose profiles were measured at home before each visit. After breakfast, the 2 hour post prandial (2h PP) glucose values were 9.7 mM and 10.6 mM during treatment with LP and HR, respectively ($p<0.001$). After the evening meal the 2 h PP glucose values were 8.6 mM and 9.3 mM for the LP and HR groups, respectively ($p=0.003$). After breakfast, the changes in the 2h PP, as a percentage of the premeal glucose, were 0.4% and +17.5% during LP and HR treatments, respectively. Similarly, after dinner the percent changes in 2h PP glucose values were -10.9% and +10.4% during LP and HR treatments, respectively ($p<0.001$). Although HR, compared with LP, lowered glucose values at bedtime, 3 am, and fasting ($p<0.005$), the hypoglycemia rate from midnight to 6 am was lower during treatment with LP compared with HR ($p<0.001$). The number and types of adverse events did not differ between the treatment groups. In conclusion, insulin lispro reduces nocturnal hypoglycemia and improves post-meal glucose control in children ages 9-18 years.

PS 32

Combination Therapy

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GLYCAEMIC CONTROL OF POORLY CONTROLLED INSULIN TREATED TYPE II DIABETIC PATIENTS IS IMPROVED BY METFORMIN THERAPY.

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Secondary failure to oral hypoglycaemic medications is common and leads to insulin therapy, often resulting in weight gain and sub-optimal glycaemic control. We hypothesised that, in this group of diabetic patients, the addition of metformin to their insulin regimen would improve glycaemic control. A double blind, placebo controlled, crossover study was employed; after a 6 week run-in period recruited patients were randomly allocated to receive either metformin 1 gm bd. or placebo for twelve weeks at which point crossover took place. At baseline and at six week intervals during the treatment periods fasting venous blood was taken for HbA1c, glucose, triglyceride, HDL and total cholesterol measurement. Results are given as medians *(quartiles)* and Wilcoxon-rank-sum tests were used for statistical analysis after an order effect was excluded.

Baseline characteristics: **n=19**, **age 64yrs** *(57&67)*, **BMI 28kg/m^2** *(26&31)*, duration of diabetes 14 yrs, **total cholesterol 5.7mmol/l** *(5.2-6.3)*, **HbA1c 8.6%** *(8.3&9.8)* and fasting plasma **glucose 10.5mmol/l** *(8.1&14.9)*. Significant improvement in glycaemic control was seen after metformin treatment; median fall for **HbA1c 0.9%** *(0 &1.9)* $p<0.003$, and for fasting plasma **glucose 4.4 mmol/l** *(6.7&-0.2)* $p=0.008$. Furthermore total cholesterol improved with a median fall of 0.5mmol/l $p=0.02$. There were no significant changes during the placebo period. We conclude that metformin is an effective treatment for sub-optimally controlled insulin treated Type II diabetes. The striking fall in fasting glucose as compared to the more modest fall in HbA1c suggests that the principal effect is to reduce hepatic glucose output.

1354

USEFULNESS OF COMBINATION THERAPY WITH SULFONYLUREA AND α-GLUCOSIDASE INHIBITOR IN NIDDM.

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Purpose: Effect of α-glucosidase inhibitor (Voglibose) alone or combined with sulfonylurea (SU) therapy in patients with NIDDM in whom SU therapy had failed was investigated.

Methods; Subjects were 71 patients with NIDDM in failure glycemic control under SU therapy. Concerning the administration method, initially, SU were discontinued or halved of daily doses. After 2 weeks administration of Voglibose alone or with SU was started. Patients were followed 20 weeks. Criteria for the effect of reducing the dose of SU were follows; SU was no longer required, the dose of SU was decreased or the SU could be changed another SU with a weaker activity.

Results; A reduction of SU was obtained highly in 76.1% (54of 71 patients). Levels of postprandial blood glucose, HbA1c and 1,5AG improved significantly from 223.4±59.5 to 184.9±53.6mg/dl from 7.8±1.5 to 7.2±1.2% and from 7.0±6.3 to 9.8±7.6μg/ml respectively.

conclusion; These findings suggest that this regimen is useful as a therapy for reducing the dose of SU, father moderate the exhaustion of pancreatic ß cell.

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GLYCOSYLATED HEMOGLOBINS ARE BETTER ON COMBINATION ORAL THERAPY WITH OR WITHOUT EVENING OR NIGHT INSULIN THAN ON TWICE DAILY MIXED INSULIN. D. Bell and M. Mayo. The University of Alabama at Birmingham, Birmingham, Alabama.
100 of 130 C-peptide positive non-insulin-dependent diabetes mellitus patients on insulin were successfully transferred to combination oral hypoglycemic therapy with glyburide and metformin. (A) - a primary failure rate of 23%. Secondary failure occurred in 17 patients (17%) after a mean of 6.4 months (range 2-16 months). Two patients restarted insulin because of cost. Of the primary failures 6 were due to side effects, 6 were successfully managed on combination oral therapy plus night or evening insulin (B)and 16 on twice daily insulin with Metformin (C). Of the secondary failures, 12 were controlled on regimen B and 5 on regimen C. There was no difference in C-peptide regimen levels between regimens A,B,or C. Glycosylated hemoglobin levels (Al N5-8%) were significantly less on A (9.8v8.3% p=0.0001), B(11.2%v9.7% p=0.001), and less on C(10.3% v 9.8% p=0.45) when compared with twice daily insulin alone. Conclusion: Improvement in glycosylated hemoglobin in insulin-utilizing non-insulin-dependent diabetes mellitus patients can be obtained on combination oral therapy alone or on combination oral therapy with once daily evening or night insulin

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THE COMBINATION OF INSULIN AND METFORMIN IN TREATMENT OF NON-INSULIN DEPENDENT DIABETES MELLITUS (NIDDM)
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Metformin is used alone or in combination with sulphonylurea in the treatment of non-insulin dependent diabetes mellitus (NIDDM). We have now examined whether in NIDDM 1) metformin with insulin can safely cause a fall in HbA_{1c} to 7% or less and 2) if this may result in weight loss and lower insulin dose when compared to insulin treatment alone. Forty NIDDM patients on insulin on their first visit to the Diabetes Center were identified by retrospective chart reviews of all the patients seen in the past year. These were divided into groups who were on insulin (Group I) or insulin + metformin (Group 2) at the most recent visit. Group 2 was subdivided into those with BMI of either ≤ 30 (Group 2A) or > 30 (Group 2B). Blood glucose, HbA_{1c}, insulin dose and weights were collected from their initial and most recent visit. HbA_{1c}decreased from $10 \pm 2.7\%$ to $7 \pm 1.1\%$ ($p < 0.01$) in Group 1 and from $9.8 \pm 2.1\%$ to $7.2 \pm 1.4\%$ ($p < 0.01$) in Group 2. However, the magnitude of decrease in HbA_{1c} was not different between the two groups. Total insulin dose increased from 45 ± 16 units/day to 56 ± 15 units/day ($p < 0.05$) in Group 1 while it decreased from 80 ± 45 units/day to 73 ± 38 units/day in Group 2. The median increase in insulin dose was 8 units in Group 1 while the median decrease was 3 units ($p < 0.05$) in Group 2. Similar decreases were noted in Group 2A. Individuals in Group 1 increased their weight from 75 ± 8.6 kg to 77.7 ± 9 kgs ($p < 0.01$) while weight decreased from 100.4 ± 23.6 kg to 98.6 ± 22.7 kgs in Group 2. A decrease in weight was seen even in Group 2A. The increase in weight was 3 ± 3.3 kgs in Group 1 while weight decreased by 2 ± 4 kgs in Group 2 ($p < 0.01$). We conclude that insulin + metformin is safe and is as effective as insulin alone in improving glycemic control in NIDDM obese and non obese patients. However, it lowers insulin dose and prevents weight gain which might be of importance in lowering the cardiovascular risk factors in these subjects.

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EFFECTS OF THREE DIFFERENT THERAPEUTICS REGIMENS ON GLUCOSE AND C-PEPTIDE RESPONSE TO A MIXED MEAL IN NIDDM PATIENTS.
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The effects of 3 different therapeutic regimens (2 months for each) with glicazida 320 mg (G), G and insulin (GI) and insulin (I) on glucose and C-peptide response to a mixed meal (317 Kcal: 55.7% CHO, 14.8% protein,29.5% fat), body weight (BW) and metabolic control was evaluated in 25 NIDDM patients (18 F), age 46.1 ± 7.6 yr, diabetes duration 10.6 ± 9.6 yr. During phase2 (GI) 6-10 U of NPH insulin (bedtime) was added, at phase3 (I) only insulin was given.The following parameters of C-peptide and glucose curve were studied: FBG, basal C-peptide (BC-p), area under the curve for glucose (AUCg) and C-peptide (AUCp), peak value for glucose (VPg) and C-peptide (VPp). Stastical analysis was done by Friedman and Wilcoxon test (Bonferroni correction). The results expressed in median (range) were:

	G	GI	I
BW (Kg)	64,8 (43,1 - 98,9)	66,7*(42,8-101,4)	65,8(42,7-104)
FBG(mg/dl)	242,5(138,5-449,5)	155 ** (86-384)	189(85-358)
Vpg(mg/dl)	290(183-535)	214*** (150-448)	278(147-479)
AUCg($x10^4$) mg/dl.min	3,0(1,18-5,97)	2,1(1,46-5,12)	2,8(1,63-5,2)
BC-p(ng/ml)	2,05(0,4-3,8)	1,9(0,6-4,8)	1,2(0,1-4,9)
Vpp(ng/ml)	2,97(0,6-5,9)	2,72(0,7-8,2)	2,54(0,7-8,2)
AUCp (ng/ml.min)	311,25 (50,4-581,7)	256,8 (56,25-648,75)	203,25 (54,75-669,75)
NPH(U)		12 **** (12-30)	18(11-60)
HbA1C(%)	9,2(6,8-13,8)	8,8(5-12,5)	8,8(5,5-13,4)

*p<0.005vsG;**p<0.001vsG and p=0.01vs I;*** p<0.002vsG and I, ****p<0.0001vsI

We concluded that combined therapy resulted in better glicemic response to a mixed meal without increment in C-peptide and with a significant weight gain.

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EVALUATION OF GLYCAEMIC CONTROL IN NIDDM PATIENTS TREATED WITH ORAL HYPOGLYCAEMIC AGENTS VERSUS COMBINATION WITH NPH INSULIN.
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In a multicentre study we compared the fasting and 2 hours post breakfast blood glucose (FBG and PBG) and HbA1c levels with the weighted mean blood glucose level as calculated from home blood glucose monitoring (HBGM) in 79 NIDDM patients (38 males) who were switched from oral (15mg glipizide and 1800mg metformin daily; ORAL) to combined oral and bedtime-NPH-insulin (COMB) therapy. Mean age was 59.6 (SD 8.4) years, diabetes duration 10.3 (11.3) years. On ORAL therapy, HbA1c was 1.37 (0.31) times the upper limit of the laboratory reference range (ULR), mean FBG 9.9 (2.2), PBG 11.8 (2.9) and mean 24 hours HBGM level 10.3 (2.4) mmol/l. On COMB, mean HbA1c was 1.28 (0.26) ULR, mean FBG 7.7 (1.8), PBG 9.8 (2.8) and mean HBGM level 9.0 (2.3) mmol/l. In a linear regression analysis, to evaluate the effect of the therapeutic regimen on glycaemic parameters, we used HBGM-value as dependent variable revealing a beta of 0.76 (CI 0.57 - 0.95) for FBG explaining 49.9% (R^2) of the variance of HBGM in ORAL treated subjects versus a beta of 0.55 (0.36 - 0.74) explaining 36.2% when on COMB treatment. PBG explained 46.4% of the HBGM variance (beta=0.56) when on ORAL treatment and 58.3% (beta=0.45) after transfer to COMB treatment. The same regression analysis revealed lower values when comparing HbA1c with HBGM: beta=5.0 with R^2 33.6% in ORAL and beta=3.6 with R^2 33.2% in COMB treatment. With HbA1c value as dependent variable R^2 was lower than 22% for all glycaemic variables. We conclude that in NIDDM patients with secondary failure to ORAL treatment the fasting and two hours post breakfast blood glucose levels reflect glycaemic control as measured with HBGM but not with HbA1c (gold-standard). When bedtime insulin is part of the therapeutic regimen, post load blood glucose levels best reflect overall glycaemic control as assessed with HBGM but not with HbA1c. Concordance between HBGM and HbA1c values is low in these NIDDM patients on ORAL and on COMB treatment.

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COMBINATION THERAPY WITH HUMAN INSULIN AND SULFONYLUREA IN INDIAN NIDDM.

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The aim of this study was to compare three different treatment regimens in 66 Indian NIDDM patients with insufficient glycemic control on maximal doses of sulfonylurea alone. The following regimens were administered for a period of 6 months:

A: Insulin only (Mixtard® 30 HM twice daily). (n=25)

B: Sulfonylurea plus insulin (bedtime Monotard) (n=23)

C: Sulfonylurea plus insulin (morning Monotard) (n=18)

Baseline values (mean, SD) were as follows:

Age: 52 years (7.7), duration of diabetes: 9 years (4.6), body weight: 64 kg (9.9) , HbA_{1c}: 10.2 (1.4).

Results after 6 months:

	A	B	C	P value
Insulin dose	57 U/d	18 U/d	22 U/d	0.0001
HbA_{1c}, change	-1.5%	-1.5%	-1.8%	0.800
Weight, change	+2.8kg	+2.4kg	+2.2kg	0.696

Conclusion: Full insulin substitution as well as the two modes of combination therapy resulted in comparable improvements in metabolic control, and comparable weight gains, in Indian NIDDM patients with unsatisfactory control on sulfonylurea only.

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BEDTIME VERSUS PRE-PRANDIAL INSULIN THERAPY FOR SULFONYLUREA FAILURE IN NIDDM.

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It is presently controversial whether bedtime insulin (BI) or multiple insulin (MI) therapy is more effective in NIDDM patients, in whom oral hypoglycemic agents have failed. To assess the effects of BI vs MI on glucose control and metabolism, and insulin sensitivity (2-step, hyperinsulinemic-euglycemic clamp, 60 and 240 $pmol \cdot m^{-2} \cdot min^{-1}$), we studied 14 NIDDM patients (10M, 4F; BMI=27.2±0.8) according to a randomized cross-over design. Following a run-in period of 2 weeks, during which body weight (BW) and fasting plasma glucose (FPG) remained stable, NIDDM subjects were assigned to BI or MI for 3 months and subsequently switched to the other insulin regimen for other 3 months. Both treatments decreased FPG (BI, -4.4±0.8 and MI, -3.2±0.4 mmol/L) and hepatic glucose production (3-^{3}H-glucose, BI, -2.61±0.79 and MI, -2.08±0.32 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) to a similar extent (p=NS). However, mean 24-h plasma glucose concentration (BI, 12.1±0.7 vs MI, 9.2±0.6 mmol/L, $p<0.05$) and Hba_{1c} (BI, 8.6±0.3 vs MI, 7.2±0.2 %, $p<0.05$) were significantly lower after MI. Moreover, 24-h plasma insulin concentration (BI, 156±12 and MI, 171±12 pmol/L), body weight (BI, 79±2 and MI, 80±2 kg) and insulin sensitivity (M, 60 $pmol \cdot m^{-2} \cdot min^{-1}$, BI, 4.43±0.53 and MI, 4.95±0.70 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; 240 $pmol \cdot m^{-2} \cdot min^{-1}$, BI, 28.69±1.50 and MI, 27.49±2.04 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) did not differ after BI or MI (all, p=NS). In conclusion, MI can be considered a better treatment in sulfonylurea failure NIDDM, because determines a greater improvement in glucose control without inducing further hyperinsulinemia and increase in body weight.

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EFFECTS OF HALVING SULPHONYLUREA DOSAGE IN CHINESE NIDDM PATIENTS ON INSULIN-ORAL HYPOGLYCAEMIC AGENT COMBINATION THERAPY FOR SECONDARY DRUG FAILURE

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Recent evidence suggests that in NIDDM patients with secondary drug failure, insulin-oral hypoglycaemic agents (OHA) combination therapy may achieve similar glycaemic control at a lower insulin dose and with less weight gain compared to insulin therapy alone. The minimum effective dose of sulphonylurea and the long term efficacy of combination therapy, however, remain unanswered. In a 2-phase randomized, open, parallel trial, we evaluate the effects of halving sulphonylurea dosage in 66 Chinese NIDDM patients on combination therapy. After a run-in period of 12 weeks whereby subjects already on combination therapy were reviewed and stabilized, they were randomized to either maximal sulphonylurea dose (Full-SU) or half the maximal dose of SU (Halved-SU) for a period of 4 weeks without change of insulin dosage, followed by a 20-week insulin re-adjustment phase. 47 subjects (duration of DM 12.5±4.7 years and on insulin-OHA combination therapy for 14.7±15.6 months) were included for final analysis. Both groups (25 Full-SU, 22 Halved-SU) had similar basal glycaemic control (FPG 7.8±3.7 vs 7.5±2.2 mmol/l, NS; HbA_{1C} 8.5±1.2 vs 8.6±1.1%, NS) with slightly lower basal bedtime insulin requirement for the Full-SU group (12.8±6.0 vs 16.7±6.8 U p=0.037). At the end of 4 weeks, the 2 groups did not show any significant difference in terms of glycaemic control, body weight parameters, blood pressure and lipids compared to baseline. In the subsequent 20-week insulin re-adjustment phase, neither group differed in their insulin requirement to achieve similar glycaemic control to baseline (increment 1.64±2.96 vs 3.10±4.22, NS). In conclusion, we confirm our previous report of the efficacy of low dose insulin-OHA combination therapy (mean Insulatard® dose of 16 U bedtime) to achieve good stable glycaemic control for a extended mean duration of 24 months. Halving the dose of sulphonylurea after stabilization of combination therapy had no deleterious effect on glycaemic control or other cardiovascular risk factors during a 6-month follow-up.

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Meal-time insulin versus glibenclamide in type 2 diabetic patients with diet failure.

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We compared a short-acting meal time insulin regime with sulfonylurea treatment during 12 months in a group of newly detected type 2 diabetic subjects, aged < 70 years at diagnosis and not adequately controlled on diet alone. The primary objective of the study was to evaluate parameters of glycemic control, insulin secretion and insulin sensitivity. A secondary objective was to study the feasibility of the treatment regimes including side effects and tolerability. The study was approved by the regulatory authorities and the local ethics committee. Fifty-six patients with persistent fasting blood glucose values ≥ 6.7 mmol/l were recruited from two centres. The subjects were randomised to either short-acting meal time insulin or glibenclamide. At randomisation there were no significant differences between the two groups regarding age, sex distribution, body mass index, waist-hip ratio, HbA1c or fasting C-peptide values. After one year, 52 patients had completed the study. Both treatments reduced HbA1c (insulin 1.16 % versus glibenclamide 0.90 %) and increased weight (1.68 kg versus 1.96 kg). In intention to treat analyses no differences could be seen between the insulin or glibenclamide treated patients in respect to HbA1c (6.47 % versus 6.51 %) and glucose values before and during a 75 g OGTT. Fasting C-peptide values were lower in the insulin treated group (p=0.05), but not so after 120 minutes in the OGTT. Insulin sensitivity as measured with an intravenous insulin tolerance test was not significantly different between the groups. Urinary albumin excretion tended to be lower in the insulin-treated group, but not in the glibenclamide group (p=0.19). No serious adverse events were reported and both treatments were equally well accepted. At the end of the study all patients felt motivated to continue the regimes to which they had been randomised. In conclusion, both short-acting insulin and glibenclamide regimes produced similar results concerning glycemic control and insulin sensitivity with a tendency for the insulin treatment to be associated with lower fasting C-peptide values. A longer follow up or a larger study cohort will probably be needed to further evaluate these findings.

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IGF-I AND INSULIN: LONG-TERM CO-THERAPY IN NIDDM.
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The potential value of the treatment of NIDDM patients with recombinant human insulin-like growth factor-I (IGF-I) and insulin has been suggested by the reports of possible abnormalities in the IGF-I axis of NIDDM patients and by reports of improvements in insulin sensitivity in IGF-I treated subjects. In the present study, we have examined long-term treatment of NIDDM by IGF-I and insulin co-therapy in nonhuman primates with spontaneous type 2 noninsulin dependent diabetes using the highest effective dose found in humans to be associated with minimal side effects (40 µg/kg b.i.d.). This dose produced a 2.5 fold rise in fasting plasma IGF-I levels (269±26 ng/ml vs 673±38 ng/ml, $p<0.0001$), and no change in IGF-I binding protein 3 (IGFBP-3) as measured by RIA. Simultaneously, insulin doses (mean±SE: 44.4±8.3 U/day or 4.6±0.8 U/kg/day) were reduced and adjusted as needed to maintain a consistent level of moderate glucose control. At weeks 18-24 of treatment, insulin dose was stable at 26.1±7.2 U/day or 2.8±0.7 U/kg/day. This average reduction in insulin dose (-32.2%) resulted in no significant increase in fasting plasma glucose levels (basal vs weeks 18-24: 231±21 vs 239±21 mg/dl; p=n.s.) and no change in body weight. Maintenance of the reduced insulin dose level after termination of IGF-I treatment at 26-30 weeks showed a significant rise in fasting plasma glucose, 24 hour urine glucose, triglycerides and other parameters. We estimate that rhIGF-I can be used as an adjunct to the clinically indicated insulin dose and reduce overall insulin requirement by 20 to 25% without reducing glucose control. Importantly, wide variation was seen in IGF-I efficacy, with some monkeys requiring nearly their full pretreatment insulin dose and others successfully maintained with a 70% reduction in insulin dose.

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What kind of patient will benefit from treatment with a combination of premixed insulin b.i.d. and sulfonyulrea?
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Although the combination of insulin (INS) and sulfonylurea (SU) has been used in the treatment of NIDDM for long, there is no information available on which patient might benefit from the combination. To address this question, 175 patients (62 F/ 113 M; age 59 ± 9 yrs, BMI 26.8 ± 3.0) in a randomized double-blind multicenter study (SISU, 8 centers) treated with glibenclamide (GB) were started on premixed insulin (Isuhuman Comb 25/75, Hoechst AG, Germany) b.i.d. (30 ± 16 U) in addition to previous GB treatment (10.5 mg/d) for 4 mo. During this period, fasting BG (13.1 → 8.7 mmol/l) was decreased by 33%, HbA_{1c} (9.63→ 7.95 %) by 25%, serum triglycerides (2.67 → 1.91 mmol/l) by 29%, serum C-peptide (0.70 → 0.59 nmol/l) by 15%, while free insulin (21 → 33 mU/l) conc rose by 50%. In order to identify the patients who really showed benefit from the combination therapy , 75% of the patients were given INS+ placebo, whereas 25% continued with INS+GB for another 4 mo. A deterioration of 40% in FBG/HbA_{1c} during insulin +PL was considered as proof of a beneficial effect (RESP). Using this definition, 152 patients could be classified as RESP or NONRESP. 67 of 113 patients (60%) who received INS+PLA deteriorated vs 5 of the 39 patients (13%) who continued with INS+GB ($p<0.001$). Therefore, about half of them were true RESP to combination therapy. Baseline measurements/recordings of insulin sensitivity (insulin tolerance test) , fasting insulin, C-peptide, blood pressure, BMI, age, duration of diabetes were included in a multivariate analysis to identify predictors of RESP (dependent variable). Neither insulin sensitivity nor insulin secretion predicted the outcome. Only a long duration of diabetes was significantly assocaited with RESP ($p=0.0008$). In conclusion, combination therapy with premixed INS b.i.d + SU is effective in about 50% of patients who have failed on SU alone, particularly in patients with long diabetes duration. To avoid unnecessary treatment, a trial of SU withdrawal is indicated after a few months of combination therapy.

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RATE OF SECONDARY FAILURE TO SULPHONYLURIA TREATMENT IN TYPE 2 DIABETICS AND FACTORS WHICH ARE RELATED TO IT.
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In order to investigate the rate of secondary failure to oral antidiabetics in type II diabetics, we followed 100 patients with mean age of 58.5 years and mean duration of diabetes 7.5 years for 10 years. From these, 65 were still on tablets for the whole study period and form Group A, while 35 had started insulin and form Group B. At the beginning the two groups did not differ in BMI 28.0vs27.6, Systolic Blood Pressure 144.4vs135.6mmHg, Diastolic Blood Pressure 82.6vs82.6 mmHg, Cholesterol 5.9vs6.0 mmol/L,Tg 1.7vs1.9 mmol/L, while the mean annual blood glucose was higher in group B 8.5vs11.3 mmol/L and diabetes duration was longer 6.5vs9.0 years. Also no difference was found in the presence of diabetes in first degree relatives 44.6vs54.2%, smoking 26.2vs31.4%, hypertension 26.2vs40.2% and proteinuria 26.1vs45.7% (Albustix positive). The rate of secondary failure was more or less the same during the 10 years, being 3-4% per year. During the 10 years follow up period the patients were examined two to three times per year. The trend of the differences concerning all the parameters studied was the same and is reflected to the comparison of the data at the end of the study. Thus comparing group A and B at the 10th years we found that group B had higher BMI 26.5vs28.3 p>.05, higher mean blood glucose 9.5vs12 mmol/L p<.001 and proteinuria 26.1vs45.7%. Morover patients in group B had increased their systolic blood pressure during follow up period for 135.6 to 144.4 this difference being statistically significant. Conclusion: 1. In type II diabetics the rate of secondary failure was 35% per 10 years. 2. Factors possibly related to this condition were a) longer diabetes duration b) higher mean blood glucose values at the beginning. 3) Insulin treatment was associated with an increase of the systolic blood pressure and an increase in the prevalence of proteinuria (Albustix positive).

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TREATMENT OF NIDDM – PROGRESSIVE REQUIREMENT FOR POLYPHARMACY TO ATTAIN GLYCAEMIC GOALS
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To prevent complications of diabetes, the American Diabetes Association have suggested additional therapy when $HbA_{1c} > 8\%$, aiming for $HbA_{1c} < 7\%$. The 5102 patients with newly diagnosed NIDDM recruited into the UKPDS, ages 25 - 65 yrs, from 23 centres across the UK, are representative of NIDDM in the general population. Patients, mean (SD) age 52 (9) yrs, BMI 29 (6) kg/m^2, median (iqr) fasting plasma glucose 11.5 (9.0, 14.4) mmol/l and HbA_{1c} 9.1 (7.5, 10.7) %, were randomly allocated to monotherapy with diet, sulphonylurea, metformin or insulin. The proportion (%) of patients who achieved $HbA_{1c} < 8\%$ (< 7%) were :

Therapy	3 years	6 years	9 years
Diet	37 (25)	17 (10)	8 (5)
Chlorpropamide	55 (46)	35 (27)	21 (13)
Glibenclamide	52 (43)	38 (25)	16 (10)
Insulin	48 (41)	35 (27)	21 (18)

In obese patients, the efficacy of metformin was similar to that of glibenclamide. In conclusion, at 3 years only half of NIDDM patients can achieve $HbA_{1c} < 8\%$ on monotherapy and at 9 years only one in five. Polypharmacy needs to be considered early if glycaemic control is to be maintained. Even with insulin therapy, continued hyperglycaemia is common.

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PANCREATIC FUNCTION AND METABOLIC CONTROL OF NIDDM PATIENTS WITH SECONDARY FAILURE TO ORAL HYPOGLYCEMIC AGENTS.

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In NIDDM patients with secondary failure to oral hypoglycemic agents (SF) insulin therapy is a current indication. We have evaluated changes in metabolic control in patients with SF treated with insulin. 43 patients (age at diagnosis:47.8±1.9yr; evolution time up to SF:10.3±1.1 yr; body mass index (BMI):26.6±0.7 kg/m^2; mean±SE) were studied. Plasma C-peptide before (BCP) and 6 min after iv glucagon (6PC) were measured at starting insulin therapy (n=38) and after two years (n=17). HbA1c(%) before treatment (9.2±0.2, n=43) decreased at one (7.4±0.2, n=43, p<0.001), at three (8.0±0.2, n=28, p<0.05) and at five years (8.5±0.6, n=10, p=ns). Cholesterol, triglycerides, HDL, LDL and uric acid levels remained unchanged during the follow-up. BMI only increased at one year (27.7±0.7 kg/m^2, p<0.01). BCP decreased after treatment (0.63±0.07 vs 0.44±0.17 p<0.05) while 6CP (0.89±0.1 vs 0.66± 0.1) and CP increment (6CP-bCP) did not change. In conclusion, insulin therapy in NIDDM patients with SF improved the glycemic control during the first three years, regardless of a progressive deterioration in pancreatic function.

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EFFICACY OF COMBINATION OF INSULIN AND SULFONYLUREA IN NIDDM PATIENTS WITH SECONDARY SULFONYLUREA FAILURE

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We studied the efficacy of combination of insulin and sulfonylurea (SU) in the treatment of NIDDM patients with secondary SU failure. 37 NIDDM patients were randomly allocated to Group A (NPH insulin in the morning), B (NPH insulin at bedtime), C (long-acting insulin at bedtime) and D (insulin only). A, B and C were given SU. Fasting blood samples were collected at 0, 2, 12 and 52 weeks. HbA_{1C} showed a trend to decrease in C but the trend didn't affect statistics. In A, B and D HbA_{1C} was significantly improved but there was no significant difference among the three groups. CPR and lipids did not change. BMI did not change significantly in A, B and C but increased at 52 weeks in D (20.70±2.70 at start vs 23.40±1.98 at 52 weeks p<0.01). The daily insulin doses were increased at 2 weeks in D, with a trend towards a further increase thereafter (16.4±4.3 u/day at 2 weeks vs 19.7±6.6 u/day at 52 weeks, p<0.001). In A the insulin doses were increased significantly but the dose gain was more pronounced in D than A. B and C needed only 6~7 u/day of insulin. In conclusion, 1) combination therapy except for C and insulin only improved HbA_{1C} and there was no significant difference among the three groups; 2) combination of bedtime insulin and SU needed only a small amount of the daily insulin dose; 3) insulin only patients needed a large daily insulin dose and weight gain was pronounced. We recommend combination of bedtime NPH insulin and SU in secondary SU failure patients.

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A STUDY OF MODES OF TREATMENT IN TYPE II PATIENTS WITH DIABETES ATTENDING OUTPATIENT CLINIC

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We studied the modes of treatment in 483 consecutive patients with Type II diabetes attending an out-patient clinic. All the patients received detailed diet and exercise prescriptions and were subjected to periodic educational programmes. In addition, Oral Hypoglycemic Agents (OHA) and/or insulin were administered as per the individual requirement. Inspite of detailed discussion on benefits of persistent good control of blood glucose and advice regarding acceptance of insulin in those whose blood glucose is inadequately controlled with Diet+OHA therapy, tremendous resistance was encountered when insulin therapy was adviced. The main causes for reluctance to accept insulin included - 1)injection phobia, 2)cost of insulin therapy, 3)fear of "addiction to insulin" and 4)fear of severe hypoglycemia. The objections and misconceptions were overcome using all the persuasive skill developed over the years. 36.3% patients were on insulin (5% on insulin alone, 31.3% were on insulin + OHA therapy. 2% were on diet + exercise while 28.8%, 7.2%, and 25.7% were on sulphonylurea(S), Biguanide (B), B+S therapy respectively. We conclude that with type II diabetes we used insulin in 36.3% patients. Even though most of the patients had strong objections to the use of insulin, they were overcome by detailed patient education and use of persuasive skill.

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TROGLITAZONE ADDED TO A SULPHONYLUREA SHOWS A DOSE-RESPONSE CONSISTENT WITH MONOTHERAPY

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Troglitazone is a novel once daily, oral anti-diabetic agent for the treatment of type 2 diabetes. Clinical studies used daily doses in the range 10 to 800 mg. Using a pharmacodynamic model, analyses of combined data from two double-blind, placebo-controlled, dose-ranging studies in type 2 diabetic patients have shown, in terms of HbA_{1c}, fasting serum glucose (FSG) and triglyceride reductions, an effective dose range of 200-600 mg od. Dose-response data from a 16-week, double-blind, placebo-controlled study adding troglitazone (T) 100 or 200 mg od to stable sulphonylurea (SU) therapy (baseline fasting capillary blood glucose ≥ 7 and ≤ 12 mmol/l) are presented. Ratio to placebo at week 12 (95 % confidence intervals) are as follows:

	HbA_{1c}	FSG	Triglycerides
T 100 mg + SU *n*=79, **n*=80	0.97 (0.93, 1.02)	0.88 (0.82, 0.94)	1.01* (0.9, 1.14)
T 200 mg + SU *n*=84	0.91 (0.87, 0.96)	0.79 (0.74, 0.84)	0.95 (0.85, 1.07)

Triglyceride and FSG values fit on the existing dose-response curves. HbA_{1c} values, sensitive to random variation, were similar and within existing confidence intervals. When added to SUs, troglitazone displays dose-response characteristics consistent with monotherapy, where 200 mg represents the minimum effective therapeutic dose.

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Insulin/Amylin Analogs

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GENERALIZED ALLERGY TO HUMAN INSULIN TREATED WITH INSULIN LISPRO

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Nowadays, insulin allergy is an uncommon complication specially in chilhood.We communicate an 11 year-old girl with an IDDM evolution of 8 months and poor metabolic control even after trying different ways of insulin therapy. She had ketoacidosis and local skin reaction at insulin injection areas(pain, reddening, swelling). Skin prick tests were carried out with positive reaction to human, porcine and beef insulins, as well as to protamine and available commercial insulin formulations. An intravenous insulin infusion (1,68 UI/kg/day) and oral antihistamines were started. Ketoacidosis disappeared, and blood glucose ranged from 200 to 300 mg/dl during 15 days. Treatment with insulin lispro was initiated (0.96 UI/Kg/day); after 48 h. blood glucose ranged from 60-100 mg/dl with symptoms of clinical hypoglycemia. Subcutaneous insulin lispro administration before the four main meals was started after 72 h, and two doses of Ultralente insulin were used to cover basal insulin requirements (total daily insulin = 1 U/Kg).After four months of treatment metabolic control was markedly improved, with HbA1c levels decreased from 13,5% to 7,8%. Insulin specific antibodies kept always high (99%,92,1%). The inflammation areas became gradually better with a posterior atrophy of the subcutaneous cellular tissue. There were no local reactions.It's important to notice that in spite of the high range of antiinsulin antibodies, insulin lispro achieve a good metabolic control. Since insulin lispro is rapidly absorbed from the cellular subcutaneous tissue, it might avoid the total inmunological destruction process.

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ACTION OF INTRAVENOUS AND SUBCUTANEOUS ACYLATED HUMAN INSULINS IN CONSCIOUS SWINE. J. Radziuk, J. Davies, S. Pye, D. Flora, L. Vignati, R. DiMarchi and R. Chance. Ottawa Civic Hosp., Ottawa, CAN; Lilly Res. Lab., Indianapolis, USA

[N^{ε}–palmitoyl Lys(B29)]human insulin (C16 insulin, n=6), [N^{ε}–myristoyl Lys(B29)]human insulin (C14 insulin, n=5) and [N^{ε}–myristoyl Lys(B28)Pro(B29)]human insulin (C14 insulin, n=6) were compared (means±sem) to regular human insulin (n=5) during a 3h i.v. infusion at 0.01nmol/kg–min in 20h fasted pigs with a concurrent infusion of somatostatin (0.3µg/kg–min) and euglycemic clamp. Total glucose requirements were similar for (a) insulin: 3.9±0.3, (b)C16 insulin: 3.8±0.7 (c) C14 insulin: 4.4±0.5 (d) C14 lispro: 4.1±0.5 g/kg. Peak total insulin (IRI) was (a) 0.28±0.02 (b) 8.0±0.7 (c) 3.3±0.7 (d) 6.1±0.4 nmol/L. Extension of action was characterized by the time after termination of the glucose infusion at which it was 10% of maximum: (a) 103±12 (b) 471±34 (c) 313±19 (d) 399±37min. A similar prototcol was implemented for 840 min after s.c. injection of 3 nmol/kg of the acylated analogs or NPH human insulin. Glucose requirements were (a) NPH: 5.4±1.1 (b) C16 insulin: 2.8±0.5 (c) C14 insulin: 6.9±0.5 (d) C14 lispro: 3.8±0.6 g/kg. Peak total IRI levels were (a) 0.16±0.01 (b) 2.40±0.29 (c)1.77±0.17 (d) 2.08±0.22 nmol/L. Peak mean glucose requirements were (a) 11.8±2.1 (b) 5.3±1.3 (c) 14.5±3.1 (d) 7.9±1.5 mg/kg–min. The ratios of final (840min) to peak glucose infusion rates were (a) 0.35 (b) 0.49 (c) 0.19 (d) 0.19 suggesting both lower peaks and longer action for NPH and C16 insulin ($p<0.05$). These data show that for acylated insulins, increased albumin binding, indicated by extension of action and higher total IRI levels after i.v. infusion, predicts a decreased bioavailability as indicated by IRI levels and glucose requirements after s.c. injection ($p<0.05$). C14 insulin is the most bioavailable and C16 insulin has the flattest response.

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C-PEPTIDE EVOLUTION IN NIDDM PATIENTS TREATED WITH LISPRO INSULIN FOR SECONDARY FAILURE TO ORAL HYPOGLYCEMIC AGENTS. MA.Gonzalo, C.Del Peso, O.Sánchez-Vilar, S.Azriel, JI.Lara, A.Rovira and JL.Herrera-Pombo. FUNDACION JIMENEZ DIAZ (UAM). MADRID. SPAIN.

In patients with NIDDM and secondary failure to oral hypoglycemic agents (SF) the administration of insulin may improve insulin secretion. We have evaluated the pancreatic ß-cell function and the metabolic control in five NIDDM patients (age:58.6±2.7 yr; evolution of disease: 10.6±1.3 yr; mean±SE) with SF before and after treatment with intensified insulin therapy (NPH and LIS(B28) PRO(B29) analogue, total daily dose of 0.65±0.07 U/kg) during four months. HbA1c values declined after insulin therapy (10.3±0,4% vs 8.4± 0.6%; $p<0.05$). Basal C-peptide and 6 min after 1 mg iv glucagon were 0.53±0.1 and 0.79±0.19 nmol/L before and 0.54±0.11 and 0.88± 0.28 after insulin treatment (p=ns). Body mass index (BMI) increased from 29.0±2.6 to 30.0±0.1 kg/m^2 ($p<0.001$). It was observed a significant improvement in triglyceride and HDL-c levels. Only two patients reported hypoglycemia once. In conclusion, the intensive treatment with human rapid insulin analogue (Lispro) and NPH insulin improves metabolic control without changes in the pancreatic ß-cell function in patients with NIDDM and secondary failure to oral hypoglycemic agents.

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ACTIVITY PROFILES OF NPH HUMAN INSULIN AND [N^{ε}–PALMITOYL LYS(B29)] HUMAN INSULIN IN SUBJECTS WITH IDDM. J. Radziuk, L. Vignati, P. Roach, R. DiMarchi, R. Chance, B.Bradley, S. Pye and J. Braaten. Ottawa Civic Hosp., Ottawa, CAN; Lilly Res. Lab., Indianapolis, USA

[N^{ε}–palmitoyl Lys(B29)]human insulin is an acylated analog of insulin which demonstrates an extended action compared to insulin alone when infused intravenously (IV) secondary to binding of this analog to circulating albumin. The acylated insulin (A) was compared with respect to duration and activity profile in a crossover fashion to NPH insulin (B) following subcutaneous (s.c.) doses of (A) 6nmol/kg and (B) 1.2 nmol/kg in 9 subjects with IDDM. After overnight IV infusion of regular human insulin, a.m. glucose was (A) 6.8±0.1 and (B) 7.1±0.1 mmol/L. After the s.c. injection, IV human insulin or glucose was infused to maintain near–basal glycemia and tracer glucose was infused for the separate assessment of hepatic glucose production (HGP). An activity profile was deduced for each study by expressing the glucose infusion rate at each time point, as a positive fraction (%) of the basal (measured) HGP, and the IV insulin infusion rate as a negative fraction (%) of the basal requirement and summing the two fractions. Basal insulin infusion rates were (A) 0.99±0.09 and (B) 1.25±0.09 pmol/kg–min and the basal concentrations were (A) 318±84 and (B) 200±45 pmol/L. Mean insulin requirement fell to < 10% of basal by (A) 200 min and (B) 150 min. During the 13h study (A) 0.42±0.06 and (B) 1.35±0.14 mmol/kg of additional glucose were needed. Mean endogenous glucose production was (A) 10.1±1.5 and (B) 7.8±0.9 µmol/kg–min (n.s). The activity profile at 3, 6, 9 and 12h was (A) 10±10, –9±7, –9±15, and –3±3% and (B) 12±10, –6±15, 27±18, 26±14% ($p<0.05$). These data indicate that both forms of insulin administration exert an extended (12h minimum) action which moreover remains stable over this period.

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REDUCTION OF SNACKS WHEN SWITCHING IDDM PATIENTS FROM REGULAR INSULIN TO LISPRO INSULIN

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The small or no improvements in HbA1c during insulin lispro treatment despite of better postprandial glucose control may be due to inadequate dietary adjustments. We tested whether reduction of snacks and increasing correspondingly the energy in the main meals results in improved metabolic control when switching to lispro treatment. We studied 141 IDDM patients, mean age 36 (SD 9) yrs, diabetes duration 14 (10) yrs, BMI 24.5 (2.4) kg/m^2. All had two daily NPH injections throughout the study. After baseline visit the patients used regular insulin before main meals thrice daily for 12 weeks. Thereafter they were switched to lispro (injections immediately before meals), and advised to transfer at least 50% from their snack carbohydrates to preceeding main meals. After the regular period and the 12-week lispro period, HbA1c was 7.81 and 7.70 % ($p=0.088$), weight 73.4 and 72.9 kg ($p<0.001$), NPH dose 25.9 and 27.2 U/day ($p<0.001$), and short-acting insulin dose 23.9 and 23.3 U/day ($p=0.035$), respectively. In those patients who diminished their snacks exactly as advised (n=67), HbA1c decreased from 7.91 to 7.66% ($p=0.014$). In patients with high HbA1c (>7.5%) after regular insulin and who reduced their snacks properly (n=46), HbA1c decreased from 8.49 to 8.13% ($p=0.004$). The number of hypoglycemias (blood glucose <2.5 mmol/l) was lower during lispro period (2.1 vs 1.4 episodes, $p=0.004$). We conclude that when switching from regular to lispro insulin, transfer of snack carbohydrates to main meals is safe, and results in improved HbA1c especially in patients with unsatisfactory metabolic control.

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PREMIXED FORMULATION OF B28ASP AND NPH-INSULIN: PHARMACODYNAMIC PROPERTIES OF A 30/70-STABLE MIXTURE

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The pharmacodynamic properties of the novel 30/70 mixture of soluble and protamin-retarded B28Asp have not been investigated yet, whereas the time-action profile of the rapid acting insulin analogue B28Asp has already been studied. We compared in a double-blind study the pharmacokinetic and pharmacodynamic properties of a 30/70 mixture of soluble and protamin-retarded B28Asp with a 30/70 mixture of soluble and protamin retarded human insulin (Actraphane HM, Novo Nordisk, Denmark). 24 healthy male volunteers (age 26±2 years, BMI 23,7±1,7 kg/m^2; mean±SD) received a s.c. injection of 0.3 U/kg body weight of either preparation into the abdominal wall on two study days during euglycaemic glucose clamps (target blood glucose 5.0 mmol/l, baseline i.v. insulin infusion 0.15 mU/kg/min). Glucose infusion rates (GIR) and serum insulin concentrations (INS) were determined the following 24 h. Injection of the premixed formulation with B28Asp resulted in an earlier and more pronounced increase in metabolic activity. The metabolic effect within the first 4 h after injection was 37 % higher, while the overall-effect was similar.

	30/70 B28Asp	30/70 Actraphane
GIR_{max} (mg/kg/min)	9.7±2.3	7.4±1.7*
t_{max} (min)	127±24	185±52*
AUC 0-240 min (g/kg x 240 min)	1.77±0.43	1.29±0.34*
AUC 0-1440 min (g/kg x 1440 min)	4.49±1.52	4.74±1.29
INS C_{max} (pmol/l)	183±12	101±8*
INS t_{max} (min)	115±3	177±13*
AUC 0-240 min (nmol/l x 240 min)	32.9±11.2	21.1±7.6*
AUC 0-1440 min (nmol/l x 1440 min)	59.3±24.7	61.9±16.1

(mean±SD), * P<0.0001

Subcutaneous injection of a stable 30/70 B28Asp protamin retarded mixture resulted in a more pronounced metabolic effect in the first hours after injection than an equivalent mixture with human insulin. The overall metabolic effect of both preparations was comparable.

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LOCALISATION OF ALBUMIN BINDING SITE(S) FOR NN304

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NN304: LysB29-tetradecanoyl des(B30)-insulin, is a new soluble, long-acting insulin analogue that is protracted by interaction with the protein albumin. Albumin is a multifunctional protein that binds and transport various endogenous molecules including fatty acids as well as certain drugs. The NN304 insulin analogue interact with albumin by binding to the long-chain fatty acid binding sites. The association constant for binding of the NN304 insulin analogue to human albumin is in the order of 10(5) M-1. The binding affinity of NN304 to albumin is lower than that of the free fatty acids. Domains of recombinant human albumin was expressed in yeast to investigate the site of interaction between NN304 and albumin as well as the difference in binding affinity between free fatty acids and NN304. Tetradecanoic acid and NN304 binding affinities to purified recombinant human domains I and III were determined. Tetradecanoic acid was found to have high affinity binding to domain III and insignificant binding to domain I. NN304 was found to bind to recombinant human domain III and to have minor binding to recombinant human domain I. We conclude that the mechanism for albumin binding of tetradecanoic acid and NN304 to be cognate and that NN304 is likely to associate with the fatty acid high affinity binding site on human albumin domain III.

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IMPROVED GLUCOSE CONTROL WITH INSULIN LISPRO DEMONSTRATED BY REDUCING GLUCOSE VARIABILITY

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Accurate assessment of daily blood glucose is important in evaluating new therapies and in estimating the sample size for a clinical trial. The variability of a multiple daily 7 point blood glucose profile was evaluated in a large 6 month randomized, multicenter crossover study comparing insulin lispro (LP) to human regular insulin (HR) in patients with IDDM (n=379) and NIDDM (n=328). A variance components analysis was performed to estimate the within and between patient variability in blood glucose measurements. A large decrease in variability was observed by increasing daily glucose readings which allows one to decrease the overall sample size of a similar study.

2hr Post-Breakfast Blood Glucose Measurements in mmol/L.

Glucose	Standard Deviation		Sample Size*	
Readings	IDDM	NIDDM	IDDM	NIDDM
1	4.25	3.76	145	113
4	3.10	2.95	77	70
% Decrease	27.1	21.5	46.7	38.5

*(alpha=0.05 two-tailed, Power=80%, detectable difference=1)

Using all 4 daily blood glucose measurements, a decrease in the mean 2 hr postbreakfast blood glucose measurement was observed during LP therapy (LP mean 8.58 sd 3.05 vs HR mean 9.75 sd 3.11, $p<0.001$).

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THE FIRST CLINICAL EXPERIENCE WITH INSULIN LISPRO IN RUSSIA

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The aim of the study was to assess efficacy, safety and acceptance of insulin lispro in patients on intensive insulin treatment. Thirty-nine IDDM patients with moderately good metabolic control (HbA1c < 10%) participated in this study. The age of the patients was 25±9.7 years. Duration of diabetes was 113±70.4 months. At the beginning of the treatment with insulin lispro, patients were instructed to use basal insulin (Humulin NPH) twice a day. The dose and number of injections of lispro insulin were adjusted by patients based on home blood glucose monitoring. Postprandial blood glucose control was assessed by 1- and 2- hour blood glucose after the standard meal. After the two month treatment period with insulin lispro significant improvement in 2-hour postprandial blood glucose was achieved compared to the prestudy values (13.5±5.2 vs. 10.9±5.4 mmol/l, p=0.032). One-hour postprandial blood glucose decreased from 15.0±5.0 to 12.8±5.9 mmol/l. HbA1c decreased from 8.4%±1.4 to 8.0±1.5% . Severe hypoglycemic episodes were not reported during the two month study period. Treatment preference questionnaire (insulin lispro vs. regular insulin) showed that 27/39 (69%) patients observed improvement in metabolic control on insulin lispro, 18/39 (46%) observed improvement in hypoglycemia, 21/39 (54%) experienced benefits in planning daily activities. When the study was completed 30/39 (75%) patients decided to continue using insulin lispro . In patients on intensive insulin treatment insulin lispro was preferred and superior in controlling postprandial blood glucose to their previously used prandial insulin.

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THE CARDIOVASCULAR SAFETY PROFILE OF INSULIN LISPRO (LP)

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Cardiovascular (CV) disease is a cardinal complication of diabetes. We examined the safety profile of LP (Humalog®), the first insulin of the rapid acting class of insulin analogs, as it related to CV disease and sudden death. Ten pivotal studies including 3634 patients (> 2000 patient years) were conducted. Adverse events were recorded at baseline and throughout the trials and assigned a COSTART term (specific for CV disease and subsets). There was no difference between LP and regular insulin (HR) in the number of patients developing a new CV disease event.

	n	LP	HR	p-value
All CV Events	3634	108	92	0.216
Cerebrovascular	3634	13	9	0.399
Cardiac	3634	45	39	0.508
Peripheral Vascular	3634	16	16	1.000

Patients were classified to a low or high risk group for developing a new CV event based upon a history of a CV event prior to entry. The highest CV risk group was identified as those high CV risk group patients who had a prolonged QTc interval at baseline. When either a newly developed CV event or an increase in severity of an existing CV event were considered, there was no statistical difference (Fisher's Exact test) between LP and HR therapy for any of the three risk groups.

Risk Group	n	Event Risk Rate	LP	HR	p-value
Low	2381	4%	54	38	0.089
High	1253	10%	65	66	0.927
Highest	80	16%	6	7	0.764

Sudden cardiac death was evaluated as observed death from ventricular tachycardia or fibrillation within 24 hours of the event. Eight cases of sudden cardiac death were reported in the controlled and long-term trials. There was no statistical difference between the LP versus the non-LP treated patients (3 vs. 5, p = 0.274).

In conclusion, there no clinical or statistical difference between LP and HR treated patients for the development of new CV events or sudden cardiac death.

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INSULIN LISPRO REDUCES HYPERGLYCEMIA AFTER A TEST MEAL BASED ON TRADITIONAL NUTRITION OF CENTRAL EUROPE

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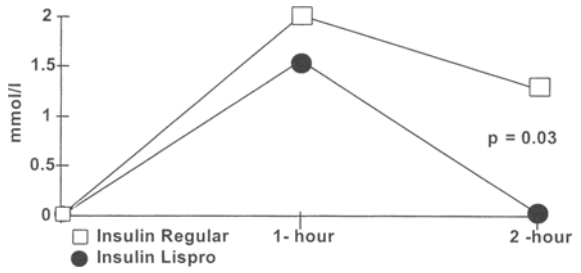
The objective of the study was to compare insulin lispro with human regular insulin in regard to glycemic control of diabetic patients on intensive insulin treatment. 62 patients (age 35.7±9.3; BMI 24.6±3.5; 55 patients Type 1) from 8 centers in the Czech Republic, Slovenia and Slovak Republic participated in a 4 month open, randomized, cross-over study. Fasting, 1- and 2-hour blood glucose after a test meal were measured for insulin lispro and regular insulin. The test meal (220-400kcal) was based on local and individual dietary habits and consistent for each patient throughout the study. Insulin lispro was injected immediately before the test meal while human regular insulin was administered 30 minutes before the meal. Fasting blood glucose value for insulin lispro was 10.2±4.2; regular insulin 10.3±4 mmol/l. Blood glucose excursions are presented in figure below. The HbA1c (7.6±1.5 vs. 7.4±1.5 %), frequency of hypoglycemia's and daily insulin dose (0.67+0.11 vs. 0.65±0.11 U/kg) did not differ significantly between insulin lispro and human regular insulin treatment groups. The study shows that insulin lispro is more effective than regular insulin in reducing hyperglycemia after a traditional Central European breakfast.



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OPTIMAL COMBINATION OF INSULIN LISPRO AND BASAL INSULIN IMPROVES GLYCEMIC CONTROL IN IDDM

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The short-acting insulin analog, insulin lispro (Humalog™) has recently become available for clinical use. We examined, if the use of Humalog will improve HbA_{1c} if basal insulin is optimally adjusted. 84 IDDM patients first used Humalog as premeal therapy for 5 months. They were then randomized for another 4 months to either continue with Humalog or to switch to human regular insulin (Humulin Regular). Insulin doses were adjusted according to glucose self-monitoring. During Humalog period, HbA_{1c} decreased from 8.8±0.1 to 7.9±0.1% (p<0.001), mean daily glucose level decreased from 9.5±0.2 to 8.6±0.2 mmol/l (p<0.001), premeal insulin decreased from 27±1 to 22±1 U/d (p<0.001) and basal insulin (NHP) dose increased from 20±1 to 27±1 U/d (p<0.001). The number of NPH injections increased from 1.4±0.1 to 2.7±0.1 per day (p<0.001). The hypoglycemia rate remained unchanged. During the second period, in Humulin group premeal insulin dose increased by 3.4±0.9 U (p<0.01), NPH dose decreased by 5.6±0.7 U (p<0.001), mean blood glucose increased by 0.7±0.1 mmol/l (p<0.02) and HbA_{1c} rose by 0.3±0.1% (p<0.01). In Humalog group, premeal and basal insulin doses, blood glucose and HbA_{1c} remained unchanged. In conclusion: 1) Humalog with optimal basal insulin lowers HbA_{1c} and mean blood glucose concentration without an increase in hypoglycemia, 2) Improved control can be maintained better with Humalog than with Humulin Regular insulin.

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COMPARISON OF THE EFFECT OF USING INSULIN LISPRO WITH ULTRALENTE AND NPH BASAL REGIMENS IN IDDM PATIENTS.

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Lilly Research Centre, Windlesham, UK[1]; University Hospital, Uppsala, Sweden[2]; Kolding Hospital, Kolding, Denmark[3].

Insulin lispro (LP) has a more rapid onset and shorter duration of activity than regular human insulin. The objective of this open label study was to compare the effect of using preprandial LP, with both NPH and Ultralente (UL) basal insulin regimens, on metabolic control. 167 IDDM patients (mean age 34.8 years, mean duration of diabetes 11.4 years) participated in this crossover study. There was a 1 month run in period (LP/NPH) and each treatment period was 3 months in duration. UL and NPH could be given in any regimen. Measurements included HbA1c, eight point blood glucose profiles, and the incidence of hypoglycaemic episodes. There was no significant difference in overall metabolic control at endpoint as measured by HbA1c (mean±SD) (7.0±0.9% [NPH] vs. 7.0±1.1% [UL], p=0.330). Breakfast premeal glucose levels were significantly lower with NPH (8.7±2.8mmol/L vs. 9.6±3.4mmol/L, p=0.007) and with ultralente before the evening meal (9.1±2.9mmol/L vs. 8.2±3.0mmol/L, p=0.003). However, 2hr postprandial blood glucose excursions were not significantly different between treatment groups after any meal. The incidence rate of hypoglycaemia (number of hypoglycaemic episodes/patient/treatment) was significantly lower between midnight and 6AM with UL (1.2±1.9 vs. 0.8±1.5, p=0.001) and between 6PM and midnight with NPH (1.6±2.6 vs. 2.9±4.5, p<0.001), but there was no difference in the overall incidence rate of hypoglycaemia (8.1±8.9 [NPH] vs. 9.1±10.9 [UL], p=0.158). The same trends were observed whether one or more basal insulin injections were used. The number of severe hypoglycaemic episodes did not differ between treatment groups (NPH, n=9 in 4 patients; UL, n=9 in 6 patients). In conclusion, LP is suitable for use in varied intensive regimens with NPH and UL basal insulins and use with UL may reduce nocturnal hypoglycaemia. The choice of basal regimen did not affect overall glycaemic control in this study.

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IMPORTANCE OF BASAL INSULIN TO IMPROVE CONTROL WITHOUT INCREASING HYPOGLYCAEMIA IN INTENSIVELY TREATED IDDM USING A SHORT-ACTING INSULIN ANALOG AT MEALS.

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In previous studies the short-acting insulin analog Lispro has improved 1- and 2-h postmeal blood glucose (BG), but not long-term BG control (HbA1c) as compared to human regular insulin (Hum-R). To test the hypothesis that Lispro improves HbA1c in IDDM only when basal insulin is optimally replaced, 12 IDDM patients on long-term intensive therapy (run-in HbA1c 6.46±0.14%) were studied. Patients were assigned to either Lispro injected at mealtime (n=6) or Hum-R injected 10-30 min prior to meals (n=6) (bedtime NPH continued on both occasions) for 3 months and thereafter crossed over. With Lispro, NPH was added to Lispro at breakfast and lunch (N=9) and also at supper (N=3) to optimize pre-meal and bedtime BG. With Hum-R, NPH was added to Hum-R at lunch (N=7) to optimize pre-supper BG. The total daily insulin units were no different in the two treatments, but in the Lispro+NPH treatment patients used 26% less short-acting insulin at meals (5.2±0.7 U/day), but 34% more NPH (4.4±0.7 U/day) vs Hum-R+NPH (p<0.05). The bedtime NPH dose was no different. With Lispro+NPH, postprandial BG was lower than after Hum-R+NPH (8.3±0.2 vs 8.9±0.2 mmol/l, p<0.05) whereas pre-meal BG was no different (8.3±0.4 vs 8.4±0.3 mmol/l); HbA1c was lower (6.19±0.16 vs 6.59±0.15%) (p<0.002), but the frequency of hypoglycaemia (BG <3.3 mmol/l) was no different (4.1±0.9 vs 3.7±0.9 episodes/patient-month, p=NS). It is concluded that, if basal insulin is optimally replaced, mealtime injection of Lispro improves the 24-h BG and, longterm, HbA1c as compared to Hum-R injected 10-30 min prior to meals. The lower HbA1c with Lispro+NPH is not associated with increased frequency of hypoglycaemia

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ALGORITHM ADAPTATION UNDER FUNCTIONAL INSULIN TREATMENT DURING SWITCH-OFF FROM REGULAR TO INSULIN LISPRO

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Aim: To investigate the necessity of dosage adaptation to pre-defined criteria during ambulatory switch-off from regular insulin ***R*** to insulin lispro ***L*** under flexible,functional insulin treatment FIT, discriminating between prandial, basal and correctional use of insulin. **Methods:** 28 insulin-dependent patients (age: 41±16, diabetes duration:16±9, FIT-duration: 5±4 years) who routinely make acute corrections of hyperglycaemic blood glucose levels, have been switched to ***L*** after a run-in period (***R***, baseline investigation 1) and were evaluated after 2 weeks (investigation 2, early adaptation) and after 11 weeks of observation period with ***L*** (investigation 3). **Results:** Basal insulin including either human insulin ultralente in the morning and NPH (51% of cases) or lente type insulin late be-fore sleeping (32%), twice daily ultralente (11%) or twice daily NPH (6%) was suitable for ***L*** with the exception of the latter option. The neces-

Algorithms \ Investigations	**1: regular**	**2: lispro,** *2wk*		**3:lispro,** *11wk*	
Basal delayed-act. ins.morning *U*	11,4±4,4	11,4±4,4	*0,60*	11,8±4,0	*0,79*
Morning short-act. component *U*	2,79±2,01	2,66±2,02	***0,05***	2,46±2,04	***0,01***
Delayed-acting evening *U*	11,4±5,2	11,3±5,3	*0,43*	11,9±4,6	*0,52*
Prandial insulin/50 kcal CHO *U*	1,8±0,7	1,8±0,7	*0,43*	1,8±0,7	*0,58*
Correction insulin: Delta BG/1U ↓	-2,4±0,8	-2,4±0,9	*0,99*	-2,4±0,8	*0,23*
Target for corr.,premeal BG *mmol/l*	5,7±0,4	5,7±0,4	*0,57*	5,7±0,3	*0,63*
Target for corr., after meals *mmol/l*	9,2±0,6	8,9±1,0	*0,06*	8,7±1,1	***0,01***
Effects: Ins.need:short-act.U/day	31,4±11	30,4±12	*0,24*	31,1±13,0	*0,65*
Delayed -acting insulin *U/day*	22,7±9,5	22,8±9,4	*0,54*	23,4±8,3	*0,69*
Mean blood glucose *mmol/l*	8,4±1,2	8,0±1,5	***0,03***	8,1±1,4	*0,17*
Mean fasting BG *mmol/l*	8,3±1,8	8,2±1,8	*0,45*	8,3±1,4	*0,71*
Mean postprandial BG *mmol/l*	9,3±2,1	8,2±2,1	***0,02***	7,9±2,0	***0,01***
% BG values <3,3 mmol/l	8,0±5,9			6,9±4,7	*0,06*
HbA1c *%, range 4,2-6,6%*	7,48±0,91			7,25±1,01	***0,05***
Interval used injection: meal *min*	9,3±8,5			3,8±7,0	***0,01***

sary algorithm adaptation was of minor degree *(intraindividual comparison, p vs investigation 1)*. **Conclusions:** FIT allows safe ambulatory switch-off to ***L***, a slight decrease in the morning dose of short-acting insulin is recommendable.

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HUMALOG REDUCES THE INCIDENCE OF SEVERE HYPOGLYCEMIA IN IDDM PATIENTS

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A short-acting insulin analog, Humalog (insulin lispro), is absorbed more rapidly, has a higher peak and a shorter duration of activity than human regular insulin. In large clinical trials premeal therapy with Humalog has reduced postprandial rise in plasma glucose, when compared to regular human insulin. There was a consistent reduction in severe hypoglycemic episodes (resulting in coma, or requiring glucagon or intravenous glucose) during Humalog therapy, although this was not statistically significant in any of the separate studies. A cumulative meta-analysis using Cochran-Mantel-Hanenzel odds ratio test was performed combining the data from all 8 large Humalog IDDM clinical trials. The studies were 6-12 months duration, 5 with cross-over design, using regular human insulin as a control. Each of these clinical trials used a similar method to collect information on hypoglycemic episodes. After combining these studies, 2327 patients used Humalog and 2339 patients regular human insulin. At least one severe hypoglycemic episode occurred during Humalog therapy in 72 (3.1%) patients, and during regular human insulin treatment in 102 (4.4%) patients (p=0.024). The mean HbA_{1c} level was similar in the two groups. In conclusion: 1) Humalog reduces the incidence of severe hypoglycemia by 30% compared to regular human insulin in IDDM patients. 2) This is probably due to a narrow time action profile of Humalog for postprandial period with less overlapping with basal insulin.

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EVALUATIONS 1- AND 2-HOUR POSTPRANDIAL GLUCOSE EXCURSIONS AS INDICES OF POSTPRANDIAL GLUCOSE CONTROL. J.R. Woodworth, R.K. Yordy, and L. Vignati, Eli Lilly and Company, Indianapolis, IN.

The use of 1- and 2-hour postprandial glucose measurements and the change of glucose from a premeal baseline (glucose excursions) can provide a means of evaluating the rapid glucose control provided by insulin and insulin lispro immediately following a meal. These measurements may also be indices to evaluate glucose control beyond 2 hours, establishing a means to evaluate an entire blood glucose profile with just one or two postprandial measurements. Two previously performed studies in which multiple postprandial glucose measurements were monitored in response to insulin and insulin lispro were used to evaluate this premise (Howey et al, *Clin. Pharmacol. Ther.*, 58, 459-69, 1995 [N=12]; Heinemann et al., *Diabet. Med.* 13, 625-9, 1996 [N=10]). Both studies were designed to monitor postprandial glucose control in patients with diabetes. The mean doses of insulin and insulin lispro were identical in both studies. The maximum postprandial excursion (GE_{max}), the time to GE_{max} (TGE_{max}), and hourly incremental areas under the glucose excursion curve (AUC_{ex}) were calculated from all data. Correlations were then attempted between these data and the 1- and 2-hour postprandial glucose excursions. Both the 1- and 2-hour excursion values correlated with the GE_{max} values, although the 2-hour postprandial excursion provided a better correlation (r^2=0.39 and 0.59, respectively). AUC_{ex} from 0 to 4 hours postprandial correlated well with the 2-hour postprandial excursion measurement in both studies, with nearly identical slopes from both studies (r^2=0.77 and 0.82, respectively). These correlations were independent of the type of insulin administered. Correlations were present beyond 4 hours, although the regression of 0-5 hour AUC_{ex} with 2-hour postprandial excursions was not as predictive as that with the 0-4 hour AUC_{ex}. TGE_{max} could not be reliably predicted. In conclusion, 2-hour postprandial glucose excursions serve as an excellent indicator parameter for evaluating glucose control allowing assessments of 0-4 hour AUC_{ex}.

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PREFERENCES FOR, AND IMPROVEMENTS IN ASPECTS OF QUALITY OF LIFE (QoL) WITH, INSULIN LISPRO IN A MULTIPLE INJECTION REGIMEN.

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The rapid action of human insulin analogue Lispro allows injection immediately before meals. 97 IDDM patients, participating in a 6 month, multi-centre, randomised, cross-over trial of Lispro injected immediately pre-meal compared with standard soluble insulin injected 30 minutes pre-meal, completed the Diabetes Treatment Satisfaction Questionnaire (DTSQ) and Well-being Questionnaire (WBQ) (Bradley, 1994). Significant treatment differences for the WBQ and 3/4 subscales, together with the DTSQ total scale and treatment convenience item score all favoured Lispro.

	Mean Diff. favouring Lispro	p	95% CI	Possible Range
WBQ Total	4.50	0.002	1.58 to 7.42	0-66
Depression subscale	-1.20	0.006	-2.06 to -0.34	0-18
Anxiety subscale	-1.50	0.001	-2.40 to -0.60	0-18
Energy subscale	0.72	0.001	0.30 to 1.14	0-12
Positive Well-being subscale	0.36	n.s.	- 0.22 to 0.94	0-18
DTSQ Total	2.89	0.001	1.31 to 4.47	0-36
Convenience score	0.71	0.001	0.35 to 1.07	0-6

There was no significant difference in HbA_1c. More detailed analyses of psychological outcomes suggest that disappointment at allocation to standard insulin contributed to the results and that experience with Lispro was followed by marked reduction in satisfaction with standard insulin. On completion of the study, 83% of eligible patients elected to continue using Lispro, supporting patient preference and QoL data favouring Lispro.

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EFFECT OF CIRCULATING FREE FATTY ACIDS ON THE *IN VIVO* KINETICS OF NN304

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The prolonged effect of the new basal insulin analogue NN304 (LysB29-tetradecanoyl des(B30)-insulin) is ascribed to its high affinity for serum albumin in the interstitial fluid at the s.c. injection site and in blood. Because NN304 is bound to serum albumin at the long chain fatty acid binding sites, variation of the concentration of free fatty acids may affect the equilibrium and hence the time action of NN304. In the present study we examined the effect of high levels of circulating free fatty acids on the kinetics of 125I-labelled NN304 injected i.v. or s.c. to pigs. Healthy subjects show serum values of free fatty acids between 0.1 and 0.6 mmol/l. Low levels of free fatty acids (0.1-0.2 mmol/l) were obtained in fed pigs (n=4, 96-108 kg), whereas high levels (1.6-2.5 mmol/l) were induced in the same pigs after i.v. infusion of the beta1-agonist, dobutamine 10 µg/kg/min for 105 min. Both the disappearance course of 125I-labelled NN304 from a subcutaneous depot (0.2 µCi and 2 U) measured by external gamma-counting, and the elimination of trichloroacetic acid (TCA) precipitable radioactivity in plasma after i.v. injection of the labelled insulin analogue (25 µCi and 0.5 U) were similar with and without elevated levels of free fatty acids. In conclusion, elevated circulating levels of free fatty acids do not alter the pharmacokinetics of NN304.

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INSULIN LISPRO CHANGES TREATMENT SATISFACTION UNDER FLEXIBLE *FUNCTIONAL INSULIN TREATMENT*

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Aim: To investigate and quantify the subjectively perceived advantages of insulin lispro ***L*** vs. regular insulin ***R*** for Functional Insulin Treatment (FIT), discriminating between basal, prandial and correctional use of insulin. **Method:** 55 FIT-patients (age: 41,8±16,0, diabetes duration:16,0±9,6, FIT-duration: 5,4±3,2 years) who routinely make acute corrections to their blood glucose levels with extra insulin as required were recruited and randomized into two study groups. Educational update in FIT was provided together with information on ***L*** pharmakokinetics. After a run-in period of 8 weeks, parallel periods of 11 weeks each (either with ***L*** or ***R***) were compared. Psychological measures included status **(S)** and change **(C)** versions of the *Diabetes Treatment Satisfaction Questionnaire (DTSQ of C.Bradley 1994)* extended with items designed for FIT. The *DTSQ(C)* relates the present treatment satisfaction of the subject to that in the preceeding phase of investigation. **Results:** While the ***R*** group did not change significantly, the ***L*** group increased *treatment satisfaction* in *DTSQ(S)* (intragroup comparison) in total satisfaction (p=0,01), and specifically in all categories related to correctional (speed: p<0,001; accuracy: p=0,001; general: p=0,001) and prandial use of insulin (efficiency to deal with blood glucose after meals; necessary timing of injections; general; all p-values <0,001). Increased predictability (p=0,047) and controllability (p=0,022) of blood glucose levels and increase in satisfaction with ability to perceive hypoglycaemia (p=0,035) were also found with lispro. DTSQ*(C)* augmented these perceived advantages with ***L*** (intergroup comparison) particularly in categories „satisfied-with-your-current-treatment" (p=0,01), convenience (p=0,047) and flexibility of treatment (p=0,008), understanding of diabetes /blood glucose course/ (p=0,043), and „wish-to-continue-this-kind-of-treatment" (p=0,006). HbA_{1c} decreased significantly with ***L*** from 7,5±0,9 to 7,2±1,0 (p=0,049), while remaining unchanged with ***R*** (p=0,9). Percentage of low blood glucose values tended to decrease with ***L*** (p=0,06). **Conclusion:** Insulin lispro improves treatment satisfaction under flexible, functional use of insulin while reducing HbA_{1c}.

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PHARMACOKINETIC AND GLUCODYNAMIC ASSESSMENTS OF N-PALMITOYL, LYS(B29) HUMAN INSULIN IN HEALTHY VOLUNTEERS.

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N-acylated insulins may provide a means of creating a sustained activity of insulin in a soluble preparation, potentially providing less inter- and intra-patient variability in absorption than current intermediate- or long-acting insulins. We performed a dose-ranging trial in healthy volunteers (N=23) with N-palmitoyl lys(B29) human insulin (C16-HI). Subcutaneous doses from 0.03 to 0.57 nmol were tested in this 24-hour, Biostator-controlled glucose clamp trial. Blood samples were collected over the 24 hourperiod for assessment of total and unbound C16-HI serum concentrations. Maximum serum concentrations (C_{max}), time to C_{max} (t_{max}), area under the curve from 0 to infinity (AUC), and half-life ($t_{½}$) were determined from the serum concentrations. Maximum glucose infusions (R_{max}), time to R_{max} (TR_{max}), and total glucose infused (G_{tot}) were documented from the clamp. AUC values were linear with dose (r^2=0.85). Protein binding of C16-HI was high, with 1.32%±0.60 (mean±SD) unbound 4 hours after dosing. Glucodynamic response was low and, although linear across doses, highly variable (r^2=0.35), reflecting a potency of 20-25% of NPH based upon published values. The table summarizes mean±SD data once normalized to a 0.48 nmol dose.

	C_{max}, pM	t_{max}, hr	$t_{½}$, hr	AUC, nM•hr	R_{max}, mg/min	TR_{max}, hr	G_{tot}, gm
bound	4259±1346	5.2±1.9	4.34	47.4±9.83	221±86.9	5.6±2.3	75.1±65.1
unbound	65.2±39.4	4.6±2.2	4.20	0.58±0.28	-	-	-

Attempts to relate G_{tot} values with total drug exposure (AUC) resulted in a minimal correlation (r^2=0.31), which improved only slightly when related to unbound AUC (r^2=0.40). In conclusion, C16-HI showed a highly reproducible and linear pharmacokinetic profile. The glucodynamic response was consistent with an intermediate-acting insulin and linear across doses, but the time-action profile was highly variable.

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COMPARISON OF THE METABOLIC ACTIVITY OF THE INSULIN ANALOGUE B28ASP WITH SOLUBLE INSULIN IN U40 AND U100 CONCENTRATION

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It is well known that rapid-acting insulin analogues like B28Asp show a faster onset and a shorter duration of action than currently available U100 soluble insulin preparations. Since this effect is mainly due to a lower concentration of slow-absorbable hexamers, the metabolic profile of human insulin in lower concentration (e.g. U40 insulin) might be more similar to that of B28Asp. Therefore, we compared the pharmacodynamic and pharmacokinetic properties of U40 soluble insulin with B28Asp (U100) and with U100 human insulin. 8 healthy volunteers (age 26±2 years, BMI 22.8±2.1 kg/m²) received at different study days s.c. injection of B28Asp and U40 insulin (0.2 U/kg body weight) under euglycaemic clamp conditions (blood glucose 5 mmol/l, basal i.v. insulin infusion 0.15 mU/kg/min). Glucose infusion rates (GIR) and serum insulin concentrations (INS) were measured the following 600 min. In a second study, U40 and U100 soluble insulin was administered to 9 other volunteers (age 25±1 years, BMI 22.6±2.1 kg/m²; N.S.) under similar conditions. No significant differences were observed between the summary measures of U40 and U100 insulin. B28Asp showed a faster onset and a shorter duration of action than U40 insulin.

	U40	**U100**	**P**	**U40**	**B28Asp**	**P**
GIR_{max} (mg/kg/min)	8.9±2.0	9.5±2.3	0.296	9.5±2.6	10.5±2.6	0.151
t_{max} (min)	144±23	156±29	0.228	146±18	108±15	0.006
early $t_{50\,\%}$ (min)	61±11	65±15	0.523	60±11	43±7	0.001
late $t_{50\,\%}$ (min)	345±68	387±68	0.088	360±75	271±35	0.008
AUC 0-120 min	515±170	533±216	0.798	556±225	789±251	0.008
AUC 0-600 min	2663±512	3011±547	0.122	3002±1084	2724±558	0.395
INS C_{max} (pmol/l)	205±62	196±41	0.612	231±76	334±42	0 018
INS t_{max} (min)	111±35	129±36	0.268	97±21	71±11	0.005
AUC 0-120 min	17.7±6.5	16.1±4.0	0.622	19.8±6.3	29.9±3.0	0.007
AUC 0-600 min	52.0±10.8	52.9±5.9	0.806	54.7±17.8	53 3±7.6	0.824

The metabolic activity of human insulin in concentrations of U40 and U100 is comparable. S.c. injection of the insulin analogue B28Asp leads to a faster onset and a shorter duration of action even in comparison to U40 insulin.

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CROSS-OVER INSULIN PUMP STUDY WITH HUMAN INSULIN ANALOGUE [LYS(B28), PRO(B29)]

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The short-acting insulin analogue LP ([LYS(B28), PRO(B29)]) is absorbed from the subcutis more rapidly than regular insulin (R) because of its monomeric structure. To compare the clinical effectiveness of LP vs. R, 11 IDDM patients with continuous subcutaneous insulin infusion (CSII) therapy (6 F, 5 M, age 30±2,5 years, diabetes duration 14±1,0 years, BMI 24,0±0,8 kg/m^2, HbA_{1C} 6,5±0,2%) were studied in an open, randomised cross-over study for 6 months (3 months LP and 3 months R or vice versa). Mean fasting blood glucose was not significantly different in both regimens (6,5±0,4 vs. 7,5±0,6 mmol/l), but during LP 2 hours postprandial blood glucose levels were significantly lower compared to the R phase (6,8±0,3 vs. 8,5±0,4 mmol/l, p<0,01). There was no significant difference concerning hypoglycaemic episodes between the two groups. No significant differences concerning HbA_{1C}-levels between the two groups at month 0 were found (LP:6,3±0,2 vs. R:6,7±0,4). Among the patients treated first with LP HbA_{1C}-levels improved significantly compared to the group treated with R (at month 3: 5,7±0,3% (LP) vs. 6,5±0,3% (R), p=0,03). In the second phase of therapy HbA_{1C} decreased from 6,5±0,3% to 6,3±0,3% under LP regimen and increased from 5,7±0,3% to 6,2±0,2% in R phase. It can be concluded that LP in CSII-treatment is safe and further improves metabolic control.

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TIME-ACTION PROFILES OF PREMIXED FORMULATIONS OF INSULIN LISPRO AND NPL-INSULIN

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Stable mixtures with insulin lispro (LP) can be prepared with the novel NPL-insulin, i.e. a protamine-retarded formulation of LP. In an open randomised study we investigated the time-action profile of pure LP and NPL-insulin and of three different mixtures: High Mixture (HM) 3:1 (LP/NPL); Mid Mixture (MM) 1:1, Low Mixture (LM) 1:3. 30 healthy volunteers (12 female, 18 male, age 27±2 years, BMI 23.0±2.3 kg/m² (mean±SD)) received a s.c. injection of 0.3 U/kg of one of these preparations on five study days, separated by at least one week. Glucose infusion rates (GIR) were registered during euglycaemic glucose clamps (target blood glucose level 5.0 mmol/l, i.v. insulin infusion 0.15 mU/kg/min) for 1440 min or 600 min (LP). Whereas the maximal metabolic activity decreased with lower LP content, the time point of maximal and of early half-maximal metabolic activity was comparable between the three mixtures. Higher proportions of LP resulted in a more rapid decline to late half-maximal activity and in higher AUCs within the first 360 min after injection. Serum insulin concentrations showed a similar behaviour. This study shows, that the pharmacodynamic and pharmacokinetic properties of insulin lispro are preserved in stable mixtures with NPL-insulin.

	GIR_{max}	t_{max}	early $t_{50\%}$	late $t_{50\%}$	$AUC_{0\text{-}360\ min}$	INS-C_{max}	t_{max}
unit	mg/kg/min	min	min	min	g/kg/360 min	pmol/l	min
LP	12.7±3.0	107±21	44±12	266±57	2.73±0.58	871±202	72±17
HM	10.2±2.7	120±25	47±13	339±76	2.45±0.52	548±99	82±20
MM	9.0±2.8	121±22	40±12	384±110	2.19±0.58	407±89	81±23
LM	7.3±2.6	141±36	44±12	557±205	1.87±0.62	206±79	94±45
NPL	4.9±2.3	252±34	70±30	941±268	1.24±0.68	124±73	160±98

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IGF-I RECEPTOR-MEDIATED SIGNALLING OF THE HUMAN INSULIN ANALOGUE HOE 901

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HOE 901 is a novel human insulin analogue with protracted action being a promising candidate for basal insulin substitution with a daily single injection. Insulin receptor signalling of this analogue is not significantly different from native insulin, however, differential interaction with IGF-I receptors may modify the mitogenic properties of insulin analogues. H9 cardiac myoblasts, a cell line expressing a high level of IGF-I receptors with undetectable amounts of insulin receptors, were used to investigate IGF-I receptor signalling of HOE 901 and to compare it to native insulin and the supermitogenic Asp(B10)-insulin. Competition experiments indicated a higher affinity of HOE 901 for the IGF-I receptor when compared to regular human insulin (IC_{50} values: 44, 70 and 101 nM, for Asp(B10), HOE 901 and insulin, respectively). However, the mitogenic activity of Asp(B10), as determined from ^{3}H-thymidine incorporation, was significantly higher than the effect of HOE 901 with the latter being esssentially equipotent to native insulin. H9 cells were then labelled with ^{33}P-orthophosphate and stimulated with the peptides for 24 h. 2D-analysis indicated the presence of three major phosphoproteins (40-70 kDa) being largely dephosphorylated in the presence of insulin and HOE 901. In contrast, Asp(B10) induced a marked increase of additional phosphoproteins not detected in the presence of HOE 901 and insulin. Postreceptor signalling was analysed at the level of IRS-1 and Shc. Tyr-phosphorylation of IRS-1 increased 2 fold for both insulin and HOE 901 both after 10 and 60 min, with a significantly higher effect of Asp(B10) at 60 min (2.6 fold, p=0.026,n=5). One Shc protein with a molecular mass of 52 kDa was detected in H9 cells. Both insulin and HOE 901 did not modify the tyr-phosphorylation of Shc, whereas Asp(B10) produced a 50% increase in tyr-phosphorylation of this adapter protein.

In conclusion, IGF-I receptor signalling by HOE 901 is essentially identical to native insulin. Activation of Shc and sustained activation of IRS-1 by Asp(B10)-insulin may explain the higher mitogenic activity of this insulin analogue. Complete understanding of the action profile of insulin analogues must include analysis of IGF-I receptor signalling.

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EFFECTS OF THE AMYLIN ANALOGUE PRAMLINTIDE ON THE GLUCOSE RESPONSE TO A GLUCAGON CHALLENGE IN IDDM

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Hepatic glycogen stores have been shown to be depleted, and glucagon stimulated hepatic glucose production (HGP) reduced in IDDM subjects, possibly contributing to deficient counter-regulatory responses to hypoglycaemia in these patients. Co-administration of amylin and insulin has been shown to replete hepatic glycogen stores in diabetic animal models. To investigate the effect of amylin replacement, employing the analogue pramlintide (Pr), on hepatic glucagon responsiveness, 13 IDDM males were studied in a double-blind placebo-controlled cross-over study after 4 weeks of subcutaneous Pr (30μg*4) or placebo (Pl) administration. Following an overnight fast plasma glucose was kept above 5 mM from 0-240 min (baseline=210-240 min), with an insulin infusion rate of 0.3 mU/kg/min. To control portal glucagon levels, somatostatin was infused at a rate of 200 μg/h, and basal GH (2 ng/kg/min) and glucagon levels (0.7 ng/kg/min) were replaced. Glucagon infusion was increased to 2.1 ng/kg/min at 240-360 min (step 1) and to 4.2 ng/kg/min at 360-420 min (step 2). Baseline mean plasma glucose (5.59 ± 0.16 vs 5.67 ± 0.25 mM) and HGP rates (1.32 ± 0.22 vs 1.20 ± 0.13 mg/kg/min) were similar and glucagon responsiveness was unaffected by pramlintide (glucose: step 1; 6.01 ± 0.31 vs 5.94 ± 0.38 mM, step 2; 6.00 ± 0.37 vs 5.96 ± 0.50 mM, HGP: step 1; 1.91 ± 0.18 vs 1.83 ± 0.15 mg/kg/min, step 2; 2.08 ± 0.17 vs $1,96\pm0.16$ mg/kg/min, Pr vs Pl). Glucose disposal rates were similar at baseline (2.44 ± 0.13 vs 2.28 ± 0.09 mg/kg/min; Pr vs Pl) as well as during the glucagon challenge ($p>0.20$). In conclusion: Co-administration of pramlintide (120 μg/day) and insulin to IDDM subjects for 4 weeks does not change the plasma glucose - or hepatic glucose production response to a glucagon challenge, following an overnight fast. In addition, pramlintide administration does not appear to alter insulin-mediated glucose disposal. Additional studies are required to assess the impact of pramlintide therapy on glucagon physiology in the postprandial state.

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Pramlintide Improves Glycemic Control in Patients with Type II Diabetes Requiring Insulin

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The effects of 4 weeks of subcutaneous administration of pramlintide, (Pr) an analog of human amylin, on glycemic control in 203 patients with Type II diabetes mellitus requiring insulin were examined in a randomized, double-blind, placebo-controlled, parallel-group trial. Statistically significant reductions in serum fructosamine concentration were observed in the Pr 30 µg QID group (17.5±4.9 µmol/L), the Pr 60 µg TID group (24.1±4.9 µmol/L) and the Pr 60 µg QID group (22.6±4.1 µmol/L) compared to placebo (PBO) (3.5±3.8 µmol/L). There also were statistically significant shifts in the proportion of patients with an abnormal serum fructosamine concentration at baseline that normalized at Week 4 within the Pr 60 µg TID group (28%) and the Pr 60 µg QID group (31%) compared to PBO (10%). Consistent with the reduction in fructosamine, there were also statistically significant reductions in HbA_{1c} in the Pr 30 µg QID group (0.53±0.07%), the Pr 60 µg TID group (0.58±0.07%) and the Pr 60 µg QID group (0.51±0.08%) compared to placebo (0.27±0.08%). Based on RBC lifespan, and assuming stable glycemic control, these reductions in HbA1c in the Pr groups should increase over the following 2-3 months. The reductions in fructosamine and HbA_{1c} were accompanied by a statistically significant reduction in fasting total and LDL cholesterol. In contrast to treatment with insulin alone, there were trends towards decreased body weight in the Pr 60 µg TID and 60 µg QID groups. Furthermore, the incidence of hypoglycemia was no greater in any Pr group than in placebo. In conclusion, measurement of similar changes in both serum fructosamine concentration and HbA_{1c} suggests that pramlintide therapy for 28 days improves glycemic control in patients with Type II diabetes mellitus requiring insulin.

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THE HUMAN AMYLIN ANALOGUE PRAMLINTIDE INHIBITED GLUCAGON SECRETION IN TYPE I DIABETIC SUBJECTS

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It has been reported that Type I diabetic patients of greater than 5 years duration have a decreased glucagon response to hypoglycemia despite elevated basal and post-prandial glucagon secretion compared to non-diabetic controls. Rat amylin has been shown to inhibit the glucagon secretion elicited by an intravenous bolus of L-arginine in non-diabetic rats. Limited data, however, exists in humans. We measured plasma glucagon from patients who were treated with the human amylin analogue, pramlintide, to determine if similar glucagonostatic effects can be seen in human patients with Type I diabetes. The study utilized a randomized, placebo controlled, single-blind, 2-visit crossover design in which 16 patients received a 5 hour intravenous infusion of pramlintide at either 25µg/hr or 50µg/hr. Patients started the infusion at T=0 and received their normal morning insulin dose subcutaneously at T=30 mins. At T=60 mins, patients were given a 355 kcal standardized liquid meal challenge (Sustacal®). Plasma glucagon concentrations were measured by RIA (Linco Research Inc.) and plasma pramlintide concentrations were measured by IEMA. Mean steady state plasma pramlintide concentrations were 116 pM (110 pM - 125 pM) and 227 pM (207 pM - 253 pM) respectively [mean (range)]. Glucagon secretion from the time of Sustacal® administration to the end of the pramlintide infusion (60-300 minutes) was significantly reduced in both groups when on active treatment. The mean ± SEM plasma glucagon $AUC_{60\text{-}300}$ for placebo (N=16) was 15994 ± 1295 pg*min/ml compared to 14873 ± 2518 pg*min/ml and 12956 ± 72.6 pg*min/ml for the 25 µg/hr (N=8) and 50 µg/hr (N=8) pramlintide infusions respectively ($p<0.001$, crossover ANOVA). Additionally, a slight reduction in fasting (pre-Sustacal) plasma glucagon concentrations was seen after pramlintide infusion. The mean ± SEM change in fasting glucagon post infusion was -0.69 ± 1.10 pg/ml for placebo compared to -3.80 ± 1.31 pg/ml and -4.79 ± 0.80 pg/ml for the 25 µg/hr and 50 µg/hr treatments respectively (p=0.11 and p=0.09, crossover ANOVA). Although these individual comparisons did not reach significance, the pooled pramlintide results vs. placebo were statistically different (p=0.02). We conclude that the administration of the human amylin analogue, pramlintide, significantly reduced the elevated basal and postprandial secretion of glucagon seen in patients with Type I diabetes. This mechanism may contribute to the lowering of the post-prandial blood glucose that has been reported in previous studies.

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PHARMACOKINETIC EFFECTS OF SYRINGE MIXING PRAMLINTIDE, ISOPHANE INSULIN, AND SOLUBLE INSULIN.

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The pharmacokinetics (AUC, C_{max}, and T_{max}) of pramlintide, free insulin and glucose following subcutaneous injections of 30 µg pramlintide, isophane insulin (I) and soluble insulin (S), given separately or syringe-mixed in various combinations within 5 min prior to injection, were compared in 28 patients with Type I diabetes mellitus. In a randomized, open-label, five-way crossover trial, there were five treatment regimens with a 1-week washout between each treatment: (1) placebo+I+S; (2) pramlintide+I, separate S; (3) pramlintide+S, separate I; (4) pramlintide, I, S, all separate; (5) pramlintide+I+S. Breakfast and lunch were given after the morning pramlintide and insulin administration, and blood samples were collected for 10 hours (5 hours for pramlintide analysis). Pramlintide was rapidly absorbed and eliminated following separate injection or various combined injections. When mixed with I, pramlintide appeared to have increased bioavailability compared to the other treatments. The free insulin profile following combined pramlintide+I+S injection was similar to that following separate injections of pramlintide, I and S. Glucose C_{max} values were significantly lower and T_{max} values were delayed following combined pramlintide+I+S injection compared to combined placebo+I+S injection. Up to 180 min after administration, the four pramlintide treatments resulted in lower glucose profiles compared to placebo treatment. Over the entire period (0-600 min) the glucose profile was clinically optimal with the combination pramlintide+I in one syringe, with or without S, compared to the other treatments. These results support the mixing of pramlintide with isophane and/or soluble insulin in the same syringe prior to subcutaneous self-administration.

PS 34
Devices

1400

RELIABLE BLOOD GLUCOSE MEASUREMENT WITH THE APEC GLUCOSE ANALYSER

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In contrast to the large variety of glucose meters for patients' use, there is only a small selection of glucose analysers available for rapid, reliable and cheap glucose measurements on the ward. The APEC glucose analyser (Ruhrtal Labor Technik, Möhnesee-Delecke, Germany) estimates glucose concentrations by measurement of the oxygen decline with a polarographic oxygen electrode employing the glucose oxidase reaction. This desk top analyser allows glucose measurements in blood, serum, plasma, urine (in 10 µl samples) and in haemolysed blood (mixture of 40 µl blood, drawn up with a heparinised capillary and 590 µl reagent) within 30 s (46 s with blood), prints out the measurement results, performs a number of self tests and prints error analyses in case of problems. The costs for a single blood glucose measurement are 0.35 DM. We evaluated the reliability of the APEC glucose analyser in comparison to the Beckman Glucose Analyser (Beckman, Munich, Germany) by parallel measurement of 429 venous blood samples. Plasma glucose concentrations measured by the Beckman analyser were corrected by subtraction of 10 % to blood glucose concentrations (5.0±2.1 (1.7-17.2) mmol/l; (mean±SD (range)). These values were comparable to the results with the APEC analyser from haemolysed blood samples (5.0±2.1 (1.1-17.3) mmol/l, p=0.129, paired t-test), the absolute difference being 0.0±0.4 mmol/l. Linear correlation gave a regression line of y=0.983x+2.07 and a correlation coefficient of r=0.982. With the error grid analysis 426 (99.3 %) of all results with the APEC analyser are in zone A/a, in which differences between the results have no clinical implications. Only one value was in zone B and two in zone D, i.e. only three results showed clinically relevant or not acceptable deviations. The APEC glucose analyser allows rapid and reliable blood glucose estimations in small blood volumes.

1401

VALIDITY OF HbA_{1c} MEASUREMENTS IN DIABETIC PATIENTS WITH A HIGH DEGREE OF HYPERGLYCEMIA

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Although HbA_{1c} is now routinely used as an indicator of long-term (2 months) glycemic status in diabetic patients, its validity in rising degrees of hyperglycemia becomes increasingly difficult to be ascertained in human subjects *in vivo* due to obvious ethical reasons. Like many tropical developing countries Bangladesh has a group of patients termed as malnutrition related diabetes mellitus (MRDM), who presents with an extreme degree of hyperglycemia (fasting blood glucose values usually above 18mmol/l and 2-hr post prandial values >25 mmol/l) and even without any treatment, they do not develop ketoacidosis. In the present study HbA_{1c} level of 16 MRDM (12 FCPD and 6 PDDM), 26 NIDDM (of same age group) and 54 age-matched nondiabetic control subjects were measured to explore the applicability of the test in MRDM patients. A three sample OGTT was also performed in each subject. Glucose was estimated by Glucose-oxidase method and HbA_{1c} was estimated by an automated HPLC technique based on cation-exchange (Variant, Bio-Rad, USA). HbA_{1c} in MRDM subgroups (%,M±SEM: FCPD: 17.1±1.12) and PDDM: 16.4±1.9) were much higher than those in Control (4.7±0.1) and NIDDM (10±0.6). The values reflect the wide difference in fasting serum glucose levels between the MRDM subgroups (mmol/l, M±SEM, FCPD: 20.2±1.8 and PDDM: 16.9±2.5) and the NIDDM group (11.4±1.0) as well as between the MRDM subgroups and Control (3.5±0.1). The mean (±SEM) postprandial 1-hr glucose levels were 6.6±0.4, 30.9±2.2, 27.1±3.4 and 21.3±1.5 mmol/l and the mean (±SEM) postprandial 2-hr glucose levels were 5.6±0.3, 32.3±2.1, 28.3±3.3 and 23.0±1.6 mmol/l in control, FCPD, PDDM and NIDDM patients respectively. Significant difference ($p<0.001$) was found between all the diabetic groups and the nondiabetic one but not between FCPD and PDDM regarding serum fasting and postprandial glucose and HbA_{1c} levels. The NIDDM patients had significantly ($p<0.001$) lower values compared to FCPD patients in all cases. HbA_{1c} correlated with fasting serum glucose in FCPD ($r=0.71$, $p<0.01$) and in NIDDM ($r=0.8$, $p<0.001$) cases.The results demonstrate that HbA_{1c} is a reliable indicator of glycemic status in all types of diabetes including those with very high blood glucose levels.

1402

LOCAL ADVERSE EVENTS DURING LONG TERM TREATMENT WITH IMPLANTABLE INSULIN PUMPS IN TYPE I DIABETIC PATIENTS

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The aim of the study was to assess the frequency of local adverse events during long term therapy using implantable insulin pump, their clinical impact and the predisposition factors. From 1989 to 1995, 352 IDDM patients were treated with either Minimed MIP 2001(n=466), Infusaid M1000 (n=52) or Siemens Promedos ID3 (n=30) devices. Local incidents, implantation procedure, patients' characteristics, usual physical activity and history of allergy were recorded in a retrospective multicentric study. Cumulative follow-up was 1180 patients.years. At least 1 local pump pocket complication occured in 84 patients after a mean implantation duration of 11.2 months (1-28). Mean rate of these events was 7.1 per 100 pts.yrs, 24% of the patients were affected.Wide variations (ns) were observed between large centers (eg>24 patients) and small centers (6.9 vs 13.9 per 100 pts.yrs, 22.5 vs 30.9%). Main local complications were inflammatory reactions (34.5%), skin erosions (44%) and infections (9.5%) requiring pump explantations in 64.4 % of these cases. None of the factors such as gender, age, BMI, diabetes duration, physical activity, history of allergy, pump model or implantation procedure was correlated with the occurence of local complications. They occured for 60.2% in patients implanted for the first time, of the non affected patients 35% were primo-implanted ($p<0.05$). No prophylactic antibiotherapy was prescribed in one of the large center with the highest frequency of local events (28.7%). Cutaneous complications were significant regarding their frequency and their consequences (pump explantation). No major causal factor involving patients characteristics or clinical procedure was found.

1403

VALIDATION OF A VASCULAR GLUCOSE MONITORING SYSTEM USING SODIUM CITRATE ANTICOAGULATION.

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The use of heparin anticoagulation in metabolic studies may be undesirable due to a coagulation disorder or, more generally, artifactual elevations in plasma FFA due to its effect on plasma lipoprotein lipase. We tested the compatibility of using localized sodium tricitrate (NaCit) anticoagulation with a recently US-FDA approved in-line vascular glucose sensor (VIA Model 121, VIA Medical, San Diego, CA) during hyper-(11.1), eu-(5.3) and hypo- (2.5mM) glycemic clamps in 16 subjects. We substituted NaCit for heparin at 1.1 mg/dl in the isolyte flush solution that was infused at 60 cc/hr. The external sensor was attached via an 18-20 gauge catheter to an arterialized hand vein. Blood was sampled at 5-10 minute intervals per our usual clamp protocol from the sensor's stop cock blood drawing port and plasma glucose measured on a Beckman autoanalyzer. We compared values for 21 consecutive studies (439 data points) with a glucose range of 2.3 to 16.1 mM. For the group as a whole, the bias (percent error) was 0.9% with a precision (standard error of the mean) of 2.25%, and an R^2 of 0.985. In conclusion, sodium tricitrate anticoagulation is effective in maintaining vascular line patency when frequent blood sampling is required during physiologic studies and this additive does not affect the accuracy of glucose measurement when using the VIA model 121 real time monitor.

1404

INTRAVENOUS INSULIN PUMP AND AUTOMATIC RATE ADAPTATION BY MEANS OF NEURAL NETWORK. PRELIMINARY STUDIES

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Intraveinous (iv) insulin infusion is often required in the management of acute situations in type I and II diabetic patients. The lack of suitable algorithm for dose adjustment in function of capillary blood glucose (CBG) represents a limitation for this treatment, especially in non diabetologic units. We developed a new pump in order to allow i) iv insulin infusion, ii) automatic rate adaptation in function of patients characteristics and discontinuous CBG measurements (10/day). The system is based on neural networks, educated by means of a bank of data (10,000 examples of rate adjustments in function of CBG, and the resulting CBG), obtained by collecting data of 711 type I or II patients (1990 to 1994). A : Retrospective safety and feasibility study, using another bank of data (similar conditions, more than 10,000 examples). Correlation between the system propositions (SP) and the mecical propositions (MP) : $R = 0.979$, $p < 0.001$. B : prospective on-site study : when SP and MP were different, the physician selected the rate to be applied. Correlation between SP ans MP : $R = 0.969$, $p < 0.001$. When SP was applied (78.3 % cases), 70.15 % of the CBG obtained were between 60 and 150 mg/dl, 4.03 % were lower, 25.46 % were upper. When the rate choosen was MP, 81.5 % CBG were between 60 and 150 mg/dl, 4.6 % were lower, 13.9 were upper. The significant correlation was observed as early as the first day of treatment. There was no severe hypoglycemia. A larger study is necessary to validate the system before its use in non diabetologic units.

1405

LONG-TERM CLINICAL APPLICATION OF WEARABLE ARTIFICIAL ENDOCRINE PANCREAS IN DIABETIC PATIENTS

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To achieve the long-term blood glucose regulation and clinical application of the closed-loop system in ambulatory diabetic patients is essential. However, two major problems should be solved for long-term application of wearable artificial endocrine pancreas.

1.Development of stable and reliable subcutaneous glucose sensor: With a ferrocene-mediated glucose sensor covered with highly biocompatible membrane, 2-methacryloyloxyethyl phosphorylcholine-co-n-butyl methacrylate (MPC-co-BMA) membrane, subcutaneous tissue glucose concentrations could be monitored precisely and continuously for up to 7 days without any in vivo calibrations. and for 14 days with one-point in vivo calibrations.

2.Development of subcutaneous insulin infusion algorithm: Considering the management and safety of insulin delivery route when wearable artificial endocrine pancreas is applied to ambulatory diabetic patients for long-term basis, subcutaneous insulin infusion algorithm has been developed by analyzing by analyzing the dynamics of subcutaneously injected short-acting insulin analogue (Insulin Lispro)by three compartment model. By applying subcutaneous insulin infusion algorithm using Insulin Lispro which is absorbed 2 to 3 times faster than usual regular insulin, near-physiological glycemic control could be achieved in diabetic patients without showing any delayed hyperinsulinemia nor hypoglycemia.

In conclusion, wearable artificial endocrine pancreas is now recognized as an excellent therapeutic tool for regulating blood glucose excursions physiologically in ambulatory diabetic patients on long-term basis.

1406

GLUCOMETER®DEX™ - A NEW SMBG SYSTEM

(J.O.Noell, M.K.Musho and K.F.Yip. Bayer Corp., Elkhart, IN, USA)

The Glucometer DEX Diabetes Care System is a convenient and accurate home use device for the self-monitoring of blood glucose (SMBG). The system consists of an instrument, reagent sensor discs and control materials. It is conceptually the same as other Glucometer products currently available for blood glucose testing. The unique aspect of the system is that the test sensors are not packaged individually. Rather tests are packaged ten at a time (individually sealed) in an aluminum formed blister (Test Sensor Disc). The user never handles the actual test sensor. The DEX test sensor is comprised essentially of electrodes capable of making an amperometric measurement and a reagent deposition of the enzyme, glucose oxidase, and the electron transfer agent, potassium ferricyanide. The DEX test sensor uses a capillary for sampling. The volume of the capillary is 3-4 µl. Testing time for each sample is 30 seconds. The DEX test sensor has a linear response to glucose from 10 - 600 mg/dL. The DEX system exhibits good precision across the whole response range (less than 3mg/dL as one sd below 50mg/dL and 3-5% CV above 50mg/dL) when tested either with whole blood samples or controls. The test results from the DEX system correlate very well with the SMBG devices currently available on the market (with correlation slope between 0.957 and 1.02, correlation intercept between -3.3 and 6.9, and standard error of estimation between 8.8 and 16.2 mg/dL). The system exhibits minimal bias from hematocrit effect, oxygen, temperature and common interference in whole blood.

1407

HUMORAL IMMUNE RESPONSE TO POLYMERIC GLUCOSE SENSOR MEMBRANES AS PARAMETER OF BIOCOMPATIBILITY TESTING

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There are numerous reports on implanted glucose biosensors showing that the loss of sensitivity to glucose after implantation is one of the major problems. One of the possible reasons for this might be the bioincompatibility of materials used for sensor preparation, resulting in thrombogenic, inflammatory and immunological responses to the implant. In a preliminary study after implantation of glucose sensors, we have detected IgG-antibodies against the outer sensor membrane consisting of cellulose acetate suggesting a specific immune response against polymeric materials. Therefore this study was aimed to compare the immunogenicity of different polymeric membranes (polyimide, pellethane, polycarbonate, PTFE) potentially suitable for covering biosensors. Sterilized pieces of 10x10 mm were subcutaneously implanted into female LEW.1A rats on days 1, 21 and 42. The time course of antibody formation was analyzed on days 1, 10, 21, 28, 35, 42 and 49. IgG-antibodies were detected by means of a specially developed enzyme immunoassay technique using the respective membrane immobilized at the bottom of a 96-well MINIFOLD I. The cut-off for antibody positivity was calculated from mean O.D.+2SD of control animals for each antigenic target. On day 49 antibodies to polymeric membranes were detectable by 40% (4/10) with the PTFE membranes, 36% (5/14) with pellethane, 20% (3/15) with polycarbonate, and 13% (2/15) with polyimide. Except polyimide the antibody prevalence against all materials could be significantly enhanced by one intraperitoneal application of complete Freund's adjuvant on day 1 (100% (4/4) with PTFE, 60% (3/5) with pellethane and 80% (4/5) with polycarbonate). All membranes tested in cell culture showed no cytotoxic effect. The results demonstrate a significant but individually varying IgG-antibody formation against different biomaterials. Polyimide has shown the lowest immunogenicity and therefore it might be highly biocompatible. We conclude that immunogenicity should be generally involved in biocompatibility testing for selection of sensor membranes to improve the functional stability of biosensors implanted.

1408

ACCEPTANCE AND EFFECT OF A COMPUTER-BASED BLOOD GLUCOSE MONITORING DEVICE IN ADOLESCENTS WITH TYPE I DIABETES.

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Adolescents with insulin-dependent diabetes mellitus (IDDM) often have problems with therapy and metabolic control. Intensive support may be useful for this age group. **Objectives:** To investigate the acceptance of a computer-based blood glucose monitoring device (Accutrend DM, Boehringer Mannheim, Germany) and to evaluate the influence of using this device on understanding of disease, metabolic control, and therapy. **Methods:** A prospective randomized study was conducted in patients (pts) with IDDM aged 12-18 years over a period of 10 months. 57 of 162 pts were interested in participating. They were randomly divided into an interventional group of 29 pts receiving blood glucose monitoring systems and into a control group of 28 pts with unchanged self-monitoring. Two pts of each group were lost to follow-up. Thus, 27 pts of the intervention group (12m, 15f, age 15.3±1.4 yrs, diabetes 5.2±4.1 yrs) and 26 pts of the control group (12m, 14f, age 15.3±1.9 yrs, diabetes 4.8±3.6 yrs) were evaluable. At begin and end of study, expectations and perceptions of pts and parents were documented. HbA_{1c}-level, frequencies of metabolic control and insulin injection, and handling and judgement of the monitoring device were registered every 3 months at regular visits. **Results:** 67% of the pts rated the handling of the blood glucose monitoring device as good, 12% had problems. 32% used the internal statistic program, and 71% reported an improved understanding of their diabetes treatment. 67% were motivated to increase the metabolic control. 65% of the intervention group and 60% of the control group performed three or more blood glucose tests per day (n.s.). Numbers of insulin injections were increased in 10 of 20 pts of the intervention group with initially less than 4 daily injections vs. 4 of 19 pts of the control group (p=.06). HbA_{1c}-levels were not significantly different between both groups at any time, but improved during the course of study in both groups. Results were not influenced by sex, age or school education. **Conclusions:** In the majority of adolescents, acceptance of the blood glucose monitoring device was positive. Within 10 months, there were either no or only small differences in frequencies of metabolic control, insulin therapy and HbA_{1c} between pts with and without monitoring device.

1409

NO DIFFERENCE IN PAIN PERCEPTION WHEN TWO TYPES OF LANCETS FOR BLOOD GLUCOSE TESTING WERE USED

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Aiming for a better glycemic control inevitably requires frequent blood-glucose testing. Previous lancets are of 23G size but a 28G lancet has recently been introduced. The aim of this study was to evaluate if a thinner lancet would reduce the finger-pricking pain. At a diabetes summer camp we performed a double-blind, crossover study with 29 IDDM children aged 9-13 years. The lancets were used only once. Pain was registered on a 10 cm VAS scale with faces and endpoints marked "No pain" and "Much pain". The mean VAS pain score was for the 23G lancets (Ames Surelite, 0.65 mm diameter, 3 mm length) 1.5 ± 1.1 cm and for the 28G lancets (Becton-Dickinson Microfine +, 0.36 mm diameter, 4 mm length) 1.6 ± 1.1 cm (n.s. t-test). The residual pain (pain when pressing the finger the next day) was for the 23G lancets 0.6 ± 0.8 cm and for the 28G lancet 0.3 ± 0.4 (n.s. t-test). 11.0% of the 23G needles needed repricking to obtain enough blood with the Glucometer GX meter (needs 30 - 40 μl of blood) compared to 2.8% with the 28G needle (p=0.055 Chi^2). 5.6% of the 23G needles needed repricking to obtain enough blood with the Glucometer Elite meter (needs 3 μl of blood) compared to 6.8% with the 28G needle (n.s. Chi^2). 27.9% of the prickings with 23G lancets needed extra wiping of blood after the test compared to 40.0% of the 28G needle (p=0.036 Chi^2). There was no difference in which lancet the children preferred using. In conclusion, we did not find any difference in pain perception or patient preference when two lancets with considerable difference in size were tested. The 28G lancet is thinner but also longer which might cancel each other out in pain perception. The longer lancet needed more wiping but also slightly less frequent repricking when used with a meter needing more blood for measuring. It seems as if the length of the lancet is more important than the diameter for how much blood is obtained when pricking. In this camp setting, where the group pressure of accepting blood glucose testing is larger than at home, the children were indifferent to the types of lancets tested.

1410

Rapid Fructosamine Test for Use in Diabetes Clinics. G. Neyer, C. Carter, S. Miller and V. Noetzel. LXN Corporation, San Diego, California, USA.

Diabetic patients achieve better glycemic control when health professionals are aware of their glycated protein levels. Now, an instrument for the determination of glycated protein (fructosamine) has been developed (LXN Corporation) which allows patients or health care professionals to use a simple procedure to obtain rapid test results from a fingerstick drop of blood. The potential of various interfering substances to affect system accuracy has been studied. System accuracy was unaffected by hematocrit values within normal physiological ranges (between 20% and 65% hematocrit). Also, no significant effect on accuracy was observed when ascorbic acid (to 1.5 mg/dL), bilirubin (to 8.0 mg/dL), hemoglobin (to 200 mg/dL), glucose (to 1200 mg/dL), uric acid (to 17 mg/dL), triglycerides (to 800 mg/dL) and glycerol (to 88 mg/dL) were tested within and above physiological ranges. These results show that the LXN Fructosamine Test is a viable alternative to laboratory-based testing.

1411

GASTRIC BANDING AND TYPE 2 DIABETES
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22 patients (7 diabetics, 15 non diabetics) indicated for gastric banding were followed for 3 years. 7 patients had less than 10 kg weight loss, 15 patients successfull weight loss, some with weight regain later - 7 patients. Anova with repeated measures found no difference between DM and nonDM in BMI: Diabetics 43.8...41.0...39.6...40.1 kg/m^2, non diabetics 45.1...41.3 ...39.6...39.0 kg/m^2. In Fisher test the only variable which had borderline significance for ability to lose weight was the family history of type 2 diabetes. Stepwise regression analysis for BMI changes was performed for every interval of weight change. The only significant result was (BMI change during whole 3 years)= -7.88 + 2.39 (BMI change in 6 months). All other initial variables: BMI, gender, history of weight oscilations, insulin, blood glucose, T3, T4, triglycerides failed to enter the models.
Conclusion: 1. Difference in weight change cannot be explained by diabetes or basic metabolic and hormonal characteristic of patients. 2. The only type of patients with greater benefit of the operation are non-diabetics with family history of DM. 3. The early weight loss can be used to predict the final result.

1412

TELEMEDICINE IN THE CARE OF IDDM PATIENTS
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Self blood glucose monitoring is important for good control in IDDM patients. The use of telematic information transmission could save time and expenses related to outpatient visits. We examined the usefulness of this method in 10 IDDM patients (age 36 yrs, rage 17–50 yrs, duration of diabetes 12 yrs, range 1–23 yrs, mean insulin dose 31 U/d, range 10–54 U/d). They all had previous experience in personal computers. A software was developed to store, transfer and print out self blood glucose monitoring data in different forms (numbers, figures). A two–way transfer of messages was possible as well. After initial education, the patients had a 3 month run–in period followed by a 12 months study. Data on home blood glucose monitoring, symptoms and large glucose excursions were transferred, and HbA_{1c} was determined at outpatient visits at 3–5 month intervals. The number of home glucose determinations varied from 206 to 1902 and telematic transmissions from 2 to 26 per patient/year. No significant changes were observed in HbA_{1c} level, total insulin dose or major fluctuations in glycemia as compared to a 6 month period before the study. No patients had any emergy visits to hospital due to hypoglycemia or ketoacidosis. 9/10 patients were pleased with the system and willing to continue it after the trial. In conclusion: 1) Telematic data transmission can be utilized in the management of IDDM patients familiar with computer technology. 2) The two–way use of this technology may enhance safety, help in every–day care and save time and expenses of outpatient visits.

1413

ACCEPTABILITY OF A 'PATCH LIKE' DEVICE FOR CONTINUOUS BASAL INSULIN SUPPLY.
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Due to their relatively short time action profiles and large variations in absorption rates, currently available basal insulin preparations are not ideal. Subcutaneous insulin infusion pumps may provide good basal insulinisation but are technically sophisticated and not suitable for many patients. The objective of this study examined patient acceptability of wearing a 'patch like' device for continuous delivery of basal insulin. 38 IDDM and 7 NIDDM insulin using patients (20 female, 25 male, mean age 38 yrs) from 2 centres tested 4 prototypes (2 different shapes and 2 different adhesives). All prototypes were non functional and did not have a needle attached. Each patient tested each prototype for a period of one week in a randomised order. Patients collected information throughout the study period in a diary and completed a series of six questions using a visual analogue scale (VAS 1-10) at the end of each week to assess acceptability. Patients continued to take their usual insulin regimen throughout the study period (pen device n=38, syringe n=7). 14 patients (31%) reported problems with fixation of the prototypes (28 reports) and 26 patients (58%) reported a prototype becoming detached (44 reports). Detachment was most commonly associated with a bath, undressing or occurred overnight. 20 patients (48%) reported minor skin reactions, including pruritis. There was no detectable device or adhesive effect on acceptability but there was an observable period effect with patients more likely to accept the prototype tested in week two. Overall, 58% of patients said that they would consider changing their current regimen to use a 'patch like' device (VAS score >5). In conclusion, there remain technical difficulties with the use of this device but if these can be overcome they may offer a feasible alternative for basal insulin delivery.

1414

ACCURACY OF HOME BLOOD GLUCOSE METERS WITH SPECIAL RESPECT TO DIFFERENT GLYCAEMIC RANGES

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AIM: Aim of this study was to investigate the accuracy of 6 home blood glucose meters with special respect to different glycaemic ranges (low range <3,89 mmol/l, acceptable range 3,89-9,99 mmol/l, high range >9,99 mmol/l.

METHODS: In total 1146 blood glucose monitor readings [Reflolux S (Boehringer Mannheim), One Touch II (LifeScan), Glucocard Memory (Menarini), Precision (Medisense), HemoCue (HemoCue) and Accutrend α (Boehringer Mannheim)] and 191 reference values (Beckmann Glucose Analyzer 2) were analysed. Arterialised blood samples were obtained from 5 subjects under controlled conditions (glucose clamp technique). The correlation coefficients for each glucose meter in total and according to the different glycaemic ranges and the percentage of measurements outside 20% were as follows:

RESULTS:

	<3,89 r	3,89-9,99 r	>9,99 r	total r	total > 20%
Reflolux S	0,60	0,88	0,62	0,96	32%
OneTouch II	0,73	0,96	0,90	0,99	7%
Glucocard M.	0,78	0,97	0,86	0,99	13%
Precision	0,61	0,94	0,79	0.98	30%
HemoCue	0,67	0,94	0,41	0,97	8%
Accutrend α	0,70	0,96	0,81	0,98	12%

CONCLUSION: Accuracy of blood glucose meters shows substantial differences according to defined glycaemic ranges. Therefore we suggest separation into different glycaemic ranges, whenever accuracy of home blood glucose meters is evaluated.

1415

On-Line Continuous i.v. Glucose Monitoring in Diabetic Patients during Hemodialysis

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Introduction: Diabetes mellitus is one of the major causes of end stage renal failure. The practice of continuous i.v. glucose monitoring during hemodialysis may show the actual metabolic state of the patient and may help correct possible hypo/hyperglycemias. Aim: To evaluate the feasibility and profits of the on-line continuously monitoring of blood glucose during hemodialysis in NIDDM using the i.v. Glucosensor Unitec Ulm. Methods: A catheter for continuous blood extraction(v=20-40µl/min) was inserted into the tubes of the artificial kidney, located immediately after the inlet of the heparin supply pump. Blood was diluted 1:5 with a heparin saline and pumped into a flow chamber, where a glucose oxidase membrane was attached to a Ag/Pt electrode polarised at 700mV. The extracted blood glucose was oxidised to H_2O_2. The latter releases e^- to the electrodes proportional to the blood glucose concentration and are measured as electric current. In this fashion blood glucose was continuously monitored in 6 NIDDM patients(2 women and 4 men) during hemodialysis. Results: Mean blood glucose was 197±83mg/dl. In 2 patients blood glucose was corrected with i.v. insulin injection during hemodialysis The sensor signal correlated very well with the reference glucose values. The major problem was the interruption of the continuous blood extraction due to blood clotting in the tubing system of the glucosensor (n=3). However, this problem could be avoided in further experiments by perfusing 2000U/ heparin in the artificial kidney before starting the monitoring. Conclusions: On-line continuous blood glucose monitoring is feasible during hemodialysis and allows a better metabolic control of diabetic patients.

1416

INFLUENCE OF HYPERBARIC OXYGENATION ON THE LEVEL OF COUNTERINSULIN HORMONS IN IDDM.

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AIM: To eualuate the possible hormonal changes in newly diagnosed IDDM after GBO therapy which was used as adjunctive to intensified insulinotherapy to improve tissue oxygenation and preserve residual insulin secretion. METHODS :Blood level of IRI ,C-peptide ,cortisol, GH ,TSH, T3, T4 were estimated by immunoassay. Urine excretion of Ad and Nad was detected by flurometry and 17- OCS by Porter-Silber method. 53 patients were treated by HBO (10 cessions duration 40 min at 2 ATA):mean age 24.6+67, disease duration mean 5.61+4.1.20 patients were matched control.RESULTS. GBO enable to compensate IDDM in short period .Just after GBO the level of C-peptid was increased from0.53+0.09 to 0.91+0.09 ng/ml. Increased Adr excretion before GBO was in 82 % of patients(109.0 +13.2nmol/l)and significantly lowered after GBO (to 84.5+10.2nmol/l). Nad excretion remained unchanged.Therefore Nad/Ad ratio became normal. Increased cortisol level preserved after GBO, but 17 -OCS excretion was in normal range, probably as a result of disturbed cortisol metabolism.GH level was increased only in group of patients with retinopathy and decrease to normal after GBO. The level of other hormons did not change significantly .Data revealed may prove the efficasy of including the GBO in the complex IDDM therapy.

1417

HABITUAL INSERTION VELOCITY OF INSULIN NEEDLES IN DIABETIC PATIENTS

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The movement characteristics during needle insertion of diabetics have not previously been described. This information is needed for optimal design of automatic needle insertion devices and to perform experimental and clinical studies investigating insertion of insulin needles - mainly related to discomfort and pain. Different movement phases were investigated in 28 patients with IDDM (12 female, 16 male, mean age 40.9±10.8 years, mean duration of IDDM 18.2±10.2 years) during 10 habitually performed insertions in each patient with G30 (0.3 mm outer diameter) 8 mm insulin needles. A motion analysis system with infra-red cameras (MacReflex System, Qualisys, Sweden) was used to describe the insertion movement. The overall mean velocity of needle insertion was 18.6 mm/s (SD 19.9), overall range: 1.0-133.3, range of individual means 2.1-43.6. The mean velocity of needle insertion in the thigh was 20.6 mm/s (21.9) and in the abdominal region 16.1 mm/s (17.0). The mean velocity of needle insertion performed with a 45 degree angle of insertion was 15.5 mm/s (16.4) and with a 90 degree angle 23.2 mm/s (23.6). Statistical analysis of variance showed significant difference between insertion with 45 and 90 degree angle ($p<0.05$). No significant difference between the velocity of needle insertion in the thigh and in the abdominal region was found. There was no correlation between the velocity of insertion and the duration of IDDM.

In conclusion these data suggest a considerable degree of individual variability of insulin needle insertion velocities. The velocity of insertion was related to the angle but not to the anatomical region of insertion.

1418

INCONSTANT RELIABILITY OF BLOOD GLUCOSE SENSORS WITH ALTITUDE.

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It is clearly established that IDDM- patients can practice even extreme sports like mountaineering. Still, reliability of blood glucose sensors with altitude has not been assessed. In this view, we have climbed Kilimandjaro (5895 meters) with 4 IDDM - patients and controlled our capillary blood glucose with different glucose sensors (One Touch Basic, One Touch Profile, Glucometer 4, Exatech Sensor) from 1500 meters to the top. We were the healthy references and visual measurement of capillary blood glucose was done each time with BM test 20800 strips. Our capillary blood glucose values during fast at 1500 meters are from 061 to 1.05 g/l according to visual measurement and much lower from 0.39 to .44 g/l at the top (visual measurement : 0.8 g/l). Diabetic patients have glycemics values with sensors near the one found with visual measurement for near normal values. Dispersion of the values when hyperglycemia occurs is much more important : one subject at the thop has 2.5 g/l with visual strip, 1.92 g/l with Glucometer 4 and 4.3 g/l with One Touch Profile ! This variability seems to be more important with height. We have not found one sensor better than the others. In conclusion, reliability of blood glucose sensors with altitude is bad and visual strips should be used during mountaineering.

1419

DEPENDENCE OF THE DELAY BETWEEN BLOOD GLUCOSE AND SUBCUTANEOUS GLUCOSE DYNAMICS ON INSULIN.

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Subcutaneous (sc) glucose sensors have the potential to greatly improve glucose monitoring by diabetic patients. However, a sensor signal reflects sc interstitial fluid not blood glucose. The extent to which sc glucose differs from the plasma is not fully known. In the present study, sc amperometric enzymatic sensors were used to monitor glucose (MiniMed; in vitro sensitivity 0.2-0.5 nA/(mg/dl), T½<30 sec). The dynamics of the in vivo sensor current were studied during hyperglycemic clamps performed in 4 normal dogs. Each dog had up to 4 sensors inserted in the neck area 3hrs prior to the clamp. During the clamp blood glucose was increased from basal to ~180 mg/dl for two hours and then allowed to return. Insulin concentration increased ~10 fold in response to glucose infusion. In a separate set of 4 experiments somatostatin was infused to suppress insulin secretion. The in vivo delay in sensor current was identified by a differential equation (dI/dt= -p2xI + p3xGlu) from which the T½=1/p2 and the sensitivity=p3/p2 were estimated by least-squares fits of the data separately for rise and fall in glucose (mean±SEM). T½ was slower during the rise than the fall (4.52±1.22 vs 0.97±0.2 min, n=14; p=0.026). With somatostatin T½ was equal to 4.71±0.8 (n=14) for the rise of glucose. The in vivo sensitivity of the sensors (0.35±0.02, n=28) was similar to in vitro values. In conclusion, the time delay T½ of ISF glucose is typically only ~5min and decreases substantially with increasing insulin (<1min). Thus, interstitial glucose kinetics obtained with sc sensors should be appropriate for glucose control and hypoglycemic warning in patients. Advantages in correcting for the time delay remain to be studied.

1420

SAFETY AND ACCURACY OF BLOOD GLUCOSE MEASUREMENT IN HEIGHTS ABOVE 3 000 METER AND TEMPERATURES BELOW 0 ° C ON A HIKING TRIP WITH INSULIN - DEPENDENT DIABETICS

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The aim of this study was to evaluate the accuracy of self testing of blood glucose on a hiking trip to the Vincent Pyramid (4215 m) and establish practical guidelines for insulin - dependent diabetics to hike safely. Six diabetic hikers , age between 26 to 45 years with a diabetes duration between 5 - 41 years and six age, sex and hiking experienced matched control persons took part in this expedition. Before and after every hike blood glucose, lactate, blood pressure, pulse rate and fluid intake was measured. On the hike the diabetics and non - diabetic control persons measured blood glucose every two hours with four blood glucose meters and collected 20 µl blood in an end to end capillary in a prepitted hemolysate sample. Below 4 000 m the average difference between the four blood glucose meters (Basic, Glucotouch, One Touch II and Profil) and the laboratory results was 13.5%. We found differences regarding the testing skills of the participants and divided all hikers in two groups regarding experience and accuracy in the use of the four Lifescan meters. The self - monitored blood glucose results differed in the experienced group by 11± % from the laboratory results and by 16 ±% in the less trained group in heights from 1 950 m to 3 650 m and temperatures up to - 15 ° C. Above 4000 m the average difference of the four blood glucose meters to the laboratory control was 46 ±%. There was no difference between the diabetic and the non - diabetic hikers regarding lactate, adaption to the height, blood pressure, fluid intake or pulse results. All diabetics reduced their short - and long - acting insulin between 20 - 50 % and increased their carbohydrate intake up to 300 %. During the whole trip no severe problems occured regarding the diabetes treatment. The meters tested in our study proofed to be reliable under those extreme conditions below 4 000 meters, but the users of the blood glucose meter have to undergo an intensive training and education programme to learn how to use their meter properly.

1421

Development of non-invasive determination of blood sugar level using the FT-IR

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To prevent the development of complication in the diabetic patients, a strict control of blood glucose level is the essential. For that purpose, measurement of blood glucose level with frequent blood sampling is necessary, which gives pains to the patients. We have developed a non-invasive determination system of blood glucose level using the Fourrier-transform infrared spectra.

[Method]Infra red spectra was measured at diamond 20(Nihon Denshi,Tokyo) using the multi-reflection method by prism. Since C-O-C structure of glucose had a characteristic absorbance at wave number 1039cm-1, glucose concentration could be determined quantitatively by applying the peak intensity. To determine the glucose level the fifth finger is adhere to the prism surface. At the same time the blood glucose level was measured by the glucose oxidase method to compare the values obtained by our method.

[Result]In in vitro examination, the absorbance of infrared rays increased depending on the glucose levels and it was indicated that the determination was possible for glucose concentration of 3.5 mM to 35mM. In the first in vivo study using the skin of finger, when the blood volume of the finger was constant, the determined values of glucose coincided with those of glucose levels measured by the glucose oxidase method, but the determined values varied when the finger was cooled or when the blood stream was blocked. In the second study we developed a non-invasive determing method of blood glucose level by obtaining the blood volume in finger using the amount of hemoglobin by the near infrared rays and correcting the determined values with the amount of hemoglobin.

1422

CLINICAL EVALUATION OF THE GLUCOMETER® DEX™ BLOOD GLUCOSE MONITORING SYSTEM

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This study evaluated a new blood glucose monitoring system in young adults with Type I diabetes and health care professionals (HCP). The meter sensor uses a glucose oxidase / electrochemical method. Five males and 15 females, 15-35 years old, participated in the study. Capillary blood results obtained with meters referenced to plasma/serum glucose values (PS) were compared to results of the Hitachi 747, a hexokinase chemistry analyzer (glucoses: 2.6-32.1 mmol/L). Whole blood referenced meter results (WB) were compared to results of the YSI 2300 Stat Plus, a glucose oxidase / electrode method (glucoses: 2.3-26.9 mmol/L). The hematocrit range was 36-50%. Linear regression results were:

Operators	Meter	N	Slope	Intercept	Std. Error	R
20 Patients	PS	105	0.94	0.50	1.01	0.98
2 HCP	PS	105	0.95	0.62	1.03	0.98
20 Patients	WB	109	0.99	0.38	0.85	0.99
2 HCP	WB	109	1.00	0.46	0.84	0.99

The pooled coefficients of variation for the paired patient and HCP meter results were 6.5% and 6.4% for the PS and WB systems, respectively. Features enhancing the clinical utility for self-monitoring included: automatic calibration, ten-test disc put in the meter resulting in less individual strip handling, only 3-4 µL of blood required, and results in 30 seconds. The meter system was found to have acceptable performance and clinical utility for self-monitoring of blood glucose.

1423

EFFECTS OF LASER THERAPY ON THE IMMUNE STATUS OF PATIENTS WITH DIABETES MELLITUS

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The goal of our study was to research effects of low energy heli-neon laser therapy (LEHNLT) on the immunological status (IS) of 78 patients with insulin-dependent diabetes mellitus (IDDM) and 26 patients with non-insulin dependent diabetes mellitus (NIDDM) (the age range: 14 - 64 years; duration of the disease: from 1 month to 19 years). The control group included 30 healthy individuals of the same age range and the comparative group consisting of 100 patients with diabetes mellitus (DM) who had undergone routine treatments. LEHNLT was performed cutaneously on the pancreas for 6 minutes and IV for 30 minutes. The treatment included 10-15 procedures. The amount of T-and B-lymphocytes, T-subpopulation and immunoregulation index, levels of IgM, IgA, IgG, the immune complex (IC), functional activity of lymphocytes (spontanious B-lymphocyte activation and proliferative activity in response to T-and B-mitogens) estimated by luminoscent microscopy were used to evaluate the immunological effects of LEHNLT. Before the treatment essential changes in the IS had been revealed only in the IDDM patients. Our findings show that those who had suffered from IDDM for five years exhibited high autoimmunity, and changes in other organs were revealed in those who had had IDDM for over 5 years. Minimal immunological changes in the patients with NIDDM resulted from metabolic disturbances at the background of decompensation of DM or overdosage of insulin. Routine treatments for DM without immunocorrecting drugs did not improve their IS. The LEHNLT improved significantly the IS in over 70% of the IDDM patients ($p<0,05$). The best immunocorrecting effect was revealed in the patients with a short-term IDDM history (up to 5 years), it was stable for 3 months, correlated with a high level clinical-metabolic remission of IDDM, and then it decreased to the initial level within 3-6 months. To sum up, LEHNLT is effective in our complex therapy for DM to correct the IS in IDDM patients.

1424

PERFORMANCE EVALUATION OF A NEW GLUCOMETER ENCORE® QA+ WORKSTATION IN NEONATAL, LABORATORY AND WARD SITES

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A Glucometer Encore® QA+ Workstation, with optical system and calibration algorithm enhancements developed to deal with a wide variety of hematocrit and glucose combinations, was evaluated in the hands of intended clinical users. Encore® reagent strips were used to test heelstick capillary bloods (4 neonatal nurses, 4 meters), fingerstick capillary bloods (2 ward nurses, 2 meters) and venous bloods (2 analysts, 2 meters) for glucose in comparison to the laboratory method at each site. Results were reported as mmol/l plasma glucose. Coefficients of variation with controls ranged from 5.3 to 9.6% (low), 4.3 to 5.4% (normal) and 3.4 to 5.5% (high). Hematocrits ranged from 25 to 68%, 26 to 49% and 24 to 60% at neonatal, ward and laboratory sites, respectively. No hematocrit effects on meter glucoses were noted with neonatal bloods. Encore® QA+ glucoses compared to laboratory plasma glucoses as follows:

	n	Regression	Syx	r	Bias	±15%	±20%
Neonatal wards	97	y=0.96x+0.03	0.36	0.951	-0.13 [c]	96.9%	99.0%
Diabetes ward	99	y=0.96x -0.23	0.83	0.986	-6.4%	93.0%	98.0%
Laboratory (lot 1)	70	y=0.96x+0.05	0.82	0.994	-1.7%	94.0%	100%
Laboratory (lot 2)	70	y=1.01x-0.006	0.75	0.995	1.6%	99.0%	100%

Plasma glucoses ranged from 0.7 to 8.0 mmol/l, 2.2 to 24.6 mmol/l and 0 to 26.3 mmol/l at neonatal, ward and laboratory sites, respectively. With Encore QA+ neonatal specimen glucoses ≤ 2.8 mmol/l, all were within 12% of the laboratory plasma glucose results. The new Glucometer Encore QA+ Workstation system performed well in these studies with neonatal heelstick bloods, adult capillary fingerstick bloods, and venous bloods from the laboratory site in comparison to the laboratory plasma glucose methods at the three sites.

1425

HELI-NEON LASER THERAPY WITH ROUTINE DRUG TREATMENTS IN PATIENTS WITH DIABETES MELLITUS

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The main aim of our study was to investigate pathogenetic efficacy of low energy heli-neon laser therapy (LEHNLT) in combination with routine drug treatments in patients with diabetes mellitus (DM). Influence of LEHNLT was researched on the basis of clinical, laboratory and immunological findings in 104 patients with DM (78 insulin-dependent and 26 non-insulin dependent DM; their age spectrum: 14-64 years; the duration of DM: 1 month - 19 years). The control group consisted of 30 healthy individuals (the age range: 17-50 years), and the comparative group included 100 DM patients who had undergone routine medication therapy (diet, intensive insulin therapy or tablet-form hypoglycemic medications or their insulin and tablet combinations). Cutaneous and intravenous laser treatments were performed. The course of treatment consisted of 10-15 procedures. It was revealed and properly estimated that LEHNLT in patients with DM is effective and can be characterized by clinical remission accompanied by decrease of average daily glycemia and total compensation in 79% of the insulin-dependent and 81% of the non-insulin dependent patients. In addition to the total compensation, decrease of daily insulin doses was 45,3% ($p<0,001$) in the patients with insulin-dependent DM, and decrease of requirements for tablet-form hypoglycemic drugs was 58% ($p<0,001$) in the patients with non-insuline dependent DM. The laser treatment resulted in increase of insulin secretion activity of the pancreas ($p<0,005$), reliable hypolipedimic effect ($p<0,05$) and reliable immunocorrecting effect in 80% of the patients with insulin-dependent DM, mostly with 5-year duration of DM. The best effect was revealed in the patients with the 2-3 year duration of insulin-dependent DM. The above-mentioned positive effects remained for 6 months. To conclude, we consider the use of LEHNLT to be a necessary component of our complex treatment of patients with DM.

1426

CALIBRATION OF HbA_{1c} DETERMINATION
BY HbA_{1c} VALUES OF NON-DIABETIC SUBJECTS

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HbA_{1c} is widely accepted as a valuable indicator of long-term blood glucose control. At present, however, inter-laboratory comparison of HbA_{1c} values is not possible and calibration is extensively investigated. Here we propose the use of HbA_{1c} values found in non-diabetic subjects as a simple mean for calibration. Oral glucose tolerance tests (OGTT) and HbA_{1c} determination in duplicate have been performed in ten Diabetes Clinics of the Marche region of Italy. Only non diabetic subjects (with normal OGTT response) have been considered. For comparison, HbA_{1c} has been assessed in a lyophilized material at low and high concentration, and re-determined again 1-2 months apart. Intra-assay, inter-assay and inter-laboratory coefficients of variation (CV) have been evaluated. HbA_{1c} has been determined by High Pressure Liquid Chromatography (HPLC). 208 non-diabetic subjects have been examined and mean (±SEM) HbA_{1c} value was 5.10±0.07% (range 3.4-6.6%). Mean intra-assay CV (duplicate samples in the same laboratory) was 2.18%, 2.13% and 1.57% for non-diabetics and for the lyophilized at low and high HbA_{1c} concentration. Inter-assay CV in each laboratory was respectively 1.98%, 2.07%, and 1.83%. Inter-laboratory CV was higher (13.04% in non diabetics; 17.41% and 13.12% in the lyophilized at low and high concentration). Calibration reduced the mean inter-laboratory CV to 5.83% wen based on the values of the lyophilized material, and to 5.77% wen based on the values of the non diabetic subjects. In conclusion, calibration of the HbA_{1c} determination greatly reduces the inter-laboratory CV and makes comparable the results obtained in different laboratories. Similar results can be obtained with HbA_{1c} values of both non-diabetic subjects or a lyophilized material, but the latter is at present not available in a stable and ubiquitous form.

1427

A HEART RATE VARIABILITY MEASUREMENT SETUP

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A purpose of this case work is to demonstrate an application of a time-voltage converter as a heart rate variablity (HRV) monitoring device. In HRV analysis technique this device detects burst-type signal and continuous activity and represents the results as rate vs. time directly. HRV fluctuates over time with a personal manner depending the balance between the sympathetic and parasumpathetic nervous control. In diabetes this fluctuation is changed because of e.g. neuropathy. However, the primary cause of the phenomenon is not known. HRV could be cured by a surgery operation if it is of anatomical origin. The heart rate measurement was realised in a convertional way. The instantaneous HRV was measured with a means of a time-voltage converter (Tektronix TVC501 TIME-VOLTAGE CONVERTER). Three volunteer subjects were measured and their HRV was analyzed based on the time-voltage converter measurement. In the healthy subjects heart rate was 0.86 and in the diabetec 2.05 beats/s. In the healthy subjects HRV was steady and in the diabetec random in supine position. The HRV clearly shows that the respirator rhythm causes finger prints which can be left also in the diabetec HRV power spectrum based on time-voltage conversion. The peaks in the HRV power spectrum move to high frequency in diabetes.

1428

INVESTIGATION OF COMMON INTERFERENCES ON HbA_{1c} USING THE A. MENARINI DIAGNOSTICS - KDK HA-8140 ANALYSER.

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The measurement of glycated haemoglobin has become of central importance in the evaluation of glycaemic control. Since the introduction of the clinically useful methods for glycated haemoglobin measurement, techniques have been refined to improve their reproducibility and more recently their level of automation. Although methods have improved significantly over recent years, the presence of interfering substances remain a major problem with associated with some techniques. A significant problem is associated with haemoglobinopathies and with increased levels of fetal haemoglobin; additionally the labile Schiff base remains a problem. A number of major haemoglobin variants were studied using the HA-8140 (A. Menarini Diagnostics - KDK), including HbS, HbC, HbE and thalassaemia. The range of HbA_{1c} results in normal individuals did not differ significantly from that found in non-diabetics with haemoglobinopathy. True peak separation was not always achieved in patients who have HbE present, although in all cases this abnormality could easily be recognised as a shoulder on the HbA_0 peak. Schiff base interference was investigated by incubating (for a limited time period) red cells in phosphate buffered saline containing a high concentration of glucose. Haemoglobin A_{1c} results obtained confirmed that this interfering compound was removed by the analyser; Schiff base is removed by incubation the sample in tetrapolyphosphate buffer, this is performed automatically on the HA-8140 analyser. The effect of fetal haemoglobin was investigated by spiking cord blood into samples collected from a number non-diabetic and diabetic subjects, this procedure resulted in an increase in HbF to 19%. Increased fetal haemoglobin had no significant affect on the measured HbA_{1c}. The results of this investigation show that the HA-8140 analyser is not affected by interfering compounds that adversely affect other systems.

1429

COMBINATION THERAPIES WITH INSULIN LISPRO IN NIDDM PATIENTS AT ORAL AGENT FAILURE.

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Insulin lispro (LP), an analog of human insulin, shows pharmacokinetics that may be advantageous in the treatment of type II diabetic patients with oral agent failure. A multinational 3 way parallel study was designed to compare the efficacy of preprandial LP when combined with once daily NPH Insulin (LP/NPH) or sulfonylurea (LP/SH), with sulfonylurea plus NPH-Insulin (SH/NPH). 57 patients in Germany were randomised to one of the three treatments (31 females and 26 males, age 58±8.1 (mean±sd) years, duration of diabetes 11±7.4 years, body-weight 81±18.5kg, HbA1c 9.8±1.6%). After 8 weeks, efficacy of the treatments was evaluated by HbA1c.

	LP/NPH	LP/SH	SH/NPH
HbA1c (%)	8.1±1.3	7.7±1.2	7.9±1.6

If at this time metabolic control did not meet strict criteria (fasting blood glucose < 7.8 mmol/l, blood glucose 2 h postprandially > 10 mmol/l) treatment was intensified by addition of NPH insulin and withdrawal of sulfonylurea. Ten patients with LP/NPH were changed to preprandial LP and NPH twice daily (LP/NPH bid); 11 patients with LP/SH and 10 patients with SH/NPH were changed to LP/NPH. After 8 weeks HbA1c was reassessed.

Treatment change	N	HbA1c (%) before change	HbA1c (%) after change
LP/NPH to LP/NPH bid	10	8.6 ± 1.8	8.0 ± 1.5
LP/SH or SH/NPH to LP/NPH	21	8.2 ± 1.2	7.9 ± 1.1

In all treatment periods prandial blood glucose excursions were lowest when LP was administered before meals.
The data indicate that LP is suitable for patients with oral agent failure, and that use of LP is associated with better prandial blood glucose excursions.

PS 35
Hypertension/Therapy

1430

Plasma ouabain concentration in Chinese NIDDM patients

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Diabetes related hypertension is associated with increased exchangeable body sodium and abnormal transmembrane sodium transport. Inhibition of the activity of the Na^+,K^+,ATPase by endogenous sodium transport inhibitor (ESTI) may lead to increased intracellular sodium concentration. The latter in turn can lead to increased intracellular pH and calcium with increased vascular tone and smooth muscle cellular proliferation. We examined the clinical, biochemical and hormonal parameters in 152 Chinese NIDDM patients with normal renal function (plasma creatinine <120 μmol/l) on 3 occasions during a 6-week period when all antihypertensive medications were withdrawn. We also measured the plasma concentrations of ESTI by 2 non-specific assays [inhibition of purified Na^+,K^+,ATPase (ATPI) and as digoxin-like immunoreactivity (DLS)] and by immunoassay using antibodies against ouabain. Hypertension was defined as a mean arterial pressure (MAP)>100mmHg. Hypertensive patients (n=69) had higher plasma Na^+ (141 vs 139 mmol/l, p<0.001), atrial natriuretic peptide (ANP) (49 vs 35 ng/l, p<0.001), serum angiotensin converting enzyme (ACE) activity (74 vs 55 U/l), ATPI (7.5 vs 5.8 mmol/l, p<0.001), ouabain (0.92 vs 0.68 pmol/l) and lower plasma renin (31 vs 49 ng/l, p=0.02) than normotensive patients (n=83). DLS were similar between the groups. Using stepwise regression analysis, MAP was independently related to plasma ouabain (r^2=29.6, β=0.42, p<0.001), plasma Na^+ (r^2=12, β=0.22, p=<0.01), ANP (r^2=4.2, β=0.23, p<0.001), ACE (r^2=2.2, β=0.16, p=0.01) and K^+ (r^2=1.9, β=–0.14, p=0.03). These findings emphasis the importance of salt repletion and abnormal sodium transport in NIDDM associated hypertension.

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EFFECTS OF PERINDOPRIL ON MICROALBUMINURIA IN HYPERTENSIVE PATIENTS WITH NIDDM

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To determine the efficiency of ACEIs-Perindopril on microalbuminuria in hypertensive patient with NIDDM. Of 20 cases were enrolled the study, 12 males & 8 females, with mean age 57.4±8.9 yrs, mean duration of hypertension & NIDDM as 6.8±6.1 yrs & 4.2±4.4 yrs respectively. Perindopril (Servier, France) were given 2 mg daily for 6 months in those patients. The mean values of MBP (diastolic BP+1/3 pulse pressure), AER (urinary albumin excretion rate), PRA (plasma renin activity),GFR,ERPF,HbA_1%, FBG & FBI (blood glucose & insulin), BU & CR (blood urea & creatinine) & serum potassium(K^+) at the start & end of the therapeutic period were compared. Perindopril therapy were associated reduce in mean MBP (from 115.9±4.6 mmHg to 97.6±4.5 mmHg, P<0.01) & AER values (from 76.1±30.1 ug/min to 54.3±30.3 ug/min, P<0.01). Within the total cohort the mean FBI & hyperfiltrative GFR were improved (18.4±7.1 uU/ml vs 12.6±6.2 uU/ml & 111.9±23.1ml/min/1.73m^2 vs 86.5±23.7ml/min/1.73m^2 respectively, P<0.01). The mean PRA value was increased (from 0.5±0.58ng/ml/h to 1.56±1.3ng/ml/h, P<0.01). There were no significant changes in mean HbA_1%, FBG, ERPF, BU & CR and K^+ levels during this study period. In conclusion, Perindopril has a significant reducing effect on urinary albumin excretion in hypertensive NIDDM patient.

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ENALAPRIL ENHANCES INSULIN SENSITIVITY IN THE OFFSPRING OF HYPERTENSIVE PARENTS.

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Insulin resistance is considered to be an underlying pathogenetic mechanism of several metabolic abnormalities increasing the risk of atherosclerosis. Familiar predisposition toward the IR syndrome may be the cause of increased cardiovascular complications also in families of hypertensive patients. The aim of the study was to evaluate the possibility of changing insulin sensitivity in a group of middle-aged men from predisposed families, after two-year lifestyle changes and subsequent administration of angiotensin converting enzyme inhibitor (ACEI). Euglycaemic hyperinsulinaemic clamp (75 μU/ml) lasting 3 hours was performed in a group of 15 offspring of patients with hypertension (OHP) at the baseline and after 6 week of ACEI administration (enalapril 10 mg/day), and compared to age-, weight- and sex-matched healthy controls (n=18). At baseline the metabolic clearance rate of glucose (MCRglu) was significantly lower in OHP compared to controls (7.98 ± 3.21 vs 9.24± 2.25 ml/kg.min; p<0.01). Enalapril administration significantly increased the MCRglu in OHP (7.98 ± 3.21 vs 9,7 ± 3,04 ml/kg.min; p<0.05) to values, which were comparable to the control group. We conclude that short-term enalapril administration improved insulin sensitivity in offspring of hypertensive patients.

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RENAL-PROTECTIVE EFFECT OF TEMOCAPRIL, A BILIARY EXCRETIVE ACE INHIBITOR, IN DIABETIC NEPHROPATHY.

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Objective There is a high incidence of hypertension as a complication of diabetic nephropathy. The recently developed angiotensin converting enzyme inhibitor (ACEI) of biliary excretive type is expected to be the agent that inhibits directly and more strongly advancement of nephropathy. We studied the effects of various antihypertensive agents on proteinuria in diabetic nephropathy.

Materials and Methods Temocapril 2 mg/day (T, 6 patients), enalapril 2.5 mg/day (E, 8 patients) or nifedipine 20 mg/day (N, 10 patients) were administered orally for 12 weeks. Blood pressure was measured every two weeks, and excretion amount of urinary protein in a day, blood sugar, renal function and lipids were measured before the administration and 1, 2 and 3 months after the administration.

Results From 2 weeks after the administration, both systolic and diastolic pressures dropped (p<0.01) in all patients. The antihypertensive effect continued thereafter. Excretion amount of urinary protein in a day decreased significantly after the administration of T and E compared to that before administration. In changing rates of urinary protein calculated with preadministration values, the rates decreased in T, E and N throughout the test periods with significant differences observed in T and E. Three months after the administration, the decreasing rate of urinary protein in T was significantly larger (p<0.05) than that in E. No side effects were observed in all patients during or after the administration of each agent.

Discussion ACEI has the stronger effect of decreasing urinary protein than the calcium antagonists. Its mechanism seems to be in lowering systemic blood pressure and correcting glomerular hypertension by inhibition of angiotensin II production. With its strong inhibitory action against ACE and the possible maintenance of effective blood concentrations without being excessive owing to biliary excretion, it is demonstrated that T has strong renal protective effect in diabetic nephropathy.

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CARTEOLOL IMPROVES BODY WEIGHT AND VISCERAL FAT WEIGHT GAINS IN OLETF RATS, A MODEL OF NIDDM WITH MILD OBESITY

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In order to examine the effect on body weight and carbohydrate and lipid metabolism, a non-selective β-blocker with ISA, carteolol, was administered to Otsuka Long-Evans Tokushima Fatty (OLETF) rats, an animal model of spontaneous NIDDM with visceral obesity and late onset of disease. A high dose of 0.02% carteolol admixed in a pellet diet suppressed body weight gain without affecting food and water consumption until the appearance of glycosuria. Carteolol tended to reduce the cummulative incidence of glycosuria after 26 weeks of administration: 55% in the control, 17% at the low dose (0.002%) and 25% at the high dose. Although plasma glucose and triglyceride levels in non-fasted rats were elevated with age, carteolol delayed the increases in these parameters. Carteolol also suppressed the increase in plasma glucose level, which indicated the diabetic pattern, in a 25th week OGTT. At the 26th week of administration, carteolol decreased visceral fat weight (retroperitoneal and epididymal adipose tissue), whereas the liver and the kidney were not affected. In the in vitro study, carteolol exhibited binding affinity (Ki=135$\pm$11 nM) for the membrane in a recombinant cell line (293/p220-B4) expressing the human β3-adrenoceptor, suggesting that the drug might act by activating the β3-adrenoceptor in rats. These findings indicate that carteolol induces improvement in body weight and carbohydrate and lipid metabolism in an obese condition. Consequently, carteolol may be useful for the treatment of hypertention with obesity in order to prevent cardiovascular events.

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BLOOD PRESSURE VARIABILITY DURING 24H BLOOD PRESSURE MONITORING AND AUTONOMIC DISORDER IN NIDDM.

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It has been known for a long time that the autonomic neuropathy represents a complication from diabetes . The increase in diurnal pressure variability and tachycardia seem to be an early sign of autonomic disorder but often it is impossible to verify this alteration with more specific tests that could investigate the vagal and simpatic cardiovascular function.In this study we have submitted to a 24h blood pressure monitoring(BPM),both sistolic(SBP)and diastolic(DBP),and to heart rate (HR), 25 individuals with NIDDM in an age group between 53±12 who have been affected by diabetes for more than 8 years,and with normal blood pressure,plus 25 individuals in a control group,age 50±12,also whit normal blood pressure. Afterwards they underwent an evalution of the autonomic cardiovascular function with the following tests:deep-breathing,standing to lying, postural hypotension.The subjects with diabetes showed, conpared to the control group, a singnificant increase($p<0,005$) in the variability (standard deviation SD) of the diurnal SBP(SD SBP day=18,1±7 vs, 14,3±3,5);a significant increase($p<0,005$)of the diurnal and nocturnal HR(HR day 84±8 vs 81±9,12:HR night 70±7,2 vs 65± 6,2), while the differences in the (SDSBP) and the differences in both diurnal and nocturnal (SDHR) were not significat between the two groups. Moreover 22(88%) of the 25 subjectes with diabetes showed at least 1 of the tests as pathological; this fact leads to the conclusion than the diurnal pressure variability and both diurnal and nocturnal tachycardia are good indication of autonomic cardiovascular alteration and they correlate in most cases(88%) with the positivity of more specifc tests that investigate the function of the vagal-simpatic nervous paths in the heart.

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LONGTERM TREATMENT WITH CANDESARTAN CILEXETIL DOES NOT AFFECT GLUCOSE HOMEOSTASIS OR SERUM LIPID PROFILE IN PATIENTS WITH MILD HYPERTENSION AND TYPE II DIABETES.

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Many hypertensive patients also have diabetes mellitus or dyslipidemia disorders, which increase the overall cardiovascular risk. The aim of the study was to assess the effects of candesartan cilexetil (cand.cil.) a novel angiotensin II antagonist selective for AT_1 receptors with long lasting antihypertensive effect on glucose homeostasis and serum lipid profile in mild hypertensives with type II diabetes.

Men and women, aged 30-75 years with mild hypertension (sitting diastolic blood pressure (BP) 90-100 mmHg) and type II diabetes (HbA1c 5.5-9.0%), both measured after a 4 week placebo run-in period were randomised to double-blind treatment with cand.cil. 8 mg once daily (o.d.) (n=83) or placebo o.d. (n=78) (dose increased to 16 mg o.d, if diastolic BP>90 mmHg). At randomisation and after 12 weeks treatment HbA1c (primary endpoint), blood glucose and serum lipid profile (total CH, LDL-CH, HDL-CH, TG, ApoA1 and ApoB) were assessed. The analysis of the differences between treatments was based on the changes from randomisation to the end of the study.

Cand.cil had no adverse effect on HbA1c, blood glucose and serum lipids compared to placebo. The median HbA1c both at baseline and after 12 weeks was 7.1% in patients on cand.cil. The corresponding values were 7.2% and 7.1% in the placebo group. The 95% confidence interval for the median difference in change between the groups was narrow (-0.250; 0.160), excluding any clinically important difference. The same held true for blood glucose (-1.10; 0.20), total-CH (-0.40; 0.20), and the other lipid parameters. Approximately 60 % of patients reached a diastolic BP <90 mmHg. Adverse events and withdrawals were similar in both groups.

In patients with mild hypertension and type II diabetes, cand.cil. 8 to 16 mg o.d. for 12 weeks does not adversely affect glucose homeostasis or serum lipid profile. Blood pressure was controlled in most patients and cand.cil was well tolerated.

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AMBULATORY BLOOD PRESSURE RELATIVE TO GUIDE-LINES FOR THE MANAGEMENT OF HYPERTENSION.

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To evaluate Canadian guidelines for the management of hypertension in diabetes mellitus, we studied 10 volunteers with diabetic retinopathy; age 26-78 y; 6 type I, 4 type II; mean (SEM) HbA1 (DCA 2000, Bayer Inc, ref .041-.053).085±.01. BP (in 8/10) and diabetes medications were continued. Conventional (conv) BP was measured 4 times prior to and after ambulatory BP (ABP, SpaceLabs, Model 90207) q30 min, x24h with 78-100 (median 95)% successful readings and analysis by day (D,0800-2200h) and night (N,2200-0800h). Conv BP (range) was 163-110/95-61 mm Hg.

Guide Class	Compromised >150/90	Acceptable ≤150/90	Optimal <140/90

mmHg

Classification of systolic BP (SBP) and diastolic BP (DBP) in 5 subjects was concordant using conv BP or ABP. For SBP, 3 cases were discordant (conv BP over-classified); for DBP 3 cases were discordant (conv BP under-classified in 2, over-classified in 1). Thus, under-classification was confined to DBP. During ABP abnormal N/D ratios (SBP >0.092, DBP>0.9) were found for SBP in 2/4, and for DBP in 3/4 subjects with optimal SBP/DBP suggesting that this qualitative problem remains to be resolved.

1438

CANDESARTAN CILEXETIL, A NOVEL ANGIOTENSIN II ANTAGONIST REDUCES MICROALBUMINURIA IN PATIENTS WITH TYPE II DIABETES MELLITUS AND MILD HYPERTENSION.

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Microalbuminuria is a predictor of nephropathy in patients with type I or type II diabetes mellitus and interventions that decrease albuminuria are likely to postpone the development of severe renal impairment. Blocking the renin-angiotensin system via ACE inhibition has been effective in this respect. The more direct route of inhibiting the effects of angiotensin II by means of an angiotensin II receptor antagonist is expected to provide at least similar reductions of albuminuria. This study assessed the effect of candesartan cilexetil (cand.cil.), a novel, long-acting angiotensin II antagonist, selective for the AT_1 receptor, on microalbuminuria in patients with type II diabetes and mild hypertension.

The analysis was performed in a subset of patients (n=35) who had microalbuminuria (10-100 mg in an overnight urine sample) at randomisation in a large double-blind, placebo controlled study, investigating the effect of cand.cil. on blood glucose homeostasis and blood lipid profile in patients with stable type II diabetes, mild hypertension (diastolic blood pressure (BP) 90-100 mmHg) and serum creatinine below 150 µmol/l for men and 120 µmol/l for women. Patients were randomised, after a 4 week placebo run-in period, to 12 weeks double-blind treatment with cand.cil. 8-16 mg (n=83) or placebo (n=78) once daily. The difference between treatments in change in albuminuria from randomisation to the end of the study was analysed.

Cand.cil. did not influence blood glucose homeostasis or blood lipid profile compared to placebo. The body weight remained unchanged in both treatment groups and in patients in whom GFR (iohexol decrease) was assessed (placebo n=32, cand.cil. n=39) there was no significant change in GFR. The median urinary albumin excretion decreased from 28.5 to 12.2 mg/12 h (57%) in patients treated with cand.cil., (n=15) while it increased from 30.2 to 32.8 mg/12 h (9%) in the placebo group (n=20, p=0.03 for the difference between treatments). In this subset of patients the mean diastolic BP reduction was 6.4 mmHg in the cand.cil. group and 3.6 mmHg in the placebo group.

In this placebo controlled study, 12 weeks treatment with the angiotensin II antagonist cand.cil. reduced microalbuminuria in patients with stable type II diabetes and mild hypertension. Thus, cand.cil. appears to have a nephro-protective potential in this patient category.

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HYPERTENSIVE NIDDM PATIENTS AND ANGIOTENSIN I-CONVERTING ENZYME: ACTIVITY, INHIBITION, AND ACE GENE POLYMORPHISM

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Accumulating data indicate influence of insertion/deletion (ID) polymorphism of angiotensin I-converting enzyme (ACE) gene on clinical benefit of ACE inhibition (ACEI) as reno- and cardioprotective therapy. The aim of this study was to evaluate hypotensive effect of ACEI Perindopril in NIDDM patients with regard to serum ACE activity (ACEA) and ID/ACE genotypes. 44 essentially hypertensive NIDDM patients with the following characteristics (mean±SD): age 53.1±6 yr; NIDDM duration 7.7±4 yr; body mass index (BMI) 31±4.3; waist-to-hip ratio (WHR) 0.94±0.07; received Perindopril during 3 months. Initial ACEA was measurated by RIA-KIT. ID/ACE genotyping was carried out using polymerase chain reaction technique. Blood pressure (BP) and glycated haemoglobin (HbA1c) were measured before and after ACEI treatment. Reduction (%) of basal values of BP was calculated and statistical differences analysed using Student's t-test. Patients were considered as II (n=8), ID (n=16), and DD (n=20) genotype carriers, respectively. We could not find significant difference in ID/ACE allele/genotype distribution in hypertensive NIDDM patients and reported earlier general Moscow population. Also, all the above mentioned characteristics did not differ signifacantly in various genotype carriers, even in homozygous for I and D alleles. Moreover, basal ACEA levels were similar in II, ID, and DD groups of NIDDM patients: 51.5; 45.3; and 52.6 ACE-units, respectively. Overall patients possessed evident reduction of BP after ACEI treatment (21.9% for systolic and 18.9% for diastolic) but decrements of systolic and diastolic BP did not, however, differ significantly in II, ID, and DD groups. The only variables to be influenced by ID/ACE gene polymorphism, as revealed by regression analysis, were BMI, WHR, ACEA and diastolic BP reduction. Only in II genotype carriers the following correlations were observed: BMI/WHR r=0.718; WHR/ACEA r=0.804; ACEA/dPB reduction r = -470. Conclusions: 1) ID/ACE gene polymorphism is not associated with essential hypertension in NIDDM Russian population; 2) regardless ID/ACE genotypes and/or serum ACEA, Perindopril is effective hypotensive drug in NIDDM patients; 3) the most evident correlation between physical, biochemical and clinical parameters were observed in II genotype carriers.

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THE EFFECT OF TEMOCAPRIL ON MICROALBUMINURIA IN HYPERTENSIVE PATIENTS WITH AND WITHOUT GLUCOSE INTOLERANCE

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Microalbuminuria may be a marker of early dysfunction of glomerulus or of intrarenal vasculatures in hypertension and diabetes mellitus. This study was conducted to examine the effect of long-term treatment with temocapril, a new angiotensin converting enzyme (ACE) inhibitor, on microalbuminuria.

The subjects were 100 hypertensive patients with or without impaired glucose tolerance (49 men and 51 women, mean age 59.7 years) who had been consuming antihypertensives either diuretics or calcium channel blockers except for ACE inhibitors. Their blood pressure was controlled 149.6/88.4 (means) mmHg. Serum creatinine (s-Cr), fasting plasma glucose (FPG) levels, and urinary excretion ratio of microalbumin (UAE) (mg/L) to creatinine (g/L) were evaluated, then the antihypertensive agents were switched to temocapril (2 to 4 mg) once daily. Of 58 the patients had glucose intolerance (IGT group) and 42 had normal glucose tolerance (NGT group) at baseline period.

After long-term (12 months) therapy with temocapril, their blood pressure and FPG were maintained as well. On the other hand, s-Cr (mg/dl) decreased significantly from 0.85 to 0.75 in IGT, and from 0.8 to 0.75 in NGT, respectively. UAE (mg/g Cr) was also significantly decreased from 43 to 28.1 in IGT, and 43.6 to 26.6 in NGT, respectively.

Long-term beneficial effects of temocapril on UAE and s-Cr are confirmed in hypertensive patients with and without glucose intolerance, despite similar antihypertensive efficacy with the previously used agents.

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THE RELATIONSHIP OF BLOOD PRESSURE TO SODIUM AND POTASSIUM EXCRETION IN THE EURODIAB IDDM COMPLICATIONS STUDY.

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The INTERSALT study reported a significant association between blood pressure and both sodium and potassium intake as measured by urinary electrolyte excretion. People with IDDM are at increased risk of hypertension but the relation between electrolyte intake and blood pressure in IDDM is unknown. Therefore the relations between 24 hr urinary electrolyte excretion and blood pressure were studied in a cross-sectional sample of 3250 IDDM patients from 31 centres across Europe (EURODIAB IDDM complications study). HbA_{1c}, urinary electrolytes and urinary albumin excretion rate (AER) were measured centrally. Relations between electrolyte excretion and blood pressure were studied within each centre using regression analysis and results were pooled to give a summary regression coefficient for all centres. For a difference of +10 mmol/24hr sodium excretion, SBP was 0.1 mmHg lower in men ($p<0.05$) and 0.1 mmHg higher in women($p=0.2$) (age adjusted). For a difference of +10 mmol/24hr potassium excretion, SBP was 0.3 mmHg lower in men ($p<0.05$) and .04 mmHg higher in women ($p=0.8$). On adjustment for confounding variables the associations between electrolytes and SBP in men were no longer significant. The analyses were repeated excluding those on antihypertensive therapy (10%) and those with micro or macro-albuminuria i.e. AER≥20 ug/min (30%), as these patients may have been expected to reduce their sodium intake following diagnosis, and no significant association was found. In conclusion, in this group of IDDM patients across Europe, neither sodium nor potassium excretion were significantly associated with blood pressure, in contrast to the findings in a random population sample in INTERSALT.

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RAMIPRIL INCREASES SPECIFIC INSULIN BINDING AND METABOLISED GLUCOSE RATE IN NON-INSULIN-DEPENDENT DIABETICS WITH HYPERTENSION

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The aim of the study was to compare the effect of ramipril with that of atenolol and placebo on glucose metabolism in non-insulin-dependent diabetics with moderate hypertension treated with glibenclamide. In euglycaemic hyperinsulinaemic clamp the specific insulin binding (SIB) at the erythrocytes receptors and peripheral metabolised glucose (MG) were examined before and after six weeks. Four groups of patients were compared: ramipril 2.5 mg/die (group A-20 patients), ramipril 5.0 mg/die (group B-20 patients), atenolol 50 mg/die (group C-20 patients), and placebo (group D-10 patients).
The results: SIB (%), the baseline level, before and after 6 weeks:
(A): 6.78±1.29 and 7.92±1.61 (+1.13±1.54); (B): 7.58±2.51 and 7.85±2.16 (+0.28±2.61); (C): 7.66±1.78 and 7.52±2.04 (-0.14±1.83); (D): 8.56±1.62 and 7.06±0.88 (-1.50±1.53). Comparison ramipril 2.5 mg (A) vs.placebo (D) was significant ($p<0.05$).
MG (mg/kg/min) between 90 and 120 min., before and after 6 weeks:
(A): 3.68±1.16 and 4.03±1.79 (+0.36±1.31); (B): 3.02±1.12 and 3.90±1.35 (+0,88±1.18); (C): 4.72±2.04 and 5.05±1.87 (+0,33±1.22); (D): 3.79±1.80 and 3.60±1.63 (-0.19±0.59). Ramipril 5.0 mg vs. atenolol 50 mg was significant ($p<0.05$).
CONCLUSION: (1) The significant difference between increase in SIB after 2.5 mg/die of ramipril and SIB decrease after placebo during the 6 weeks was achieved. (2) The increase in MG in the group (B) was significant as compared to atenolol 50 mg. These findings favour the usage of ramipril in hypertensive diabetics treatment.

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OSCILLOMETRIC MEASUREMENT OVERESTIMATES BLOOD PRESSURE IN NON-COMPLICATED IDDM.

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In the management of IDDM accurate control of blood pressure (BP) is of great importance. As such the method of BP measurement deserves more attention. Measurement by office sphygmomanometry (sphygmo) is increasingly replaced by new methods. We performed a study in which we compared intra-arterial BP (i.a.), sphygmo BP, 24 hours BP (ABPM, Profilomat®, auscultatory) and oscillometric BP (dinamap®) in non-complicated IDDM (DP) and healthy controls (C). The i.a recordings were used as golden standard. We studied 42 C and 51 DP. I.a. BP was registered for 5 min. (>300 registrations) in supine position. Sphygmo BP was measured 3 times after 10 min. in supine position. ABPM was recorded on a working day. BP between 10.00-22.00 was averaged as "day BP". BP between 02.00-06.30 was averaged as "night BP". Dinamap® registration was performed during 10 min. in supine position. Results are expressed as means±SE. Data are analyzed by *t*-test.

	Controls			Diabetic Patients		
	SBP	DBP	MAP	SBP	DBP	MAP
I.a	115.6±1.2	63.2±0.9	83.4±1.1	116.2±1.2	61.7±0.8	82.8±0.9
Sphygmo	116.5±1.5	67.8±1.3		117.7±1.3	69.8±1.3	
APBM						
day	120.4±1.5	83.7±1.0		120.9±1.2	84.4±0.9	
night	103.4±1.5	69.1±1.3		102.4±1.2	69.3±0.9	
Dinamap	113.4±1.4	65.0±1.0	83.3±1.0	*118.6±1.3	66.6±0.7	**85.6±0.7

* $P=0.01$,** $P=0.05$ DP versus C.

There was no difference in i.a., sphygmo or ABPM BP between C and DP. Dinamap BP was significantly increased in DP. In C dinamap MAP was not different from i.a. MAP (mean difference -0.10 mmHg) whereas it exceeded i.a. MAP by 2.8 mmHg (95% CI 0.7 to 4.5 mmHg) in DP ($P<0.01$). It is concluded that i.a. BP is similar in non-complicated DP versus C. Results of BP measurements are dependent on the method used. Auscultatory methods were comparable in C and DP (sphygmo and ABPM). In contrast the oscillometric device (dinamap®) overestimated BP in DP. We hypothesize that this difference may be caused by changes in vessel wall characteristics in non-complicated IDDM.

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UNCHANGED INSULIN SENSITIVITY WITH VERAPAMIL SR PLUS TRANDOLAPRIL IN LOW-DOSE COMBINATION IN NIDDM WITH STAGE 1 HYPERTENSION.

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Objective: Angiotensin converting enzyme (ACE)-inhibitors have been shown to increase both skeletal muscle blood flow and insulin sensitivity. Calcium antagonists also increase muscle blood flow without affecting insulin action. Since muscle blood flow might link insulin sensitivity and blood pressure, combination therapy with both substances might have additive effects on both haemodynamics and insulin sensitivity. **Research Design and Methods:** This was a randomized double-blind placebo-controlled parallel group study in 46 insulin resistant subjects with NIDDM and mild essential (predominantly stage 1) hypertension. The effect of sustained release (SR) verapamil (180 mg) and the ACE-inhibitor trandolapril (1 mg) during 8 weeks monotherapy or combination on insulin sensitivity and blood pressure was compared with placebo using the isoglycemic glucose clamp technique and both conventional and ambulatory blood pressure monitoring. **Results:** Concerning blood pressure lowering, there was no difference between the treatment groups, no additive effect of the combination, and no difference from placebo. Insulin sensitivity (MCR) increased by 47% with trandolapril ($p=0.0015$) and remained unchanged in all other groups. **Conclusion:** For the first time in a controlled study in a homogenous insulin resistant group of caucasian subjects with NIDDM and predominantly stage 1 hypertension, combined treatment with a heart rate-moderating calcium antagonist plus an ACE-inhibitor in apparent sub-pressor dose is shown to be neutral in terms of clamp-derived insulin sensitivity. The insulin -sensitizing effect of ACE-inhibitor monotherapy with its theoretical risk of hypoglycemia is completely neutralized in the combination.

1445

TREATMENT OF LEFT VENTRICULAR DYSFUNCTION WITH PROLONGED QT INTERVAL IN ELDERLY PATIENTS WITH DIABETES TYPE II .

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Elderly patients (PS) with diabetes II and asymptomatic diastolic left ventricular dysfunction (LVD) show an increased risk of cardiac death. In these PS the lenght of QT interval may be considered a target for prevention of arrhythmic death.The aim of this study was to identify high risk elderly diabetic PS and evaluate Ramipril capacity of reducing the lenght of QT interval and of improving diastolic LVD.18 elderly diabetics PS (mean age 58±7 years), both males and females, having Doppler - Echocardiographic evidence of diastolic LVD and prolonged QT interval, were studied by an open study with a parallel randomized group design. After two weeks of pharmacological wash out, PS were randomly recruited to either Ramipril 5 mg /day or placebo, and followed for 12 weeks. All patients were investigated at the beginning of the study and after 4, 8 and 12 weeks of follow up with blood samples for hematology, urea, electrolites, creatinine.12-lead ECG was carried out and QT interval was corrected with Bazett's formula and QT dispersion was calculated.Chest X-ray and Doppler- 2 Dimensional Echocardiography were performed at the beginning and at the end of the study.Exercises tollerance was evaluated using bycicle ergometer during the run-in period and after 6 and 12 weeks of therapy. All PS completed the study.Ramipril improved significantly ($p< 0,05$) diastolic ventricular function parameters and improved exercise tollerance after 12 weeks of therapy.Finally in 9 PS we observed the correction of QT dispersion.

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THE EFFECTS OF NITRENDIPINE AND ENALAPRIL ON LEFT VENTRICULAR MASS IN HYPERTENSIVE PATIENTS WITH NIDDM.

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Hypertension is a main factor promoting left ventricular hypertrophy (LVH), and there is also a well documented increased cardiovascular risk in hypertensive diabetics. It has been suggested that ACE-inhibitors are more effective than other antihypertensive drugs in decreasing left ventricular mass (LVM). This randomized, placebo-controlled trial was designed to evaluate the effects of nitrendipine and enalapril on LVM-index in patients with NIDDM and hypertension.

The study population comprised of patients with NIDDM treated by general practitioners with diet and/or drugs. Inclusion criterion for blood pressure was a diastolic blood pressure (DBP) ≥ 90 and ≤ 115 mmHg and a systolic blood pressure (SBP) ≤ 200 mmHg, and not using blood pressure lowering drugs for 3 weeks. LVM was measured by M-mode echocardiography. 121 patients were randomly allocated to receive nitrendipine (n=40, 10 or 20 mg b.i.d.) or enalapril (n=40, 10 or 20 mg o.d.) or placebo (n=41). If DBP remained ≥ 110 mmHg, acebutolol was added in either treatment group. The treatment period was 48 weeks.

Mean age was 63 ± 9 years. Nitrendipine and enalapril resulted in a significant and almost identical reduction of SBP (157 ± 2 vs 154 ± 3 mmHg, mean ± SE) and DBP (84 ± 1 vs 84 ± 2 mmHg). In the placebo group there was no change in SBP or DBP. Nitrendipine resulted in a significant decrease of LVM-index from 146 ± 6 to 132 ± 5 g/m^2. Enalapril did not change LVM-index. In the placebo group LVM-index increased from 140 ± 5 to 151 ± 5 g/m^2. The difference between nitrendipine and placebo remained statistically significant after adjusting for age, gender, LVM-index baseline, SBP baseline and difference in SBP after 48 weeks.

These results indicate that in patients with NIDDM and hypertension nitrendipine reduces LVM-index. Enalapril appears not to induce regression, but may prevent progression with an effect that is intermediate between nitrendipine and placebo.

1447

CLINICAL EVALUATION OF AMLODIPIN EFFECT ON HBP FOR PEOPLE WITH DIABETES

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The authors are presenting the results of retrospective study including 42 diabetic subjects with HBP, aged 37-42 years, taking a unique dose of Amlodipin as monotherapy over a 24 months period. In 92,4% of the cases, the subject showed an evaluation of a 4 year period for the HBP and a 6 year evolution for the diabetes. The majority of patients have undergone several hypotensive therapies in polytherapy with no real respect of sodium retriction.

The retrospective study of the 42 cases of patients taking Amlodipine allows us to note following facts :

1- stabilization of HBP with no evolutive accident ,
2- a better metabolic balance with any given hypoglycaemic drugs,
3- an excellent Amlodipin tolerance with the observance of the treatment in the majority of cases, no particular secondary effect has been noted such as orthostatic pressure, vertgoes cephalic troubles,
4- a monthly cost of the treatment reasonably supported by the majority of patients.

The authors insist on the advantage of Amlodipin use as an antagonistic remedy of calcium in the treatment of the HBP for diabetic people, with the prevention of coronary insufficiency ; the one doses monotherapy is as well adapted in the case of diabetes as a chronic invalidating and costhy disease which often necessitates other associated therapies along with the hypoglycaemic treatment.

1448

EFFECT OF TEMOCAPRIL ON INSULIN SENSITIVITY AND MICROALBUMINURIA IN HYPERTENSIVE NIDDM PATIENTS

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In contrast to other ACE inhibitors, the metabolism of temocapril seems to be independent of renal function. In addition, temocapril has been found to be more potent in lowering blood pressure (BP) and to have a faster onset of action than other ACE inhibitors. To investigate the metabolic and antihypertensive effects of this new ACE inhibitor in diabetic hypertensives, 30 NIDDM patients with mild to moderate hypertension (diastolic BP 90 to 115 mmHg) and without azotemia (plasma creatinine <180 µmol/l) were evaluated during a randomized double-blind treatment with either temocapril, 20 mg daily (n=19), or placebo (n=11) for 6 weeks. Insulin sensitivity index (SI), determined by the Minimal Model method of Bergman, serum lipoproteins, plasma renin activity, fibrinogen and microalbuminuria were assessed at the end of the placebo run-in phase and the double-blind treatment phase, respectively. Temocapril treatment produced a significant decrease in supine BP (152/92±5/3 vs. 162/98±5/2 mmHg, p<0.01). SI tended to increase on temocapril (1.44±0.4 vs. 0.95±0.2 x10^{-4}/min/mU/l), although the difference did not reach statistical significance. During administration of temocapril or placebo, no significant changes in fasting plasma glucose, insulin, serum levels of total triglycerides, cholesterol or lipoprotein cholesterol fractions and fibrinogen were observed. Microalbuminuria decreased significantly on temocapril treatment (48.6±10 vs. 78.6±16.5 mg/24 h, p<0.01) but not on placebo. These findings demonstrate that in hypertensive NIDDM patients, temocapril is at least neutral with regard to insulin sensitivity, insulin, glucose, and lipoprotein metabolism and significantly reduces microalbuminuria.

1449

PRESENCE OF NEPHROPATHY INFLUENCES THE CHOICE OF ANTI-HYPERTENSIVES IN INSULIN-DEPENDENT DIABETES.

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During one-year period we have examined anti-hypertensive, anti-proteinuric and metabolic effects of calcium channel blockers (nifedipine or nisoldipine) or angiotensin converting enzyme (ACE) inhibitors (captopril) in 45 hypertensive type I diabetes patients. Among them 15 patients were normoalbuminuric **(A)**, 20 were microalbuminuric **(B)** and 10 were macroalbuminuric **(C)**. Only in patients with overt nephropathy **(C)** compared with healthy controls (n=10) statistically highly significant (p 0.01) decrease of plasma renin (0.17±0.07 vs. 0.28l±0.08 pmol/L/sec) and aldosterone (0.12±0.05 vs. 0.29±0.09 nmol/L) with insufficient response to upright posture (renin: 0.34±0.11 vs. 1.3±0.52 pmol/L/sec; aldosterone: 0.26±0.15 vs. 1.03±0.34 nmol/L). In this group of patients tendency toward hyperkalemia during captopril treatment was demonstrated as well as significant, although transient rise of albuminuria. At the same time calcium channel blockers express optimal antiproteinuric effect particularly in this group (633.8±284 mg/24h before treatment; 266.2±146.2 mg/24 h after one-year treatment). Meanwhile, ACE inhibitors were the treatment of choice in incipient nephropathy.

1450

A COMPARISON OF CARVEDILOL AND ATENOLOL IN PATIENTS WITH NIDDM AND HYPERTENSION

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The aim of this study was to compare the metabolic and cardiovascular effects of carvedilol, a beta-blocker with vasodilating properties, and atenolol in patients with NIDDM and hypertension. This was a randomised, double-blind, 24-week trial with parallel groups. After a 4-week run-in placebo period, 42 NIDDM patients with a diastolic blood pressure (dBP) between 90 and 105 mmHg were assigned to carvedilol (25-50 mg/day) and atenolol (50-100 mg/day). Systolic and diastolic blood pressure and left ventricular mass significantly decreased with both drugs, without difference between the groups. Total glucose disposal (euglycemic clamp) increased by 9.5 umol/kg·min (95% CI, 3.5 to 15.5, p=0.02) in the carvedilol group more than in the atenolol group. After carvedilol, triglyceride levels decreased by 0.57 mmol/L (95% CI, -1.02 to -0.12, p=0.01) and HDL-cholesterol increased by 0.12 mmol/L (0.02 to 0.22, p=0.04) more than atenolol. After carvedilol, lipid peroxidation (thiobarbituric acid reactive substances) decreased by 0.24 umol/L (95% CI, -0.44 to -0.04, p=0.04) more than atenolol. Glucagon and epinephrine responses to insulin (0.15 U/kg) were similar before and after treatment with both drugs. Carvedilol may offer advantages in patients with NIDDM and hypertension by improving glucose and lipid metabolism and by reducing lipid peroxidation.

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α-LIPOIC ACID ENANTIOMERS AND GLUCOSE METABOLISM IN INSULIN RESISTANT RAT SKELETAL MUSCLE.

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The racemic mixture of the biological antioxidant α-lipoic acid (ALA) enhances insulin-stimulated glucose metabolism in insulin-resistant humans and animals. The present study determined the individual effects of the pure R-(+) and S-(-) enantiomers of ALA on glucose metabolism in skeletal muscle of an animal model of insulin resistance — the obese Zucker (*fa/fa*) rat. Obese rats were treated intraperitoneally for 10 days with either 30 mg/kg/day of R-(+)-ALA or 50 mg/kg/day of S-(-)-ALA (maximally effective doses). While chronic R-(+)-ALA treatment significantly ($p<0.05$) reduced fasting plasma insulin (-17%) and free fatty acids (-35%) relative to vehicle-treated obese animals, chronic S-(-)-ALA treatment further increased fasting plasma insulin (+15%) and had no effect on fasting plasma free fatty acids. Insulin-stimulated glucose transport activity in the isolated epitrochlearis muscle was increased by 35% in obese animals treated with R-(+)-ALA, whereas S-(-)-ALA treatment resulted only in a 16% improvement. Chronic R-(+)-ALA treatment elicited a 26% increase in insulin-stimulated glycogen synthesis and a 37% enhancement in insulin-stimulated glucose oxidation. No significant increase in either of these parameters was observed following chronic treatment with S-(-)-ALA. These data indicate that chronic in vivo treatment with the biological antioxidant ALA enhances in vitro insulin-stimulated glucose transport and non-oxidative and oxidative glucose metabolism in insulin-resistant rat skeletal muscle. Moreover, the R-(+) enantiomer is much more effective than the S-(-) enantiomer in bringing about these beneficial adaptations in skeletal muscle glucose metabolism.

1452

EFFECTS OF THREE NEPALESE PLANTS ON SERUM GLUCOSE LEVELS OF NORMAL AND DIABETIC MODEL RATS

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We have recently published an experimental approach for screening the oral hypoglycemic effects of plant materials in rat models. The experimental result can also indicate possible mode of action of an active extract. Following this apprach 3 medicinal plants (Asparagus resemusus, Allium wallichi and Scorparia dulcis), used by the traditional practitioners in Nepal, were tested on nondiabetic and, both IDDM and NIDDM model rats. Following procedures standardized in our laboratory, freeze-dried juice (250 mg/rat) of the plants were fed at various prandial states. Serum glucose was measured by glucose-oxidase method. No extract showed any effect on the fasting serum glucose levels of any rat models. In nondiabetic rats, when the extracts were fed simultaneously with glucose no effect was seen. However, when fed 30 min before oral glucose load A resemusus showed significant antihyperglycemic effects (sum of increments of over basal value, M+SEM: 4.87+0.73 in the Control vs 3.81+0.55 in the A resemusus groups, p=0.004). In NIDDM model rats, in the postprandial state, when the extract were fed simultaneously with glucose, A wallichi showed highly significant effect (sum of increment over basal value, M+SEM; 32.14+2.29 in the Control vs 6.13+1.77 in the A wallichi group, $p<0.001$). In this condition, A resemusus also showed a marginal effect, but S dulcis had no effect. In IDDM model rats, A wallichi showed significant antihyperglycemic effect (p=0.019); other two extracts did not effect the serum glucose. The results suggest that these three Nepalese plants may contain different principles which may serve as potential sources of antidiabetic agents.

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IN VITRO AND IN VIVO EVALUATION OF POLYAMINES INHIBITORY EFFECT ON OXIDATIVE STRESS IN EXPERIMENTAL DIABETES MELLITUS

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Streptozotocin (ST) and aloxan (AL) as a selective β-cell toxins, are used for induction of experimental diabetes, acting through a common mechanism including generation of oxygen-derived free radicals. The occurrence of high contents of polyamines, particularly of spermidine in rat islets, as well as the fact that pancreas contains relatively low activities of radical scavenging enzymes, compared to the other rat's tissues, suggests that polyamines may play a role in stabilizing islet β-cell against oxidative destruction. Based on these findings the protective radical-scavenger effect of polyamines in vitro and in vivo was investigated.The inhibitory effect of polyamines on in vitro induced lipid peroxidation in xanthine-xanthine oxidase system, as a source of superoxide radicals, was observed in a dose-dependent manner measured by luminol-dependent chemiluminescence. With a concentration of spermidine (Spd) and spermine(Sp), similar to those found in pancreatic islets the inhibition was in range of 65-75%. For in vivo investigation, the rats were allocated to the following groups:I-control; II-treated with single dose of ST(50mg/kg BW) or AL(170mg/kg BW); II-treated simultaneously with Spd (50 μmol/kgBW) was given ip. 1h before administration of ST and AL as wll as continually with single daily dose following 15 days; IV-treated with the Spd only in mentioned dose.

VARIABLES	ST	AL	Spd-ST	Spd -AL	Control
glucose(mmol/l)	30.2±7.84***	33.8±5.64***	9.36±2.7 °°°	6.87±1.82 °°°	6.55±0.9
MDApl(μmol/l)	16.49±2.3*	17.7±1.29*	12.9±2.05 °°	12.8±2.02 °°	11.83±1.8
MDApancreas (nmol/mg prot.)	13.5±1.32**	15.7±2.47**	11.88±1.48 °	12.08±2.0 °°	9.9±1.17

*p<0.05; **p<0.001; ***p<0.001compared with the controls

° p<0.05 °°p<0.001 compared with the ST or AL diabetic groups

Administration of Spd alone didn't alter any of the investigated parameters. Obtained results demonstrated that pretreatment the animals with Spd abolished the rise in MDA and glucose induced by ST or AL. The results suggest that Spd might provide antioxidative protection in the recovery of ST/AL-damaged islets β-cells in vivo. It could point to possible examination of therapeutic significance.

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CHARACTERIZATION OF THE HYPOGLYCEMIC EFFECTS OF DIFFERENT EXTRACTS FROM FIVE ANTIDIABETIC PLANT MATERIALS

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As a part of an attempt to characterize the chemical nature and the possible mode of action, the cold and hot water extracts and soluble dietary fibre (SDF) from *Plantago ovata, Spirulina platensis, Trigonella foenumgraecum, Syzizium cumini* and *Musa paradisiaca* were tested for antihyperglycemic effects on NIDDM model rats following procedures standardized in our laboratory. All the test materials were fed simultaneously with glucose and blood samples were collected at 0, 30 and 75 minutes. The cold water extracts of *S platensis, S cumini* and *M paradisiaca* had no significant antihyperglycemic effect, but the same extracts of *P ovata* and *T foenumgraecum* were equally highly potent ($p<0.008$-0.001). The hot water extracts of all the plants showed effects exactly parallel to those of cold water extracts. Since SDF is known to delay the absorption of glucose from the gut, it was invesigated whether the antihyperglycemic effects of these extracts were due to SDF fractions. Although there was a definite tendency in case of SDF from *P ovata* and *T foenumgraecum*, no SDF from the five plant materials showed any significant antihyperglycemic effect. The results showed that the hypoglycemic effects of *S platensis, S cumini* and *M paradisiaca* found in this laboratory are not due to water soluble compounds. Whereas, *P ovata* and *T foenumgraecum* may act as sources for antidiabetic agents and although SDF of *P ovata* and *T foenumgraecum*, may contribute to their hypoglycemic properties, other water-soluble active principles are present in these two plants.

1455

LONG-TERM IMPACT OF L-ARGININE ON GLYCEMIC PATTERN AND OXIDATIVE STATUS IN DIABETIC RATS

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The imbalance between pro- and antioxidants in diabetes provides the justification for antioxidant treatment. It has been shown that L-arginine (L-Arg) possesses superoxide scavanging properties in vitro. The aim of the present study was to determine effect of long-term treatment with L-Arg on glycemic pattern and oxidative status in diabetic rats. Male Wistar rats were given i.p. streptozotocin (STZ) at dose 65 mg/kg. One group of diabetic animals was untreated to act as control. The other group received L-Arg (50 mg/kg/day) in the diet for 4 weeks. Oxidative status was assessed in plasma by spectrophotometric determination of malonic dialdehyde (MDA), diene conjugates (DC) and reduced glutatione (GHS) levels. Administration of L-Arg decreased basal hyperglycemia (7.9±0.8 mmol/l vs 14.7±0.9 mmol/l, $p<0.01$), increased plasma immunoreactive insulin level by 40 % ($p<0.02$) and protected from body weight loss (160.0±3.8 g vs 130.0±5.1 g, $p<0.05$) compared to non-treated diabetic animals. After 4 weeks treatment with L-Arg DC content was significantly reduced (133.0±14.1 μmol/ml vs 173.2±13.5 μmol/ml, $p<0.05$) and GHS level was increased 2 fold ($p<0.01$) in comparison with diabetic control. However, MDA concentration was not changed in any groups. The results allow the conclusion that long-term treatment with L-Arg improves metabolic control and suppresses oxidative stress in diabetic rats. We suggest the use of L-Arg may have potential therapeutic benefit in the diabetes treatment.

1456

RETARDATION OF INTESTINAL CARBOHYDRATE ABSORPTION BY HOT WATER EXTRACT OF *TRIGONELLA FONUMGRAECUM* IN RATS

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Trigonella foenumgraecum has been shown to possess hypoglycemic activity in both animal models and human diabetic subjects, but the exact chemical nature of the active principle(s) and the mechanism of the effect is still unclear. Previous reports from our Group suggest that hot water extract of *T foenum graecum* and its main constituent SDF suppressed flattened the postprandial rise of blood glucose in both IDDM and NIDDM model rats were investigated. In the present study the effects of the hot water extract on carbohydrate digestion and absorption in NIDDM model rats. Carbohydrate absorption was evaluated in NIDDM model rats by measuring the amount of sucrose remaining in 6 different parts of the gastrointestinal tract of rats after oral feeding of sucrose. Sucrose solution (2.5 g/kg body weight) was fed to 20 hrs fasted rats with or without extract (500 mg/kg body weight). The GITs of rats, sacrificed at 60 min, were removed and washed with ice-cold normal saline. The wash-out fluid, acidified by H_2SO_4 and neutralized by NaOH, was analyzed for its glucose content (by enzymatic-colorimetric methods) liberated from sucrose (estimated by back calculation of glucose). Sucrose loading in control rats sharply increased the blood glucose levels and was accompained by rapid disappearance of sucrose from the upper small intestine. In the Control Group sucrose loading led to a rapid increase of blood glucose (mmol/l, fasting 7.12±0.13 and 1 hour 14.21±0.32) whereas in the Extract Group the blood glucose response was considerably flattened (fasting 7.79±0.21 and at 1 hour 9.19±0.41). The change in the blood glucose were inversely related by the gastrointestinal sucrose content of the 2 groups of rats [Sucrose, 1 hour, mg, M±SEM, stomach (2.96±0.72), upper 20 cm (0.52±0.17), middle small intestine (0.90±0.05), the lower 20 cm (0.60±0.12), cecum (0.44±0.09) and large intestine (0.98±0.01) in Control group vs 20.22±0.91, 2.86±0.38, 2.52±0.20, 0.62±0.05 and 0.68±0.04 respectively in Extract Group]. The results reconfirms the hypoglycemic effect of trigonella fonumgraecum and they also indicate that this effect may be related to the delaying of glucose absorption from the gut by agent(s) present in the hot water fraction, probably SDF. Further experiments with different timings (30, 60, 120 and 180 min) and also experiments with oral glucose load seems to strengthen this conclusion.

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EFFECTS OF *STACHYS RECTA* ON METABOLIC CONTROL AND OXIDATIVE STATUS IN DIABETIC RABBITS

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Stachys recta (tuber) is widely used in traditional medicine as hypoglycemic agent. The aim of the study was to assess the impact of *S.recta* powder (S) on glucose pattern and oxidative status in rabbits with absolute insulin insufficiency. Male chinchilla rabbits were made diabetic by i.v. injection of dithizone (35 mg/kg). One group of diabetic animals was untreated to act as control (D) and other group received S (200 mg/kg per os) for 4 weeks. At the end of study fasted rabbits were subjected to a glucose tolerance test (GTT, 2 g/kg i.v.). Plasma samples were collected from ear vein at timed intervals (0,3,5,10,30,60 min) and analysed for glucose by glucose oxidase method and for insulin by RIA. The treatment with S decreased basal hyperglycemia by 30% ($p<0.05$), increased basal plasma insulin level (160.1±10.2 pmol/l vs D: 100.3±9.2 pmol/l, $p<0.02$) and protected from body weight loss ($p<0.05$). Administration of S improved glucose tolerance (integral glycemia over i.v. GTT was 80.0±4.7 mmol/l vs D:119.2±6.2 mmol/l, $p<0.02$). Oxidative status was assessed in plasma by spectrophotometric determination of diene conjugates (DC), vitamins A and E contents. S has been shown to suppress oxidative stress reducing DC levels (0.9±0.1 μmol/l vs D:2.8±0.2 μmol/l, $p<0.01$) and increasing 1.5 fold ($p<0.02$) vitamin E contents. However, vitamin A concentration was not changed by S. The results indicate that S possesses hypoglycemic and antioxidant properties in diabetic rabbits and may serve as potential source of antidiabetic agents.

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POTENT AND SPECIFIC ACTIVATION OF GLYCOGENESIS IN RAT SKELETAL MUSCLE BY LITHIUM: COMPARISON TO INSULIN.

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Lithium is frequently used for the treatment of mood disorders, but also affects glucose metabolism. To elucidate lithium's antidiabetic potential, its stimulatory effects on glucose metabolism of isolated rat soleus muscle were compared to those of insulin. Increment over basal glycogen synthase activity induced by 20 mmol/l lithium chloride was 4.8-fold more pronounced than that induced by 10 nmol/l insulin (nmol/l UDP-glucose incorporated into glycogen in synthase activity assay/g/min: lithium, +22.1±1.8 *vs.* insulin, +4.6±3.9; $p<0.01$). In parallel, lithium was less efficient than insulin in stimulating glucose transport (cpm ^{3}H-2-deoxyglucose/mg/h: lithium, +211±19 *vs.* insulin, +311±57; $p<0.05$) and anaerobic glycolysis (μmol lactate released/g/h: lithium, +1.0±0.5 *vs.* insulin, +3.9±0.5; $p<0.01$), while similar increments in glycogen synthesis were induced (μmol glucose incorporated into glycogen/g/h: lithium, +3.32±0.43 *vs.* insulin, +3.46±0.47; n.s.). Hence, lithium differs from insulin in that it much more specifically activates the glycogenic pathway. Further evidence for differences in mechanisms of action was derived from experiments demonstrating additivity of stimulatory effects and divergent dependency on phosphatidylinsositol 3-kinase activation (relative decrease in glycogen synthesis induced by 100 nmol/l wortmannin, an inhibitor of phosphatidylinositol 3-kinase: basal, -27±8%; lithium stimulated, -21±5%, n.s. *vs.* basal; insulin stimulated, -80±3%, $p<0.01$ *vs.* basal). Lithium may therefore be regarded a candidate for the treatment of forms of diabetes mellitus associated with primary deficits in the glycogenic pathway.

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GLP-1 RECEPTOR / Gs - COTRANSFEKTION : A NEW TOOL IN SCREENING FOR GLP-1 AGONISTS.

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The GLP-1 receptor is a very exiting new target for the treatment of NIDDM. Unfortunately, GLP-1 is a 30 amino acid long peptide that has to be administered by injection. Therefore, an agonist for the GLP-1 receptor is a very interesting drug candidate. Today, functional screens where cAMP is measured directly after adenylyl cyclase stimulation of plasma membranes expressing the receptor, are difficult to use because of a rather small fold induction in an assay. This study aimed at producing a new cell line with an higher fold of induction for screening purposes. Human GLP-1 receptor was transfected into a Baby Hamster Kidney cell line previously transfected with the rat long form of the Gs-protein. Cells were transfected using the lipofectamine method. Clones were selected by western blotting with an antibody against the Gs-protein. A cell line expressing a high level of Gs-protein were transfected with the GLP-1 receptor. GLP-1 receptor expressing clones were selected on high induction following GLP-1 stimulation in a whole cell cAMP assay. The pharmacology of the related peptides glucagon and GLP-1(1-37) on the GLP-1 receptor / Gs cell line matched that of the GLP-1 receptor expressed in the same type of cell line. When using plasma membranes from this cell line in a microtiter plate format functional assay,a 25-fold (24.5 ± 3.72) induction was obtained. Plasma membranes from a normal GLP-1 receptor cell line in a similar setup only had an 8 (7.9 ± 2.1) fold induction. This cell line will make screening for agonists for the human GLP-1 receptor much more feasible. The higher induction in the assay will decrease the error on the samples and results in less false positive and false negatives. Thus, the chance of finding agonists for this very important target will be increased.

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DISTINCT CATABOLIC ACTION OF THE INSULIN SENSITITZER BM 13.0913xNa ON ISOLATED RAT SKELETAL MUSCLE.

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BM 13.0913xNa ("**BM**"; from Boehringer Mannheim, Germany) belongs to a new class of compounds referred to as activated carbon acids and exhibits distinct antidiabetic effects, when orally administered to hyperglycemic mice (ED_{50}-plasma levels: ~70 μM). The direct acute effects of **BM** on insulin-stimulated (10 nM) glucose metabolism of isolated soleus muscle strips obtained from healthy Sprague-Dawley rats were investigated. A concentration of 10 μM **BM** in the incubation medium increased the rate of insulin-stimulated ^{3}H-2-deoxy-glucose transport (cpm/mg/h: control, 778±36 *vs.* **BM**, 1033±48; $p<0.005$). In contrast to actions expected from an insulin-mimetic and/or insulin-sensitizing agent, elevated glucose transport was associated with blunted glycogen synthesis (glucose incorporated into glycogen, μmol/g/h: control, 4.58±0.36 *vs.* **BM**, 3.42±0.21, $p<0.025$), and with a distinct glycolytic response (μmol lactate released/g/h: control, 16.8±1.1 *vs.* **BM**, 26.1±1.3, $p<0.001$; CO_2 released from μmol glucose/g/h: control, 0.41±0.04 *vs.* **BM**, 1.70±0.11, $p<0.001$). At 300 μM **BM**, decreased rates of glucose transport (cpm/mg/h: control, 783±55 *vs.* **BM**, 184±31; $p<0.001$) and glycogen synthesis (μmol/g/h: control, 4.81±0.35 *vs.* **BM**, 0.19±0.03; $p<0.001$) were observed resulting in markedly decreased muscle glycogen content after 90 min of exposure (μmol glucosyl units/g: control, 12.9±0.4 *vs.* **BM**, 2.9±0.3; $p<0.001$). It remains to be elucidated, whether such acute catabolic potential of **BM** is instrumental for its long-term antidiabetic action *in vivo* or is to be regarded as an independent and potentially negative effect.

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POTENT ANTIHYPERGLYCAEMIC EFFECT OF AN IMIDAZOLINE DERIVATIVE S-22068 IN A RAT MODEL OF TYPE II DIABETES.

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Several recent data suggest that some imidazoline derivatives can lower glycaemia in experimental animal models of diabetes. We studied the activity of the imidazoline derivative S-22068 in a rat model of type II diabetes obtained by i.v. injection of a low dose of streptozotocin (35 mg/kg). These rats, called STZ rats, exhibited moderate basal hyperglycaemia, glucose intolerance and impaired glucose-induced insulin secretion. We performed glucose tolerance and insulin tolerance tests to study a possible effect of the compound. Glucose tolerance and insulin secretion were measured as the ΔG and the ΔI, i.e. the respective increase over the basal value in the glycemia and insulinemia over 30 to 120 min after the glucose load. The insulinogenic index, was calculated as the $\Delta I/\Delta G$ and the rate of glucose disappearance as the K. After an i.p. injection of S-22068 (24 mg/kg), ΔG (mmol/l/min) was decreased (91.67 ± 5.83 vs 120.5 ± 3.65; $p<.001$), whereas K was increased (1.74 ± 0.09 vs 1.18 ± 0.05; $p<.001$). Insulin secretion was not improved: ΔI and $\Delta I/\Delta G$ were similar in the two groups. Oral administration of the product was even more effective on glucose tolerance and a chronic treatement (15 days) with S-22068 increased its efficiency. In both cases, glucose tolerance was improved and the basal hyperglycaemia was much lowered. Morever, even used at a high dose, the product did not provoke hypoglycaemia. In the view of these data, S-22068 can be considered as a potential antihyperglycaemic agent in diabetes, probably acting through an extra-pancreatic effect.

1462

DOSE-DEPENDENT EFFECT OF MOXONIDINE ON GLUCOSE TRANSPORT IN INSULIN-RESISTANT RAT MUSCLE.

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High blood pressure (BP) is frequently associated with insulin resistance. Moxonidine (MOX) lowers BP centrally by modulation of I_1-imidazoline receptors and a decrease in sympathetic outflow. We investigated the chronic effect of two doses of MOX — a higher dose (6 mg/kg) that decreases BP and a lower dose (2 mg/kg) that does not — on insulin action in the obese Zucker rat, a model of glucose intolerance and insulin resistance. Animals were treated orally for 21 days with either vehicle, 2 mg/kg MOX, or 6 mg/kg MOX. In the 6 mg/kg MOX-treated animals, fasting plasma insulin and free fatty acids were 17% and 36% lower ($p<0.05$), respectively, compared to obese controls. During an oral glucose tolerance test (OGTT), the glucose response (area under the curve) was 47% lower in the 6 mg/kg MOX-treated group, while the insulin response was 19% less. Glucose transport activity (2-deoxyglucose uptake) in isolated epitrochlearis muscle stimulated by insulin (13.3 nM) was 39% greater than control. In contrast, the lower dose of MOX did not decrease significantly fasting plasma insulin or free fatty acids. While the glucose response of these animals during the OGTT was 42% lower, this was likely partly related to the slightly greater (+11%) insulin response. Insulin-stimulated muscle glucose transport activity was not increased significantly following treatment with 2 mg/kg MOX. Therefore, in this animal model of insulin resistance, the ability of MOX to improve glucose tolerance, lower plasma insulin and lipids, and enhance insulin-stimulated skeletal muscle glucose transport is dose-dependent and may be related to its sympatholytic effects.

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TREATMENT OF OBESITY AND DIABETES IN ob/ob AND db/db MICE BY DOPAMINERGIC AGONISTS

P. Scislowski, S. Phaneuf, E. Tozzo, Y. Liang, M. Lubkin, R. Prevelige and A. Cincotta. ErgoScience Corp. Boston, MA, USA.

We examined the combined effectiveness of SKF38393 (SKF), a D_1 receptor agonist, and bromocriptine (BC), a D_2 receptor agonist, in treating obesity and diabetes in *ob/ob* and *db/db* mice. Daily drug injections were administered to female C57BL/6J *ob/ob* and C57BL/KsJ *db/db* mice 1 hr after light onset for 14 days. Drug treated groups received BC (16 mg/kg) plus SKF (20 mg/kg), whereas pair fed groups (food adjusted to drug treated groups' intake) and control groups received the vehicle. Oxygen consumption was measured in metabolic cages on day 11 or 12 of treatment. Plasma glucose, FFA, and insulin levels, were measured on day 14. In the *ob/ob* mice statistically significant results included: controls gained 6.9 ± 1.3g of body weight, while the treated mice lost 7.4 ± 0.4g. The average daily food consumption of controls was 6 ± 0.2g versus 2.8 ± 0.1g of treated. Oxygen consumption for controls and treated was 1277 ± 240 ml/kg/hr and 1623 ± 230, respectively. Plasma glucose levels were 471 ± 42 mg/dl in controls, and 164 ± 13 in treated. FFA levels were 1.27 ± 0.1 mM in controls, and 0.37 ± 0.05 in treated. Plasma insulin were 63.5 ± 17 ng/ml in controls, and 37.3 ± 6.6 in treated. Similar statistically significant results were observed in *db/db* mice: controls gained 6.6 ± 0.4g, of body weight versus 3.4 ± 1.3g in the treated. The average daily food consumption of controls was 10.7 ± 2.8g versus 5.9 ± 0.5g of the treated. Oxygen consumption for control and treated was 898 ± 250 ml/kg/hr and 2322 ± 283, respectively. Plasma glucose levels were 485 ± 29 mg/dl in controls, and 390 ± 55 in the treated. FFA levels were 1.49 ± 0.2 mM in controls, and 0.45 ± 0.04 in treated. Plasma insulin were 9.4 ± 1.3 ng/ml in controls group, and 46.7 ± 8.1 in treated. Results from pairfed animals (in both *ob/ob* and *db/db* mice) indicate that the above drug-induced metabolic changes are not primarily the consequence of decreased food consumption. Our data strongly suggest for the first time that, hyperphagia, hyperglycemia and hyperlipidemia in animals lacking either leptin (*ob/ob*) or a functional leptin receptor (*db/db*) can be treated with the combined administration of D_1 and D_2 receptor agonists.

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STEREOSELECTIVE ANTIHYPERGLYCAEMIC EFFECTS OF THE BENZOIC ACID DERIVATIVE AG-EE 388 IN VIVO.

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AG-EE 388 ZW [(±)-2-ethoxy-4-[2-[[3-methyl-1-[2-(1-piperidinyl) phenyl]butyl]amino]-2-oxoethyl]-benzoic acid] is a racemate and was shown to be a potent insulinotropic and glucose lowering compound when tested in vitro and in vivo. Within this class of compounds enantioselective effects have been described in vitro. It was the aim of the present study to investigate the antihyperglycaemic effects of the S-enantiomer, AG-EE 623 ZW, and the R-enantiomer, AG-EE 624 ZW, after oral as well as intravenous administration to fasted Wistar rats. In both experimental settings, AG-EE 623 ZW turned out to be a potent antihyperglycaemic agent (ED_{50} 3.4 µg/kg (i.v.) and 9.9 µg/kg (p.o.)) whereas AG-EE 624 ZW displayed only weak glucose lowering activity (active in doses > 0.1 mg/kg (i.v.) or > 1.0 mg/kg (p.o.)) in rats. Since the glucose lowering effects of AG-EE 624 ZW cannot be explained by residual AG-EE 623 ZW (which was 0.005 - 0.007 % (Annemarie Reinhardt Varming, Novo Nordisk A/S)), AG-EE 624 ZW itself must exert antihyperglycaemic activity. It is concluded that the glucose lowering activity of the racemate AG-EE 388 ZW mainly resides in the S-enantiomer AG-EE 623 ZW which displays, in the rat, a ≥ 100 times higher potency than the R-enantiomer AG-EE 624 ZW. For this reason the S-enantiomer AG-EE 623 ZW (repaglinide) has been proposed for further development.

1465

THE EFFECT OF CILOSTAZOL ON INSULIN RESISTANCE IN A RAT MODEL OF NON-INSULIN DEPENDENT DIABETES MELLITUS

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The effect of cilostazol treatment on insulin resistance in Wistar Rats with streptozotocin(STZ)-induced, non-insulin dependent diabetes mellitus (NIDDM) was examined. Two day old neonate rats were injected intraperitoneally (i.p.) with streptozotocin (STZ) and maintained for six months at which time they were compared with age-matched control rats for glucose tolerance, insulin secretion in response to i.p. glucose loading (i.p. glucose tolerance test, i.p. GTT) and for insulin resistance during glucose infusions (GINF) in euglycemic, hyperinsulinemic glucose-clamp studies. In the i.p. GTT studies plasma glucose levels of STZ-induced diabetic rats were significantly higher and plasma insulin levels significantly lower than those of age- matched control rats. Insulin resistance was increased in STZ-induced diabetic rats since the GINF rates of these animals were significantly lower than those of age-matched control rats. After a four week treatment with cilostazol (100 mg/kg/day) in the feed chow, the plasma glucose and insulin levels of the STZ-induced diabetic rats after the i.p. GTT were not significantly different from those of age matched control rats. Similarly, cilostazol treatment reversed the decreased insulin resistance of STZ-induced diabetic rats since the GINF rates observed during the glucose-clamp experiments were not significantly different from those of the control rats. It is suggested that the improvement in glucose tolerance and insulin resistance by cilostazol in STZ-induced diabetic rats is due to the vasodilatation and accompanying increase in peripheral blood flow brought about through the phosphodiestrase inhibitory effects of cilostazol on peripheral vascular smooth muscle.

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POTENTIATION OF THE INSULINOTROPIC AND HYPOGLYCEMIC ACTIONS OF GLIQUIDONE BY SUCCINIC ACID ESTERS

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Esters of succinic acid are currently under investigation as potential insulinotropic tools for the treatment of NIDDM. The methyl esters of succinic acid, first examined in this perspective, may lead to the undesirable generation of methanol by intracellular hydrolysis. In recent studies, however, several esters, not susceptible to generate methanol, were found to remain potent insulinotropic agents. We have now explored whether these novel esters are able to potentiate the insulinotropic and hypoglycemic actions of the hypoglycemic sulfonylurea gliquidone. The monoethylester, monopropylester and monoisopropylester of succinic acid were administered intravenously (2.0 μmol/g body weight) together with gliquidone (0.2 mmol/g body weight) to fed male Wistar rats. At the 2nd min of the test, the increment in plasma insulin concentration above paired basal value averaged 17.5 ± 1.3, 13.3 ± 1.6 and 16.8 ± 2.0 ng/ml when gliquidone was administered together with the monopropyl, monoethyl and monoisopropyl ester, respectively, as distinct from only 8.2 ± 1.2 ng/ml after injection of gliquidone alone. Over 30 min observation, the hypoglycemic action of gliquidone was not significantly affected by either the monopropyl or monoethyl ester of succinic acid, but almost doubled by the monoisopropyl ester. These observations reinforce the idea that succinic acid esters could be used to improve the insulinotropic potential of hypoglycemic drugs. Moreover, the finding that some of these esters may potentiate the hypoglycemic action of antidiabetic agents indicate that their possible role as gluconeogenic precursor in the liver is not an insurmountable obstacle to their use in a therapeutic perspective.

1467

BROMOCRIPTINE/SKF38393 TREATMENT IMPROVES PANCREATIC ISLET FUNCTION IN THE *db/db* MOUSE.

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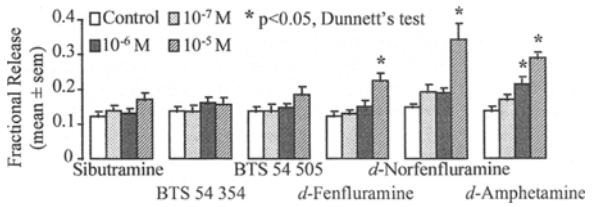
Pharmacological intervention with bromocriptine improves glucose and lipid metabolism in NIDDM animals and patients. The influence of such treatment on pancreatic islet function has not been investigated. Here we studied the effect of D_2/D_1 receptor agonists—bromocriptine/SKF38393 (BC/ SKF) on islet function in this diabetic model. Female *db/db* mice (30±1g) were treated daily for 2 weeks at 1 hour after light onset with 1) BC (16 mg/kg) plus SKF (20 mg/kg), 2) vehicle only (controls), or 3) vehicle plus feed restriction to match the reduced food consumption of treated mice (pair fed). The BC/SKF treatment reduced blood glucose (347±28 vs. 606±31 mg/dl in controls, P<0.01) and plasma free fatty acids (0.6±0.1vs.1.1±0.1 mM in controls, P<0.01) levels, and increased plasma insulin level by 3-fold compared with that in controls (49±5 vs. 16±2 ng/ml, P<0.01). In pair fed mice there was a more modest (30%) reduction (P<0.01) of blood glucose but no change in plasma insulin and a 20% increase in plasma free fatty acids compared with control levels. The insulin release response of pancreatic islets to secretagogues was tested in vitro. Insulin release from incubated islets stimulated by glucose (8 and 15 mM), arginine (10 mM) and acetylcholine (10 μM) was each 3-4 fold greater in the treated group compared with that in controls (P<0.05). Contrariwise, secretagogue-induced insulin release from incubated islets of pair fed mice were similar to those in controls. Furthermore, similar BC/SKF treatment had no effect in normal mice. Addition of BC/SKF directly to the islet incubation buffer did not enhance insulin release from db/db mouse islets. These results demonstrate that BC/SKF given in vivo markedly enhance islet function in the *db/db* but not the normal mouse. This effect is not attributable to either a direct action on islet function or inhibition of feeding. Available evidences from our lab suggests that this treatment ameliorates abnormalities within the hypothalamic-neuroendocrine-axis regulating metabolism.

1468

SIBUTRAMINE AND ITS ACTIVE METABOLITES DO NOT RELEASE [^{3}H]-NORADRENALINE FROM RAT BRAIN SLICES *IN VITRO*.

Aspley S, Prow MR, Martin KF and Heal DJ. Knoll Pharmaceuticals Research & Development, Nottingham, NG1 1GF, UK.

Sibutramine is a noradrenaline (NA) and serotonin reuptake inhibitor which is an effective weight-loss agent. In overweight, non-insulin dependent diabetics sibutramine improves glycaemic control and reduces weight, while in obese hyperglycaemic (ob/ob) mice repeated sibutramine improves glycaemic control without weight loss. Sibutramine acts *in vivo* predominantly via its secondary and primary amine metabolites (BTS 54 354; BTS 54 505). The aim of this study was to determine whether sibutramine and its metabolites release central NA *in vitro* and to compare their effects with those of other weight-loss agents. Rat frontal cortical slices, loaded with [^{3}H]-NA in the presence of pargyline, were superfused with Krebs buffer with or without drug (sibutramine, BTS 54 354, BTS 54 505, *d*-amphetamine, *d*-norfenfluramine or *d*-fenfluramine, 10^{-7}-10^{-5} M). Sibutramine, BTS 54 354 and BTS 54 505 did not evoke release of [^{3}H]-NA (Figure 1). *d*-Fenfluramine and *d*-norfenfluramine enhanced [^{3}H]-NA overflow at 10^{-5} M, whilst *d*-amphetamine dose-dependently released this monoamine (Figure 1).



These results provide clear evidence that sibutramine, BTS 54 354 and BTS 54 505 are not CNS NA releasing agents. These data clearly support the hypothesis that CNS NA release does not contribute to the pharmacological action of sibutramine and its metabolites. These data also suggest that sibutramine will be a safe, efficacious treatment for obese diabetic patients.

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A NOVEL HUMAN β3-ADRENOCEPTOR AGONIST AD-9677

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β3-adrenergic receptor (β3-AR) plays a role in regulating energy balance. β3-AR agonists are shown to increase energy expenditure by stimulating lipolysis, and to normalize hyperglycemia and hyperinsulinemia in diabetic rodents. Thus the β3-AR selective agonists that were discovered by screening for anti-obesity effects in rodent are potential anti-obesity and anti-diabetic drug. However, due to structural differences between human and rodent β3-ARs, many of these agents are poor agonists against the human receptor. To discover agents with potent activity to human β3-AR, we have established CHO cell line stably expressing human β3-AR as well as rat β3-AR. Among hundreds of compounds screened by measuring intracellular cAMP accumulation, AD-9677, which showed the strongest activity to human β3-AR, was selected. The EC_{50} values of AD-9677 to human and rat β3-ARs were 0.062 nM and 0.016 nM, respectively, and therefore the compound is seems to be one of the most active β3-AR agonist so far known. We also have established human β1- and β2-AR-expressing CHO cell lines to evaluate β3-AR selectivity. As cAMP accumulating activities of AD-9677 in human β1- and β2-AR-expressing cells were quite low, AD-9677 is a highly selective β3-AR agonist. Preliminary experiment of chronic administration of AD-9677 to obese-diabetic rodents certified that AD-9677 had anti-obesity and anti-diabetic effects *in vivo*. Thus, AD-9677 will be a useful agent for treatment of type II diabetes and obesity.

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D-FENFLURAMINE-INDUCED DEPLETION OF RAT BRAIN 5-HT IS PREVENTED BY SIBUTRAMINE OR FLUOXETINE PRETREATMENT.

Aspley S, Butler SA, Prow MR, Martin KF and Heal DJ. Knoll Pharmaceuticals Research & Development, Nottingham, NG1 1GF, UK.

Sibutramine (SIB) is a serotonin (5-HT) and noradrenaline reuptake inhibitor, and weight-loss agent. Weight-reducers which release 5-HT, viz. fenfluramine; *d*-fenfluramine (*d*FEN), cause profound brain 5-HT depletion in both rodents and non-human primates. This study compared the effects on rat brain 5-HT of *d*FEN with those of SIB and the selective serotonin reuptake inhibitor, fluoxetine (FLU). Male SD rats (80-100g) received vehicle, *d*FEN 10 mg/kg p.o., SIB 9 mg/kg p.o. or FLU 10 mg/kg i.p. for 4 days, *b.i.d.*, alone or in combination (SIB or FLU 1 h prior to *d*FEN); 14 days later, brain tissue 5-HT content was determined by HPLC-ED. *d*FEN decreased 5-HT levels in all regions. In striking contrast, FLU and SIB did not alter brain 5-HT levels (Table 1) and actually prevented the *d*FEN-induced decreases in 5-HT in the majority of areas (Table 1).

	Frontal cortex	Hippocampus	Striatum	Hypothalamus
Saline/H_2O	509 ± 29	420 ± 32	446 ± 40	947 ± 55
FLU	513 ± 29	446 ± 33	596 ± 54	912 ± 53
*d*FEN	176 ± 10 **	151 ± 11 **	250 ± 22 **	673 ± 39 **
FLU/*d*FEN	507 ± 29 ††	448 ± 34 ††	534 ± 48 †	873 ± 50
H_2O/H_2O	675 ± 39	525 ± 30	401 ± 25	802 ± 43
SIB	644 ± 38	552 ± 32	346 ± 22	756 ± 42
*d*FEN	234 ± 14 **	214 ± 12 **	220 ± 13 **	579 ± 31 **
SIB/*d*FEN	534 ± 31 * ††	480 ± 28 ††	354 ± 22 ††	704 ± 39 †

5-HT levels (ng/g wet tissue weight) mean ± s.e. mean (n=8-18). Comparisons with vehicle, *p<0.05 **p<0.01, Williams' test. interactions between drugs, †p<0.05, ††p<0.01, Multiple t-test.

These data confirm that *d*FEN persistently depletes brain 5-HT, but the monoamine reuptake inhibitors, SIB and FLU do not. Taken together with evidence from microdialysis studies which show SIB and FLU inhibit *d,l*FEN from releasing 5-HT, the data argue that SIB and FLU prevent the 5-HT depleting effects of *d*FEN by blocking its entry into 5-HT nerve terminals.

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JTT-501 IMPROVES INSULIN RESISTANCE INDUCED BY GLUCOSAMINE AND TNF-α IN CULTURED MYOTUBES FROM HUMAN NIDDM SKELETAL MUSCLE.

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Insulin resistance in skeletal muscle has been regarded as one of the most important pathogenic factors of NIDDM. In this study, the effect of a novel hypoglycemic agent, JTT-501(JT), on glucose transport was investigated in cultured myotubes from human NIDDM subjects from the view point of insulin signal transduction system and intracellular Ca^{2+}. Glucose transport rate (GTR)was expressed by 2-deoxy-D-[^{3}H]glucose uptake, and the number of cell surface facilitated glucose transporters (GTN) was evaluated by [^{3}H]-cytochalasin B binding capacity. Glucosamine(G) significantly reduced the basal and insulin (Ins)-stimulated GTR (Basal: 100±3.2%, G: 86.4±6.9, Ins: 143.5±10.3, Ins+G: 108.7±8.5, $p<0.05$). JT significantly ameliorated G-induced decrease in GTR (G+JT: 94.7±5.8, Ins +G+JT: 122.3±9.8, $p<0.05$). G significantly reduced GTN, and JT ameliorated the reduction of GTN in the presence of Ins. Wortmannin, a PI3-kinase inhibitor, abolished the effects of Ins and JT on GTR and GTN, however dantrolene, an inhibitor of Ca^{2+} mobilization, had no effects. Ins and JT demonstrated similar effects on insulin resistance induced by TNF. These results suggest that JT exerts its action by ameliorating not only the translocation of glucose transporters mediated through the activation of PI3-kinase, but also the expression of glucose transporter mRNA, and that JT is a useful agent for the treatment of NIDDM with insulin resistance.

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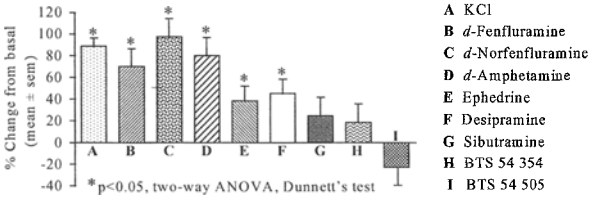
SIBUTRAMINE AND ITS ACTIVE METABOLITES DO NOT RELEASE [^{3}H]-NORADRENALINE FROM RAT HEART SLICES *IN VITRO*.

Aspley S, Broughton D, Martin KF and Heal DJ. Knoll Pharmaceuticals Research & Development, Nottingham, NG1 1GF, UK.

The noradrenaline (NA) and serotonin reuptake inhibitor, sibutramine, is an efficacious weight reducing drug which acts *in vivo* mainly via its metabolites, BTS 54 354 and BTS 54 505. In overweight, non-insulin dependent diabetics sibutramine improves glycaemic control and reduces weight, while in obese hyperglycaemic (ob/ob) mice repeated sibutramine improves glycaemic control without weight loss. In contrast to other weight modifying agents, sibutramine and its metabolites do not release NA from brain tissue. The present study examined the effects on NA release from rat heart *in vitro* of sibutramine and its metabolites, in comparison with other weight-loss agents. Rat heart slices, loaded with [^{3}H]-NA in the presence of pargyline, were superfused with Krebs buffer with or without drug (50 mM KCl, *d*-fenfluramine, *d*-norfenfluramine, *d*-amphetamine, ephedrine, desipramine, sibutramine, BTS 54 354 or BTS 54 505, all 10^{-5}M). Sibutramine, BTS 54 354 and BTS 54 505 had no effect on [^{3}H]-NA release (Figure 1). KCl (50 mM) and *d*-fenfluramine, *d*-norfenfluramine, *d*-amphetamine, ephedrine and desipramine all enhanced [^{3}H]-NA overflow at 10^{-5} M (Figure 1).



These results clearly support the hypothesis that sibutramine, BTS 54 354 and BTS 54 505 are not sympathomimetic drugs and, in contrast to other weight-loss agents (*d*-amphetamine; *d*-fenfluramine) the NA effects of sibutramine are mediated via uptake inhibition, not release. These data also suggest that sibutramine will be a safe, efficacious treatment for obese diabetic patients.

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STUDY ON THE MECHANISM OF HYPOGLYCEMIC EFFECT OF OLEANOLIC ACID IN STREPTOZOTOCIN DIABETIC RATS

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To study the effect of oleanolic acid(OLA) on the glucose metabolism, insulin secret and the glucose transporter 4 gene expression in streptozotocin diabetic rats.The animals were divided into the following experimental groups of 10 animals: ① Control ② STZ-induced diabetic ③ OLA-treated STZ-induced diabetic(OD). After STZ was injected seven days OD group was injected OLA(50mg/kg.d) subcutaneous for 7d. In the 14th experimental day all animal were killed. The whole blood were collected and the soles muscle from each leg was removed intact, clamp-frozen in liquid nitrogen, and stored at -80 ℃ until analysis. Plasma insulin concentrations were determined by RIA. Plasma glucose were detected by Reflolux IIM (Germany) glucose meter. Total tissue RNA was extracted using a guanidine thiocyanate method. Using the GLUT4 cDNA as probe , the GLUT4 mRNA content in skeletal muscle in STZ diabetic rats was detected by Dot blot analysis. The GLUT4 mRNA contents of skeletal muscle decreased 27%($P<0.05$) in STZ-induced diabetic rats. In OLA treated STZ-induced diabetic rats blood glucose decreased from 22.20±2.53 to 16.92±1.41mmol/L($P<0.01$), plasma insulin increased from 25.40±8.23 to 30.67±7.01pmol/L ($P<0.05$), the GLUT4 mRNA increased 15.2% in the skeletal muscle($P<0.05$) The mechanism of hypoglycemic effect of oleanolic acid in streptozotocin diabetic rats was probably due to the improvement of insulin secretion and /or due to the relieved of whole-body insulin resistance.

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SUCCINIC ACID ALKYL ESTERS-NEW GENERIC ORAL ANTI-DIABETIC AGENTS IN ALLOXAN AND STZ DIABETIC RATS

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In 1988 McDonald with Fahien discovered the insulin secretagogue activity of succinic acid monomethyl ester(SAMME) and Malaisse with coll.reported in 1993-95 further new effects of SAMME and other alkyl esters in STZ diabetic rats.Author has tested the hypoglycemic activity of SAMME in alloxan diabetic rats in acute and chronic stage with hyperglycemia between 12 and 25mmol/l.In the acute stage with glycemia over 17mmol,SAMME was ineffective in 14 rats,but in mild acute diabetes 1mmol/kg of SAMME by gavage depressed glycemia of 9 to 4-5mmol in one and two hours.In the chronic stage of diabetes,1mmol/kg of SAMME administered by gavage,depressed hyperglycemia in 4 groups of rats,each of ten,determined at one,two and four hour:In the first group from 9 up to 2.9,in the second from 13.6 to 7.6,in the third from 16.9 to 9.6, 9.3 and 7mmol/l and in the fourthfrom 25.2up to 8.9mmol.SAMME administered in drinking water or by gavage exhibited no signs of toxicity or depression of food intake.Conclusion:SAMME has hyperglycemia and glycosuria depressing activity in alloxan and STZ diabetic rats at unchanged food intake and without signs of toxicity and succinic acid alkyl esters could be tried as generically new antidiabetic agents in human diabetes.

1475

CATALASE INACTIVATION IN THE PRESENCE OF 3-AMINO-1,2,4-TRIAZOLE:EFFECT OF DIABET AND NICOTINAMIDE.

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In investigation of irreversible catalase inactivation by 3-amino-1,2,4 triazole (AMT) was used the model of streptozotocine induced diabetes of rats. Sharp catalase inactivation started by AMT - concentration 20 mM for control rats and 10 mM for streptozocine diabetic rats. After nicotinamide injections (250 mg/kg during 14 days) catalase inactivation started by AMT - concentration 10 mM but was not strong.

With blood haemolysates it could be shown that incubation with 50mM AMT did not cause any diminution of the catalatic activity in comparison with liver suspension. However, the addition of ascorbic acid could effectively inhibition of catalase in the presence of AMT.

Increase of catalase inhibition in the erythrocytes of streptozotocine diabetic rats from 40±1,2% to 54±0,9% by concentration of ascorbic acid according from 25 to 100 μM in comparison to control rats: from 11±0,8% to 19±1,3%. Injections of nicotinamide to streptozotocine diabetic rats dicreased the catalase inhibition (from 16±0,6% to 36±1,4%).

Rat liver suspension and blood haemolysates exposed in the presence of AMT undergo AMD-dose dependent and ascorbic acid-dose dependent inactivation of catalase. Effect of nicotinamide was realised during descrease of catalase ihnibition of streprozocine diabetic rats.

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VANADATE CANNOT REPLACE INSULIN IN ALLOXAN,STZ OR BB DIABETIC YOUNG OR ADULT RATS

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Aim:To show how erroneous may be estimation of effectivity of an antidiabetic agent from results in vitro and extrapolate them to whole organism or of diabetes from glycemia only neglecting food intake and output of glucose in urine.Author administered 1,2 up to 4mmolar ammonium metavanadate(V+5 NH4VO3) in drinking water to 12 young(90-110g)and 12 adult ALL,21young STZ and 10 BB diabetic adult rats,both sexes aa,and determined daily,up to one month,their glycemia,glycosuria,food and water intake,volume of urine and glycosuria,body mass and compared these parameters with intermittently administered insulin.In all these three types and age groups of diabetic rats with hyperglycemia 18-22mmol/ldaily food intake 15-25g,V+5containing water intake of 40-90ml/d,urinary volume 30-70ml and glycosuria 3-4g/d,the drinking of V+5 water caused a decrease in food intake and glycemia but not an increase in glucose utilization,calculated from ingested carbohydrate and eliminated glucose.On the contrary,intermittently administered insulin increased food intake,assimlation of glucose,depressed glycemia,glycosuria,water intake and urinary volume ,promoted growth of young rats while V+5 retarded it.Conclusion:Although more than 11 authors on the basis of experiments in vitro in unphysiological media and high concentrations of vanadate,noxious to rats,claimed insulin-like effect of V+5,my results show that vanadate cannot replace insulin in diabetic rats.

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TUNGSTATE NORMALIZES DIABETIC STATUS BY INCRESING BETA CELLULAR MASS.

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It has been described that administration of tungstate (T) to an animal model of NIDDM, the neonatally STZ-injected diabetic rats, normalize glycemia with concomitant increase in insulin-secretion and insulin-content. Moreover, T treatment increase the B-cell mass in the pancreas. The aim of the study is to investigate whether this increase in B-cell mass is accompained by B-cell regeneration from pre-existing B-cells or from ductal cells.The animals treated with T were given a solution of 2 mg/ml of sodium T in distilled water during 1 month. Morphometric studies were made using indirect immunofluorescence. Beta-cell replication rate was estimated from in vivo incorporation of 5-bromo-2'-deoxyuridine (BrdU) in B-cell and double immunofluorescence with anti-BrdU and anti-insuline antibodies. **Results**: T normalized glycemia in diabetic animals (4.8±0.1 mmol/l v.s 8.3±1.2 mmol/l; $p<0.05$) and this normalization in glycemia was correlated with an increase in blood insulin levels (18.8±6.7 uU/ml v.s 60.0±5.1 uU/ ml; $p<0.005$). Concomitantly a recovery in the number of B-cell in the pancreas was observed (0.86±0.07% v.s 1.67±0.31; $p<0.05$). The BrdU experiments are described in the following table:

	Intra-islet (%)	Extra-islet (%)
Healthy rats		
Untreated (4)	4.52±0.07[a]	9.23± 0.90[b]
Treated (4)	17.54±2.99	33.89±2.92
Diabetic rats		
Untreated (4)	6.60±1.2	6.60±0.92
Treated (4)	3.03±0.03[c]	59.26±7.94[d]

[a] $p<0.001$ v.s H.Treated; [b] $p<0.001$ v.s H.Untreated; [c] $p<0.001$ v.s H.Treated; [d] $p<0.001$ v.s H.Treated.**Conclusions**:Tungstate-induced normalization of metabolic status,in diabetic animals, is associated with an increase in beta-cell replication from extra-islet precursor cells.

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IN MODEL DIABETIC CATARACT, PROTECTION BY R-α-LIPOATE INVOLVES MAINTENANCE OF LENS GLUTATHIONE.

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Aims: In previous work dihydrolipoic acid (DHLA) levels in rat lenses treated in an *in vitro* model of diabetes with R-, S- and Racemic-α-lipoic acid (LA) were identical, although only the R-α-LA reduced the cataractous damage (opacity and leakage of lactate dehydrogenase). This work extends the work to monitor glutathione (GSH) levels and medium lactate in this model.

Methods: Lenses from 200 g female Wistar rats were incubated in 5.5 and 55.6 mM glucose with added R-, S-, or racemic-LA. Glutathione levels of lens homogenates were determined by DTNB, lactate by an enzymic method, and DHLA and LA by HPLC.

Results: At 24 hr the GSH levels of lenses incubated with R-LA (1292 ± 116 μmol/mg) were not significantly different from normal controls (1222 ± 213 μmol/mg) but were significantly greater than for lenses incubated with S-LA (778 ± 112 μmol/mg) or racemic LA (738 ± 254 μmol/mg). At 24 hr, the levels of LA and DHLA were not significantly different in lenses treated with R, S or racemic-α-LA. By 48 hr Lactate levels in the medium were elevated above the 24 hr level but no significant differences between control and LA-incubated lenses were observed.

Conclusions: This result is consistent with specific protection of glutathione by R-α-LA.

Supported by Asta Medica, Frankfurt.

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INSULIN RELEASING EFFECTS OF SOME PURE COMPOUNDS FROM *HEMIDESMUS INDICUS* ON ISOLATED RAT ISLETS

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Hemidesmus indicus roots are used by traditional practitioners for the treatment of diabetes. Alcohol extract of *H indicus* root was tested on nondiabetic, IDDM model and on NIDDM model rats for hypoglycemic effect and was found to have significant antihyperglycemic effect. Four compounds (HI01, HI02, HI06 and HI07), isolated from *H indicus* root extracts by repeated chromatography and identified by spectroscopic methods, were tested for insulin secretion on islets from male Long-Evans rats isolated by collagenase digestion. Batches of 7-10 islets were studied under static incubation at 37oC for 60 minutes in 400 ml of HEPES buffered medium supplemented with 1mg/ml bovine serum albumin. The compounds, dissolved in DMSO, were used at a final concentration of 1mM and the media contained either 3 or 11mM glucose at a pH of 7.4. Insulin concentration in the supernatant was measured by an ELISA technique and the protein content of the islets were measured by a detergent compatible protein assay kit. The results were expressed as ng insulin secreted per mg of protein and the values of the experimental groups were compared with the control by Mann-Whitney test. The median value of the insulin at 3mM glucose concentration was 4.22 (Min 1.059-Max 15.213) and it increased to about 5 times in response to 11mM glucose (median 21.35, Min 12.791 - Max 39.362). None of the compounds had any significant effect on insulin release at 3mM glucose concentration. However, at 11mM glucose, compounds HI01 and HI07 were found to possess a significant stimulating effect on insulin release ($p<0.04$ and <0.004, respectively). The results showed that *H indicus* roots have hypoglycemic properties which may at least partly be due to the insulin releasing properties of these compounds.

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EFFECTS OF VANADATE AND INSULIN ON THE ACTIVITY OF AMINO TRANSFERASES IN THE RETICULOCYTES OF DIABETIC RATS.

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The enzymes of gluconeogenesis and protein catabolism increased in diabetes leading to an increase in the amino acid pool and TCA cycle intermediates in the cell. Alanine and aspartate aminotransferases play an important role in the degradation of amino acids. A significant increase in the activity of both alanine and aspartate aminotransferases was observed in reticlocytes isolated from diabetic animals. The administration of insulin and vanadate was found to reverse the activity of these enzymes almost to the control values. The present results show that there is an enhanced process of ageing in diabetes as shown by the increase in activity of the enzymes of protein catabolism. Vanadate is shown to be a potential insulin mimetic and antidiabetic agent and was found to normalize the altered state of diabetes.

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APPROACHES TO THE THERAPY OF DIABETIC CHEIROARTHROPATHY
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According to our data limited Joint mobility(LJM)was found in 10.7% of examined children. There is no specially developed treatment for diabetes mellitus(DM)patients. Adequate and timely dose of Insulin lessens vascular disorders, prevents LJM progress, improves their general condition. Taking into account pts' complaints, clinical manifestations and microcirculatory disturbances, for the first time 40 pts were treeated with Plidol (soluble acetylsalicilic acid with buffer of "Pliva" company and Trental). Control group consisted of 15 pts. Pts had Plidol 0.3 x 3 times a day for 10 days and then 0.3 x 2 times a day for 1.5-2 months.Pts were repeatedly examined in 3-6 months after treatment,which resulted in decreasing of pain syndrome and constraining of motions, increasing of their functional ability. Comparing to the control group practically all children demonstrated increasing of initial blood circulation,decreasing of sensitivity to sympathetic stimulation and response to local heating increased, though not in all cases these indices coinsided with the norm. There 65% of cases where we found that constrained motions and discomfort decreased. Active and passive joints motion increased.
Conclusion:Adequate Insulin therapy combined with the above additional methods of treatment results in noticeable improvement of clinical symptoms of diabetic cheiroarthropathy.

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EFFECT OF YOGA ON INSULIN SENSITIVITY.
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In order to examine the effect of Yoga on insulin sensitivity, a demonstrative study was conducted on five male NIDDM subjects of mean(±SE) age 54.1(±2.74) years and having detected diabetes for 7 7±2.2 years. Clinical examination, anthropometry, 3 hour OGTT and hyperinsulinemic euglycemic clamp studies at serum insulin levels of about 100uU/ml (CV plasma glucose 5.24±0.37) were performed before and after three days intensive Yoga training camp and home practice (30 minutes/day) for 8 weeks which had been reinforced by a follow up camp after 15 days. The mean insulin stimulated glucose uptake (M values) were 2.92±0.72 mg/kg/min and 3.69±0.8 mg/kg/min respectively (p=0.29). The fasting glucose (170.2±15.7 and 176.5±14 mg/dl, p=0.59), and insulin levels (16±3.4 and 24±7.1 uU/ml, p=0.07) or the glucose and insulin response to 3 hour OGTT (752.3±28.2 and 787.5±46.2 mg.h/dl, p=0.25; 58.4±10.2 and 99.8±27.5 uU.h/ml, p=0.07 respectively) were not significantly different. The changes in BMI (25.8±1.2 and 25.6±1.3 kg/meter square, p=0.358), waist hip ratio (1.05±0.02 and 1.06±0.02, p=0.86) and triceps and subscapular skin fold thickness (35.8±4.5 and 36.1±5 mm, p=0.86) were not significant. There was a marginal decline in the diastolic and systolic blood pressures (4±1.16 mm Hg, p=0.01 and 3.67±1.96 mm Hg, p=0.11 respectively). Though Yoga intervention tends to induce beneficial effects, the significance will have to be proven by a randomized trial.

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THE BENEFICIAL EFFECT OF NICOTINAMIDE ON BETA CELLS FUNCTION IN INSULIN DEPENDENT DIABETICS
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It has been shown previously that a different treatment in recent onset IDDM patients might have beneficial effect on beta cells function.Nicotinamide may induce clinical remission, as it was shown.The aim of this study was to estimate residual beta cells function(by measuring C-peptide before &6 min.after 1mg Glucagone)during the first year of IDDM duration.Investigation were performed in IDDM patients before and after clinical remission:12 were treated with insulin pump and with 250mg/day Nicotinamide (group A)and 10 were treated with insulin pump only(group B). Patients from group A were ages 20,28+/-4,16 yr. and from group B 16,78+/- 5,12 yr. Glucagon test were performed at time of diagnosis,at time of remission, and in 3rd,6th and 12th month after. Duration of clinical remission in group A was 4,3+/-3,6months and in group B 4,6+/-2,3 months.Mean C-peptide values(nmol/l) in 0 and 6th minutes for both groups are shown on the table:

Group	Time of Dg	Remission	3mo	6mo	12mo
A	0 min	0,33+/-0,13	0,39+/-0,03	0,48+/-0,14	0,39+/-0,17	0,33+/-0,12
B	0 min	0,22+/-0,09	0,43+/- 0,11	0,39+/-0,14	0,24+/-0,18	0,14+/-0,12
	p	<0,05	>0,05	<0,05	<0,05	<0,05
A	6 min	0,56+/-0,23	0,68+/-0,13	0,67+/-0,13	0,57+/-0,23	0,47+/-0,20
B	6 min	0,36+/-0,10	0,70+/-0,22	0,57+/-0,21	0,35+/-0,23	0,17+/-0,15
	p	<0,05	>0,05	>0,05	<0,05	<0,05

Our results suggest that in spite of similar duration of remission, patients from group A who were treated with small doses of Nicotinamide had better preservation of beta cells function during the first years of duration of IDDM(basal & after stimulation Glucagon).

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RHIGF-I IMPROVES CORONARY RISK FACTORS IN TYPE II DIABETES.
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Type II diabetics suffer from an insulin resistance syndrome which predisposes them to premature atherosclerosis. rhIGF-I increases insulin sensitivity in patients with impaired glucose tolerance and overt hyperglycemia. Effects of rhIGF-I on known cardiovascular (CV) risk factors were investigated in clinical trials. In one trial, 212 patients taken off prior therapy (insulin/ oral agents) were randomized to receive placebo or rhIGF-I: 10, 20 40 or 80 µg/kg BID for 12 weeks. There was a statistically significant, dose-dependent decrease in fasting triglyceride levels (p=0.0013). Despite significant dose-dependent decreases in HbA1c there was no increase in body weight. In a second trial, 139 insulin-requiring type II patients instructed to alter their insulin regimen to meet aggressive glycemic goals were randomized to receive co-therapy with placebo or rhIGF-I: 20/20, 40/40 or 80/40 (am/pm) µg/kg. Preliminary results demonstrate that despite a significant decrease in HbA1c in all rhIGF-I treated groups vs placebo, weight did not increase. However, visceral fat, determined by CT scan, decreased by 14.7% in the 20 µg/kg BID dose groups as compared to a 0.14% increase in the placebo (p=0.023) group . PAI-1 and fibrinogen levels decreased in all rhIGF-I treated arms; these trends did not achieve statistical significance. Insulin use decreased in the 2 highest rhIGF-I dose groups. The most commonly reported adverse events related to drug treatment in both studies were edema and jaw pain. There was no increase in incidence of CV events (angina, arrhythmia, myocardial infarction or stroke).

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LATE PHASE II STUDY OF A NOVEL ORAL HYPOGLYCEMIC AGENT, A-4166 IN NIDDM SUBJECTS IN JAPAN

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A new oral, non-SU, hypoglycemic agent, A-4166, was found to evoke a more rapid and short-lived insulin release than SU and to decrease the postprandial rise in plasma glucose. In a randomized double-blind four arm comparative clinical trial, the optimal dose and safety of A-4166 were studied in 184 NIDDM subjects, whose glycemic control was not adequately controlled (FPG≥120mg/dl) with diet therapy. They were randomly assigned to receive either 30, 60, 90 or 120mg A-4166. The tablets were administered 5-10min before each meal for 3 months. After treatment, the mean postprandial plasma glucose level was lowered at all time points and the decrements were progressively greater up to 60min (90mg; -57.2±37.7mg/dl at 60min). Fasting plasma glucose level and HbA1c were reduced over the treatment period with the two higher doses (90mg; -15.5±23.0 mg/dl and -0.77±0.95 % at 12 wks). The plasma insulin level was raised to twice the baseline value at 30min, with progressively smaller increments thereafter (90mg; 38.1±24.0 μU/ml at 30min vs baseline; 17.6±10.0 μU/ml). The decrease in plasma glucose response (AUC) and HbA1c and the increase in plasma insulin response were maximal with 90mg A-4166 and over. No accumulation of the drug was found in the blood. No alteration in body weight was observed during the treatment period. 21 possibly drug-related adverse events were observed. They were reversible and not severe, and half of them (5% of the patients studied) were probable hypoglycemic symptoms. In conclusion, these results indicate that administration of 90mg A-4166 before each meal is optimal in treatment of NIDDM.

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Effect of an aldose reductase inhibitor on membrane abnormalities of erythrocytes in NIDDM.

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We examined alteration in erythrocyte membrane fluidity in non-insulin dependent diabetic patients and the effects of aldose reductase inhibitor (ARI) on these changes by means of electron spin resonance(ESR) and spin-label technique.Electoron spin resonance spectra foa a fatty acid spin label-agent(5-nitroxy stearate) in membranes were obtained. The values of hyperfine splitting and order parameters of the spectra were significantly higher in erythrocyte from diabetic patients than non-diabetic controls.(hypersplitting 60.66±2.38 VS 55.21±1.60 $P<0.05$, order parameters 0.778±0.009 VS 0.620±0.01 $P<0.05$)
The effects of aldose reductase inhibitor on the erythrocyte membrane fluidity in non-insulin dependent diabetic patients without nephropathy and anemia were also studied.(aldose reductase inhibitor 150mg/day × 28days) Aldose reductase inhibitor decreased the values of the hyperfine splitting and order parameters of the spectra. (hyperfine splitting pre-treatment 60.66±2.38 → 58.71±1.10, order parameters pre-treatment 0.778±0.009 → 0.743±0.010) These findings indicate that the erythrocyte membrane fluidity might be decreased in diabeic patients and aldose redutase inhibitor might improve thease membrane fluidity.

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RHIGF-I REDUCES GROWTH HORMONE SECRETION, LIPID LEVELS & INSULIN REQUIREMENTS IN ADULTS WITH IDDM.

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Insulin dependent diabetes mellitus (IDDM) is associated with elevated circulating levels of growth hormone (GH) but reduced insulin-like growth factor-I (IGF-I). The effects of subcutaneous recombinant human IGF-I (rhIGF-I) on glycaemic control, insulin requirement, plasma lipid concentration and GH secretion were studied in 6 adults (35±4, years, mean±SEM) with IDDM. Patients received either placebo or rhIGF-I (50 μg/kg BID) for 19 days in a randomised, double blind, placebo controlled trial. Overnight GH profile, serum free insulin, IGF-I, fructosamine and lipid profile were assessed at regular intervals during rhIGF-I therapy. RhIGF-I therapy increased IGF-I concentration (111.7±14.2 v 257.1±41.2, ng/ml, $p<0.01$, day 1 v day 20). Plasma fructosamine concentration was unchanged (439±32 v 429±35, μmol/l) and insulin requirement decreased by approximately 45% (0.67±0.08v 0.36±0.07, U/kg/day, $p<0.005$) following 19 days of rhIGF-I treatment. After 4 days of rhIGF-I therapy, there was a decrease in free insulin levels (8.38±1.47 v 4.98±0.84, mU/l, $p<0.05$), mean overnight GH concentration (12.6±3.3 v 3.8±2.1, mU/l, $p<0.05$) and total cholesterol and triglycerides (4.68±0.31 v 4.25±0.35, mmol/l, $p<0.05$, 1.27±0.19 v 0.95±0.21, mmol/l, $p<0.001$, respectively). This study demonstrates that subcutaneous administration of rhIGF-I decreases insulin requirements and improves the plasma lipid profile while maintaining glycaemic control in adults with IDDM. GH secretion is also decreased by rhIGF-I therapy. Exogenous rhIGF-I therapy may have a role in the treatment of adults with IDDM particularly in the setting of abnormal lipids and a high insulin requirement.

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ERGOSET IMPROVES PLASMA GLUCOSE AND LIPID PROFILE AND BODY COMPOSITION IN WEIGHT MAINTAINED OBESE WOMEN.

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Bromocriptine, a sympatholytic dopamine D_2 agonist has been shown to reduce obesity and glucose intolerance in animals in part by resetting circadian hypothalamic neural activities known to regulate metabolism. Ergoset, a quick release formulation of bromocriptine, in combination with a moderate hypocaloric diet has been demonstrated to improve glucose tolerance and reduce body weight in obese subjects when compared to individuals treated with diet alone. The present study evaluated the metabolic response of obese patients to Ergoset while they were on a weight maintaining diet. Following a two week run in period, thirteen obese (BMI= 33.2 ± 0.8 kg/m^2), hyperinsulinemic, nondiabetic women were given Ergoset once daily (1.6-4.8 mg) at 0800 for 8 weeks and were instructed to follow a weight maintaining diet throughout the entire study. Prior to the initiation and following termination of treatment, body composition was determined by hydrodensitometry and circulating levels of glucose, insulin, triglyceride and free fatty acid (FFA) were measured hourly over a 24 hour period with standardized meals eaten at breakfast, lunch, and dinner. Ergoset treatment reduced fasting plasma glucose (5.6 ± 0.1 vs 5.0 ± 0.1 mM) fasting triglyceride (2.4 ± 0.5 vs 2.0 ± 0.4 mM) fasting cholesterol (5.2 ± 0.3 vs 4.8 ± 0.2mM) ($P< 0.05$) and cholesterol to HDL cholesterol ratio (5.9 ± 0.6 vs 5.1 ± 0.5) ($P=0.06$). In addition, plasma levels of glucose, triglyceride, and FFA measured hourly over a 24 hour period were reduced on average by approximately 5%, 15%, and 10%, respectively ($P<0.05$). Plasma insulin levels were unaffected by treatment other than an approximate 30% decrease in fasting and post-lunch values which did not quite reach statistical significance. Furthermore, body density (lean to fat mass ratio) increased following treatment (0.981 ±0.002 vs 0.982 ± 0.002 kg/L) ($P<0.05$) associated with a decrease in body fat (43.7 ± 2.2% vs 43.2 ± 1.4%) even though body weight was maintained throughout the study as intended (74.2 ±1.3 vs 73.9 ± 1.3 kg). The results of this short term (8 weeks) study indicate that chronic treatment with Ergoset could have substantial multiple benefits in various clinical conditions of obesity, hyperglycemia, and/or dyslipidemia independent of dietary intervention, which however, may amplify these effects.

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THE EFFECTS OF DEXFENFLURAMINE ON CARBOHYDRATE METABOLİSM IN OBESE NIDDM PATIENTS

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To observe the effects of dexfenfluramine, two groups of obese NIDDM cases with poor glycemic regulation under treatment with either diet alone or sulfonylurea along with appropriate diabetic diet were investigated.After an eight week period during which the patients in both groups were observed for the state of glycemic regulation, dexfenfluramine in group 1 (16 patients, 12 females and 4 males; mean age 50,1 ± 7,1 years) and placebo, in group 2 (14 patients; 11 females and 3 males; mean age 48,4 ±8 years) was added to treatment and the patients were followed for 24 weeks and evaluated every six weeks.In group1,as compared with pretreatment values, significantly lower mean values were obtained after treatment with dexfenfluramine for body mass index (BMI) (33.3±4.6 v.s. 30.1±5.0 kg/m^2;$p<0.001$), HbA_{1c} (8.9±1.2 v.s. 6.7±0.5%;$p<0.001$),fasting plasma glucose (FPG) (10.6±2.6 v.s. 7.07±0.8 mmol/L;$p<0.001$) and two hours postprandial plasma glucose (PPG) (12±3.4 v.s. 7.7±1.3 mmol/L;$p<0.001$) levels, serum total cholesterol (TC) (12.9±1.4 v.s. 10.9±1.1 mmol/L;$p<0.001$) and serum triglyceride (TG) (12.1±4.3 v.s. 8.1±1.7 mmol/L;$p<0.001$) levels. In group 2, no significant change was observed, following treatment with placebo, in the mean levels of FPG (9.6±1.1 v.s. 9.3±1.6 mmol/L;$p<0.05$), PPG (10.9±2.5 v.s. 11.2±1.5 mmol/L;$p>0.05$), HbA_{1c} (8.8±1.4 v.s. 8.3±1.5 %;$p>0.05$) and TC (12.5±1 v.s. 12.3±1.1 mmol/L;$p>0.05$), while there were significant decreases in the mean BMI (32.1±2.7 v.s. 30.7±2.6 kg/m^2;$p<0.001$)and serum TG (11.4±5.3 v.s. 10.8±4.6 mmol/L;$p<0.05$) values. No significant changes were found following treatment in the fasting and 2 h. postprandial serum insulin levels which could be measured in 4 patients from group 1 (20.3±12.4 v.s. 18.2±10.4 pmol/L;50.5±23.9 v.s. 46.7±22.4 pmol/Lp>0.05,respectively)and in 6 patients group 2 (16.3±9 v.s. 14.9±7.6 pmol/L;51.4±27.9 v.s. 46.9±27.1 pmol/L,p>0.05,respectively). Although the two groups were similar with respect to the mean BMI, FPG, PPG, HbA1c,TC and TG values before treatment, significant differences came about between groups with respect to the mean FPG, PPG, HbA_{1c} and TC levels ($p<0,01$).Our results suggest that dexfenfluramine can help in glycemic regulation and in the correction of lipid anomalies in NIDDM.

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THE EFFICACY OF PIRENZEPINE IN REDUCING POSTPRANDIAL GLUCOSE LEVELS IN PATIENTS WITH NIDDM FOLLOWING TWO WEEKS OF THERAPY

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Acute cholinergic muscarinic blockade with pirenzepine (PIR) induces dose-related reductions in plasma glucose (PG) and insulin after mixed meals in normals and patients with NIDDM. We have investigated further the effects of PIR in NIDDM. 24 patients (12 M, 12 F), on no oral hypoglycaemics for at least 2 weeks, received PIR (50 mg b.d.) or placebo for 2 weeks (randomised, double blind, crossover with 2 week washout period between treatment arms). At the beginning and end of each treatment period, blood was sampled over 12 hours during which the patients received standard breakfast, lunch and dinner. The mean (± SEM) AUC (145 ± 9 vs 155 ± 9), peak (16.5 ± 0.9 vs 17.7 ± 1.0) and nadir (9.1 ± 0.6 vs 9.9 ± 0.7) PG levels (mmol/L) were all significantly less ($p < 0.05$) after PIR for 2 weeks compared with placebo whereas insulin was unchanged.. The drug was well tolerated. In conclusion this low dose of PIR causes a small, but significant and sustained reduction in PG levels in NIDDM, unaccompanied by any rise in insulin levels. On the basis of previously reported acute dose-response studies, higher doses of PIR will probably produce greater lowering of plasma glucose and significant reduction of insulin levels in NIDDM. These studies should now be undertaken.

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EFFECT OF VITAMIN E (E) AND PLACEBO (P) SUPPLEMENTATION (SUPPL) ON PLATELET AGGREGABILITY(PA) AND LIPID PEROXIDE (LP) LEVELS IN TYPE-1 DIABETIC PATIENTS (D).

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Hyperaggregability of platelets is a major risk factor in the development of thrombotic disease in diabetic patients. This study examines whether E suppl has any effect on PA and LP levels in D. After written informed consent, D were suppl with DL-α-tocopherol (E) capsule (orally,100 IU/day) or P for 3 months in a double-blind clinical trials. Alternate D were assigned to E or P during regular visits to the clinic. Fasting blood was collected before the start and after the E or P suppl from each D. Plasma E and LP (as MDA, a product of LP) were determined by HPLC; PA by the competitive ELISA immunoassay of the stable thromboxane analogue (TxB_2); and platelet counts by Coulter Counter. After the analyses and code opening data were analyzed using the paired 't' test on 12 D on E and 12 D on P suppl. Level (±SE) after E suppl versus before suppl are as follows: E 29±1.8 vs 17±1.2 nmol/ml, $p<0.001$; LP 0.33±0.03 vs 0.42±0.05 nmol/ml ($p<0.05$);TxB_2 771 vs 1569 pg/ml ($p<0.03$); there was no differences in these parameters after P suppl vs before suppl. There was no effect on platelet counts after P or E-suppl in D. There were no differences in the ages (12.7±1 vs 12±1yrs) or duration of diabetes (5.7±1vs 4.7±0.8 yrs) between P- and E- groups. This study suggests that E suppl significantly lowers cellular oxidative damage and platelet aggregation, and may be beneficial in reducing risk of thrombotic disease in diabetic patients.

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EFFECTS OF SEROTONIN RE-UPTAKE INHIBITORS IN NIDDM.

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Considering the role of serotoninergic systems in the control of food intake of obese patients, we studied the effect of: 30 mg/d of dexflenfluramine (Df), 20 mg/d of fluoxetine (Fx) and 75 mg/d of venlafaxine (Vx) on metabolic control in 3 groups of 10 obese NIDDM for 3 months by the Kruskal-Wallis test. There were side effects that led to the interruption of treatment in the following cases: 1 patient in the Df group (sickness); 2 in the Fx group (anxiety and sleepless) and 8 in the Vx group (vomiting, anxiety, hypertension). Dx significantly reduced ($p<0.05$): body weight (9.6±2 Kg versus 7.2±1 for Fx and 2.2±1 for Vx), plasma triglycerides (62±12 mg/dl versus 11±2 for Fx and 0 for Vx), insulin requirement (10.5±2% versus, 5.1±1% for Fx and 0 for Vx) and C-Peptide plasma levels (1±0.5 ng/ml versus 0.2±0.8 for Fx and 0 for Vx). The reduction in blood pressure was not significant. Plasma levels of 3-OH butirate and aceto-acetate were within normal ranges. The reduction of C-Peptide plasma levels to normal ranges appears after the first month with only 3±1 Kg weight loss. In conclusion, this appears to indicate important differences between different types of serotoninergic drugs in anorexic treatment.

1493

EFFECT OF MEDIUM CHAIN TRIGLYCERIDE AND UNIQUINONE ON METABOLIC PHASE.

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This experiment was performed to examine a metabolic effect of medium chain triglyceride and unique upon diabetes millitus. A test diet, MCT diet which is containing 28.05g of MCT was administered to the subjects after for 12 hours. Blood samples were collected before, 30, 50, 90, and 120 minutes after the diet was administered, and observed blood lenels of sugar, acetone body, NEFA, and total cholesterol. Thus the administration of MCT diet to patient of disbetics seems to aggravate an acidotie tendency uhiquinone was administered to the diabetes patients for checking a ketotic tendency which may be caused by MCT administration. The MCT tolerance test was restrained by the daily administration of 120mg of Co. Q. by the oral route. In the normal cases after administration of MCT diet with Co. Q., changing rates of blood sugar, acetone body, total cholesterol were not increased, but NEFA was increased. In the case of diabetes millitus an increase in changing rate of blood sugar was observed, where as the rates of acetone body and total cholesterol were decreased, but the rate of NEFA was no difference. It can be assumed that therapeutic effect of MCA with Co. Q., administration may be significant for the supply of dietary fat to mild diabetics.

1494

BRADIKININ AS A FACTOR OF THE IMPROVEMENT OF INSULIN RESISTANCE IN NIDDM, HYPERTENSIVE PATIENTS.

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In the larger group of NIDDM patients the dominant defect is an impairment of the tissue sensitivity to insulin. The insulin resistance syndrome which includes obesity, NIDDM, hypertension, hyperinsulinemia and dislipidemia is a major and increasing cause of morbidity and mortality. The reasons why all these disorders co-exist so frequently are still the subject of intensive research. We have examined 51 patients with NIDDM and essential hypertension on different hypoglycaemic therapy. All of them were comparable at age, BMI, duration of NIDDM and hypertension, without renal failure. We evaluated parameters of β-cell function and tissue sensitivity by measuring of C-peptide, IRI, glucose levels in serum (fasting, 60, 120 min after carbohydrate nutrition) during standard test with meal stimulation. We also have studied a possible contribution of plasma kallikrein, prekallikrein, ACE activity, proteas inhibitors, elastase-like activity, thromboxan B_4 in connection with improvement of insulin sensitivity by ACE-inhibitor. All these tests were repeated after 3 months treatment by Perindopril 4-8 mg. We determined that all the patients had high levels of basic hyperinsulinaemia, as well as after meal stimulation which were accompanied by high C-peptide and glucose levels, high activity of ACE, high blood pressure, as well as activity of kallikrein-kinin system (KKS) and increased of elastase-like (1,7 times) in plasma. Furthermore, we evaluated the influence of ACE-inhibitor Perindopril on tissue insulin sensitivity using hyperinsulinaemic euglycaemic clamp test in 7 patients which improved markedly after 60 min taking it per os. The activation of KKS certainly means increased concentration of bradikinin in plasma, which destroys quickly if the ACE activity is high. After Perindopril administration ACE activity sharply decreased but activity of KKS preserved at the same level, so as a result bradikinin effect prolonged that was linked with decreasing of blood pressure and concomitant improvement of glucose utilisation can be the consequence of insulin-like action of bradikinin itself both during clamp test and after 3 month treatment. Improvement of insulin sensitivity was proved by marked decreasing of hyperinsulinaemia (IRI, C-peptide, glucose levels) and HbA1c.

1495

THE EFFECT OF DIABETIC COMPLICATIONS AND DRUGS FOR Na-Li COUNTERTRANSPORT ACTIVITY IN RED CELLS.

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A growing body of evidence suggests that sodium-lithium countertransport (SLC) in red blood cells (RBC), a marker of risk for essential hypertension (HT), can serve as a maker for the risk of renal disease in IDDM. However, the findings of SLC in NIDDM are conflicting. The purpose of this study was to evaluate the relation of SLC activity and diabetic complications (nephropathy, neuropathy, retinopathy, hypertension) in Japanese NIDDM. We also investigated that the effect of insulin, antihypertensive drugs (ACE inhibitor: captopril, lisinopril, Ca antagonist: diltiazem, barnidipine, nicardipine, amrodipine, α β blocker: amosulalol, α_1 blocker: doxazosin) and protein kinase C (PKC) inhibitor (calphostin C) for SLC activity in vitro. The levels of SLC, BP, albminuia, TG, total cholesterol (Tch), LDL, HDL, VLDL in 142 subjects; 24 normal controls (C), 27 non diabetics with HT, 32 diabetic without HT (DM), 59 diabetics with HT (DM-HT) were assessed. SLC values was significantly higher in DM-HT (0.39±0.02 mmol/lRBC/hr) than in the C (0.19±0.002, P<0.0001) or in the DM (0.22 ±0.01, P<0.0001). SLC were related to TG (P<0.005), systolic BP (P<0.0001), and to diastolic BP (P<0.005). No statistical association were seen between the levels of SLC and the levels of Tch, HDL, or VLDL. SLC was statistically higher in NIDDM patients with retinopathy (0.4±0.02) than in those without retinopathy (0.22±0.02, P<0.0001), however, there was no difference in neuropathy. SLC was higher in the NIDDM with microalbuminuria (0.36±0.17) than in those without microalbuminuria (0.29±0.15, P<0.05). In *in vitro* study, only Ca-antagonist, diltiazem and PKC inhibitor, calphostin C significantly lowered SLC activity. These findings suggest that over activity of SLC is associated with a higher blood pressure in NIDDM and SLC may serve as a useful marker for the risk of renal disease and retinopathy in patients with NIDDM. Ca-antagonist diltiszem and PKC inhibitor has an effect to lower SLC activity.

1496

OPEN STUDY OF CILOSTAZOL AMONG NON-INSULIN DEPENDENT DIABETES MELLITUS PATIENTS WITH ARTERIOSCLEROSIS OBLITERANS.

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Cilostazol is a new antiplatelet agent and also acts as a direct arterial vasodilator. The purpose of this study is to determine the clinical effectiveness, safety and usefulness of Cilostazol in NIDDM patients with chronic arterial occlusion.

NIDDM patients diagnosed as having arterioslerosis obliterans with a most frequent manifestation of Intermittent Claudication and doppler Ankle Brachial Index of less than 0.9 were included. Patient were given Cilostazol 100 mg PO BID for 12 weeks, Primary outcome measures included walking performance by getting the Initial Claudication Distance (ICD) and Absolute Claudication Distance (ACD) on standardized treadmill testing. Hematologic exams were done every 2 weeks as well as urinalysis. Repeated measures Analysis of variance ANOVA was used to compare clinical test results over time.

This interim report of 19 patients 12 of which are male and 7 female age ranged from 41 to 69 (mean 59.4 SD 7.4) showed significant improvement in treadmill walking performance (ICD and ACD). Presenting symptoms of pain, numbness and cold sensation were ameliorated.

Cilostazol was considered safe in 18 patients (one patient had elevated liver function test which improved on discontinuation of the medicine) Inspite of the adverse experience Cilostazol was still evaluated as usefull and safe for this kind of disease.

1497

HAEMOSTASIS SYSTEM IN DIABETIC PATIENTS ON BACKGROUND OF TREATMENT WITH TOPINAMBUR

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The opportunities of corrections of infringements in varios links of haemostasis system among IDDM and NIDDM patients , with topinambur , included in conventional treatment complex investigated. The patients took topinamibur 150-200 g as a salad at breakfast and lunch. Duration of diet therapy-21 day. 198 patients who took topinamibur were examined. 79 were IDDM patients, 64 NIDDM patients had obesity, 55 NIDDM patients didn't have it. Analogous groups of 105 patients were control. Adding topinamibur had a clear clinical effect, after the course of diet therapy all paticnts noted improvement : decrease or vanishing of diabetes' symptoms. Fasting plasma glucose decreased from 9,2 mmol/L to 5,6 mmol/L. HbA1c level - from 6,66% to 5,04% . Several IDDM patients had their insulin dose diminished. Lipids level IDDM - from 5,76 to 5,66 g/L, NIDDM - from 5,01 to 4,84 g/L. Cholesterol level IDDM - from 6,47 to 5,51 mmol/L ($p < 0,05$), NIDDM - from 7,2 to 5,5 mmol/L($p < 0,05$). B-lipoproteids level NIDDM - from 43,6 to 27,1 mmol/L ($p < 0,05$), IDDM - from 59,5 to 42, mmol/L($p < 0,05$). Adding topinamibur led to a decrease of the daily insulin dose for 10 of 16 paticats for 4-6 IU. In the control group a decrease of the insulin dose was possible only for 5 patients , 4 patients had it increased. General lipids and cholesterol's level during topinamibur treatment diminished from 5,2 to 5,7 mmol/L and from 5,52 to 5,15 mmol/L. Adding topinamibur to the patients' ration resulted in weakening of initial thrombocytes hypercoagulation and hyperagregation and in fibrinolysis' normalisation. The course of treatment led to an increase in coagulation time. The level of fibrinogen in blood plasma was higher than that in the control group. The treatment resulted in decrease fibrinogen level to a norm. The therapy had a decrease of dissolved fibrin-monomer complexes level as a result. Initially decreased a number of trombocytes increased during the course.Including topinambur in medical complex doesn't just improve the general condition, but also promotes weakening of initial thrombocytes ' hypercoagulation and hyperagregation and fibrinolysis' activation. Received data allow to recommend wide usage of topinamibur during diabetes.

1498

THE EFFECT OF NORMOBARIC HYPOXIA ON OXYGEN-TRANSPORT SYSTEM IN PATIENTS WITH DIABETES MELLITUS.

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The tissue hypoxia and disturbance of oxygen - transport influence on development of complications of diabetes mellitus. The aim of the work was to evaluate the effect of interval hypoxic training (IHT) on oxygen-transport system and index of tissue hypoxia in patients with non-insulin-dependent diabetes mellitus (NIDDM).The examination was carried out before and after the IHT course. The IHT (10-11,5% O_2) was performed for 19-21 days using a hypoxicator. 21 patients 35-55 years old (2-m, 19-f) with mild and moderate NIDDM,with good metabolic control were examined. All patients were treated with oral hypoglucemic agents,dosages remained unchanged during the IHT. The control group consisted of 21 healthy women (35-55 years).
After the IHT course in NIDDM patients increase of hemoglobin Hb (g/l) 120,2±3,42 - 138,0±4,31 ($p<0,05$),reticulocytes 1,2±0,13 - - 13,9±0,33 ($p<0,001$); decrease of anomalous fraction of hemoglobin HbF (%) 2,04±0,08 - 1,11±0,06 ($p<0,001$), HbA_{fc}(%) 8,2±0,94 - 7,6±0,53; decrease of index tissue hypoxia: lactate (mmol/l) 2,5±0,16 - 1,7±0,12 ($p<0,01$), pyruvate (mkmol/l) 75,6±0,64 - 57,1±0,9 ($p<0,001$)were observed. The data as compared to the control group were statistical significant. Therefore the IHT is a non-drug method, which exerts a beneficial effect on oxygen-transport system and tissue hypoxia in NIDDM patients.

1499

TREATMENT OF IDDM WITH IMMUNOMODULIN

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Clinical imvestigations of immunomodulin efficiency in the treatment of newly diagnosed IDDM has been carried out. Immunogram, fasting and postprandial glucoses, HbA1c, serum C-peptide concentration were examined immuno-modulin is a preparation which consists of 16 highly purified natural peptides (molecular weight 1-6 kDa), prepared from lambs fetuses' and infants' thymus. 10 patients (aged 29,5±14) took immunomodulin at the dose of 1,0 ml 0,01% sol - 10 injections for a course (first 5 injections - in a day, the rest - twice a week). Clinical criteria corresponded to IDDM. The diabetes duration 1 week - 3 years. The daily dose of insulin 36±5 IU, fasting plasma glucose - 10.7±3.8 mmol/L, 2-h postprandial glycaemia - 11.6±4.5 mmol/L. After the course of treatment with immunomodulin patients with diabetes duration up to 6 months (8 patients) had been noted to have regular hypoglycaemia, a new daily dose of 20±8 IU was enough to compensate diabetes. Insulinotherapy for 2 patients was abolished. The patient with diabetes duration 1,5 yrs (daily dose 38 IU didn't conmensate diabetes, fasting plasma glucose 13,6 mmol/L, 2-h postprandial glycaemia - 21,6 mmol/L noted night hypoglycaemia, accordingly has insulin dose been reduced to 35 IU. The fasting plasma glucose had decreased to 6,1 mmol/L, 2-h postprandial glycaemia up to 6,0 mmol/L. A female patient with diabetes duration of 3 yrs hadn't had her daily insulin dose changed but for the 1st time compensation had been reached (fasting plasma glucose 5,4±1,1 mmol/L, 2-h postprandial glycaemia- 6,4±1,1 mmol/L). The HbAlc had reduced on the average from 6,9±0,41% to 5,8±0,5%. Serum C-peptide concentration had increased from 0,078±0,035 mg/ml to 0,28±0,17 mg/ml; the earlier the treatment had started the higher increase was (patients who started to take immunomodulin straight after manifestation had their Serum C-peptide concentration more than 5 times increased, 2 patients had it returned to normal, a patient with diabetes duration of 3 yrs had it insignificantly decreased). Immunogram indexes had improved. All patients have been examined for more thar 6 months, their condition hadn't changed glycaemic level suits limits of diabetes complication criterias, insulin dose is the same. Thus, accordind preliminary data, the immunomodulin usage for IDDM has positive effect on carbo-hydrate exchange, insulin secretion and immune system.

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TREATMENT INEFFECTIVENESS OF RECTAL GLUCAGON DELIVERY IN HYPOGLYCAEMIA.

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Introduction: Glucagon secretion in response to hypoglycaemia is absent in patients with more than a ten year history of insulin-dependent diabetes mellitus (IDDM). Previously we have shown that witepsol H15 base suppostories containing 100mg indomethacin and 1mg glucagon produce significant plasma glucose increments in fasting healthy volunteers. We assessed the usefulness of rectal glucagon delivery for treating insulin-induced hypoglycaemia in IDDM patients. **Method:** Five male patients with uncomplicated IDDM of at least 10 years' duration (age 21-38 years) were studied supine after an overnight fast on two separate occasions at least 14 days apart. After 45 minutes rest, baseline blood samples were taken for measurement of concentrations of glucose, glucagon and catecholamines Hypoglycaemia was induced by intravenous insulin infusion (2.5 mU/kg/min) which was stopped when plasma glucose concentration reached 2.5 mmol/l As soon as subjects described hypoglycaemic reaction (R), they inserted a suppository containing 100mg indomethacin (placebo) or 100mg indomethacin plus 1mg glucagon (glucagon) into their rectum. Measurements were repeated at intervals for 120 minutes. Local ethics committee approval was granted for this study. **Results:** (Mean[sem] plasma concentration; paired t-test used to compare between-group differences. **p=0.006).

	Glucose nadir (mmol/l)	Peak glucagon (ng/l)	Glucose recovery rate (mmol/l/hr) Over 60 minutes	Over 120 minutes
Placebo	1.8[0.3]	99.2[9.4]	0.63[0.11]	1.47[0.15]
Glucagon	2.1[0.5]	175.8[12.8]**	0.63[0.18]	1.69[0.42]

Summary & Conclusion: Rectal glucagon administration at R induced plasma glucagon concentrations of the order seen following hypoglycaemia in healthy volunteers. However, this had no effect on glucose recovery rates. We conclude that rectal delivery of glucagon is ineffective in treating insulin-induced hypoglycaemia in patients with IDDM

1501

INDUCIBLE INSULIN EXPRESSION IN A HUMAN HEPATOMA CELL LINE

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Somatic gene therapy is one strategy being considered to correct patient blood glucose concentrations in Type I diabetes. An earlier study by this group revealed that the stable transfection of insulin cDNA under the control of a constitutive promoter into a liver cell line (HEP G2) resulted in synthesis, storage and acute regulated release of insulin. The aim of the present study was to investigate insulin expression and secretion under the control of inducible promoter systems. A cDNA for human insulin (pC_2) has been inserted into the dexamethasone (dex)-inducible mammalian expression vector pMAM-*neo* (HEP G2-mam cells) and an ecdysone-inducible promoter pSP72-EcRE-insulin (HEP G2-EcR cells). The ecdysone promoter isolated from *Drosphilia melanogaster* has been shown to be inducible in CHO cells by exposure to the ecdysone analogues ponasterone A (pon) and muristerone A (mur). The HEP G2 cells were transfected by electroporation and stable transfectants were selected following exposure to selective antibiotics. In the presence of 1 μM dex (pro)insulin (ins) secretion of HEP G2-mam cells increased from 0.33 ± 0.05 pmoles/ 10^5 cells/ day to 2.1 ± 0.4 (n=6). Increasing concentrations of dex produced no rise in the level of ins secretion. The ins content of HEP G2-mam cells grown in the presence of 1 μM dex (3.2 ± 0.2 pmoles/ 10^5 cells) was significantly ($P < 0.01$, n=5) higher than in its absence (0.8 ± 0.1), indicating that both the transcription and translation of the insulin gene have been stimulated. Gel mobility shift assays indicated that HEP G2-EcR cells were capable of expressing biologically functional ecdysone receptor protein which bound to EcRE DNA in a hormone-dependent manner. HEP G2-EcR cells grown in the presence of 50 μM pon or mur overnight indicated that mRNA transcription was induced by the presence of the hormones, none being detected in unstimulated cells. Our results indicate that the introduction of insulin cDNA under the control of an inducible promoter system results in transcription and translation of the insulin message.

1502

THE EFFICACY OF D-400 IN THE TREATMENT OF NON-INSULIN DEPENDENT DIABETES MELLITUS

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D-400 is an indegenous herbomineral formulation. To evaluate its efficiency in controlling blood sugar in NIDDM, an open clinical trial was conducted. The study group comprised of freshly deducted cases between 30 and 60 years, without any complications (n = 15, 7 males and 8 females) who failed to respond to meal plan and exercise for a period of 3 months. D-400 was given at a dose of 2 tablets three times a day for 6 months. D-400 significantly reduced ($p<0.001$) fasting blood sugar from 186.67 ± 3.6 mg/dl to 109.5 ± 5.4 mg/dl and post prandial blood sugar from 279.13 ± 0.9 mg/dl to 176 ± 9.4 mg/dl. Reduction ($p<0.001$) in Glycated haemoglobin was observed from 9.4 ± 0.22% to 7.0 ± 0.16%. There was an increase in fasting and post prandial plasma insulin levels. Reduction in LDL and Serum Cholesterol ($p<0.001$) level was seen. There was no change in HDL and triglyceride levels. No alteration was seen in Body Mass Index, blood pressure, renal and liver function tests. No adverse effect has been reported. It can be concluded that D-400 is an useful first line drug in treating NIDDM patients who failed to respond to diet and exercise.

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1503

DIET AND THE ENDOCRINE PANCREAS

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Endocrine cell volumes of pancreases of Vervet monkeys (*Cercopithecus aethiops*), maintained on an atherogenic diet (Group A), were compared with those of monkeys on a stock diet (Group S). Endocrine cell types in pancreas sections were immunolabelled for the four major pancreatic peptides. Using Global Lab image software, the area of immunoreactive cells was traced for each cell type in an average of 300 islets per monkey. It was assumed that the ratio of the area of each endocrine cell type to the total endocrine cell area would be equivalent to the ratio of the volumes and these data were used for statistical analysis. A significant reduction was noted in B cell volume ($p<0.02$) in Group A monkeys (20.65±1.31) compared with Group S (34.72±2.59). A significant increase in A cell volume ($p<0.05$) was found in Group A monkeys (55.72±5.18) compared to Group S (29.52±4.61), interestingly accompanied by a significant decrease in PP cells ($p<0.004$). The concomitant volume increase in A cells with decrease in PP cells supports our hypothesis that A and PP cell volume changes represent differential gene expression of a body of cells that are immunoreactive for both glucagon and PP. The decrease in B and increase in A cell volumes in Group A monkey pancreases reflects a pattern of events that could lead to non-insulin dependent diabetes.

1504

DIABETES NUTRITION AND TARGETS OF DIABETES MANAGEMENT

The Diabetes and Nutrition Study Group of the Spanish Diabetes Association (GSEDNu). Spain

In order to know the nutritional pattern of people with diabetes mellitus in Spain, their adherence to international nutrition recommendations and the relationship with the targets for metabolic control 337 diabetic patients, 144 IDDPts (M/F 70/74) and 193 NIDDPts (M/F, 81/112), satisfactorily completed the 7-day food record diaries from May 1993 to December 1994. The median energy intake of Spanish diabetic subjects was between 2217 and 1453 Kcal/day distributed as follows: CHO between 38-40%; Protein between 19-23%; Fat between 36-41% (SFAs between 11-13%, PUFAs between 4.6-5.8% and MUFAs between 19.7-21.9 %). More than 90 % of diabetic subjects achieved the recommended PUFAs intake but only 45% consumed a proportion of CHO and MUFAs >60%, 20% consumed <10% from SFAs, and 25% consumed <300 mg/day of cholesterol. In spite of it, 69% of patients had a cholesterol level <5.6 mmol, 97% had a HDL-cholesterol level >0.9 mmol, 85% had a triglycerides level <1.7 mmol, while less than 36% of patients had an HbA1c value >8%. In conclusions, despite poor adherence to nutrition recommendations our patients have a near-optimal serum lipid levels and maintain a reasonably good blood glucose control.

1505

DIFFERENCES IN THE DIETS OF TWO POPULATIONS WITH HIGH AND LOW PREVALENCE RATES OF DIABETES MELLITUS IN RURAL INDIA

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We have reported a high prevalence of diabetes mellitus in rural to rural migrants compared to indigenous populations(9.1% *vs* 2.2%) in South India. However, the causal factors for this are unknown. We, therefore conducted a study to know the differences in the diet and nutritional status in these heterogeneous populations living together in villages in Gangavathi Taluk in Karnataka State. In a house to house survey all the dietary components were measured in weighment and 24 hr recall methods. Heights and weights of one adult from a family were taken. *Results* (M±SE)- Indigenes:n=154, Age=40.2±1.1yrs, high income (>3000Rs/mo)=4% and hard physical activity=27%. Migrants: n=152, Age=40.3±0.9, high income=17% and hard physical activity=1%. Migrants had higher BMI than indigenes:21.2± 0.3 *vs* 18.9±0.2, t=6.3, p<.001). Dietary components calculated per Consumption Unit based on physical activity, age, sex and physiological status (eg., for sedentary adult male C.U=1) in the indigene and the migrant are-Energy:1843±18.1 *vs* 2221.8±18.5 Kcal, p<.001;Carbohydrates:355.6± 3.7 *vs* 381.9±4.5gms, p<.001, Protein: 48.3±0.5 *vs* 54.3±0.6gms, p<.001, Fat:19.1±.2 *vs* 40.4±0.1gms, p<.001 respectively. Migrants consume refined cereals. The indigenes were using unpolished cereals and millets and hence had significantly high fibre in their diets, i.e.,18.2± 0.2 *vs* 4.6±0.1gms, p<.001). Since, multiple logistic regression failed to identify migration as a variable in the causation of diabetes, these differences in the dietary intakes and nutritional status are important and might account for the higher prevalence of diabetes noted in these populations rather than the stress of migration.

1506

The effect of dietary carbohydrates and lipid lowering agent, probucol, on opioid peptide receptors in SHR/N-cp rat - a genetic model of obesity, diabetes and hypertension. S.J. Bhathena, O.E. Michaelis, IV and C.T. Hansen. Agricultural Research Service, Beltsville, Maryland and Nation al Institutes of Health, Bethesda, Maryland, USA.

The obese SHR/N-cp rat, a model of type II diabetes, devrelops severe hyperlipemia. The lean phenotype is hypertensive but is not diabetic and does not develop hyperlipemia. Hyperlipemia in obese rats is worsened by dietary sucrose compared to starch. Opioid peptides acting through their receptors have been shown to regulate carbohydrate and lipid metabolism. Probucol has been shown to lower hyperlipemia. We tested the hypothesis that the hypolipidemic effect of probucol may be via changes in opiate receptors and that different opiate receptors may show differential response to either dietary carbohydrate and/or probucol. Weaning male SHR/N-cp lean and obese rats were fed either 54% starch or sucrose with and without 50 mg/kg/day probucol for 32 weeks. Total and μ, δ and κ receptors were measured from cerebral cortex membranes using specific ligands. Scatchard and competition-inhibition plots were constructed to quantify receptor number and affinity. Dietary carbohydrates showed differential response on opiate receptors in lean and obese rats in that dietary sucrose compared to starch lowered total as well as μ, δ and κ receptors in lean rats. In obese rats, dietary sucrose increased total opiate receptors as measured by etorphine binding and δ receptors but not μ or κ receptors. Probucol showed small but significant increase in total opiate receptoirs as measured by naloxone binding and μ receptors in obese sucrose fed rats but not in starch fed rats. The data show that dietary carbohydrates have differential effect on opiate receptors in different phenotypes and that though opioid peptides are involved in lipid metabolism, the effect of probucol on lipid metabolism is apparently not via changes in opiate tone.

1507

NUTRITIONAL INTAKE AND QUALITY OF LIFE IN THE DÜSSELDORF-PARIS-NAPLES (DPN) INTER-VENTION TRIAL IN NIDDM PATIENTS.

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97 NIDDM patients (43 males, 54 females; age: 42-69 years; diabetes duration: 1-24 years; 41 from Düsseldorf, 25 from Paris, 31 from Naples), insufficiently controlled (HbA_1>8%) by oral antidiabetic drugs, were included in a six-month educational intervention program, which aimed at modifications towards a healthier lifestyle. The present study evaluated to what extent changes in dietary intake (4-day dietary records) and aspects of quality of life (four validated scales) could be achieved. At the end of the intervention period patients had significantly reduced their energy intake (1772±614 vs 1587±636 kcal; p=0.0001) and their body mass index (29.8±3.5 vs 29.3±3.6 kg/m²; p=0.0001). For patients from Naples and Düsseldorf significant changes were observed in intakes of saturated fat (12.7±4.5 vs 11.3±4.7 % of energy; p=0.0048), cholesterol (318±182 vs 237±135 mg/day; p=0.0001) and fibre density of the diet (13.8±6.1 vs 16.5±7.9 g/1000 kcal; p=0.0001). Pattern of nutrient intakes differed between the three centres, both at baseline and after six months, reflecting local eating habits. After six months patients reported a better management of their diabetes in daily life (25.3±8.3 vs 22.4±8.7, p=0.002) and a more internal control over diabetes-related health outcomes (30.9±8.3 vs 28.7±8.1, p=0.007). Mental and physical well-being improved significantly (10 (6,16) vs 9 (4,14), p=0.03 and 16 (6,25) vs 12 (5,21), p=0.0001, respectively) in patients from Düsseldorf and Naples (no data from Paris). In conclusion, NIDDM patients from Düsseldorf, Paris and Naples, treated with an education programme involving regular attention by a physician, managed to reduce their energy intake and body weight within a period of six months. Overall, modifications of nutritional intake towards a more favourable pattern were achieved. These lifestyle modifications were accompanied by positive effects on the overall well-being

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NUTRITIONAL PROFILE OF NIDDM IN URBAN-WESTERN INDIA

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Nutritional profile of 622 NIDDs seen during 1995-96 was studied. 424(68.2%) patients were lacto-vegetarians (LV) and 198(31.8%) were non-vegetarians(NV). Most people in NV group partook of 1 serving of 50-100 gm of lamb meat or fish on alternate days. The average kcal/d and %kcal from carbohydrate, protein and fat intake in the preconsultation period vs prescribed diet were 1391 vs 1458, 65.5 vs 67.8, 14.3 vs 13.9 and 20.2 vs 18.3 respectively. Upward and downward revision of diet by 500 kcal was called for only in 8.2% and 5.5% of patients respectively. The most common diet prescribed was about 1500 kcal/d. The BMI of 5.3,43.8, 34.4,10.1 and 6.4% of LV group and 4.6,42.1,32.1,14.2 and 7.0% of NV group was <19,19.1-25,25.1-30,30.1-35, >35 respectively.

Only 7.5% of patients were controlled on diet alone. The treatment modalities, the metabolic control (total GHb, normal < 8%; mean ± SD, LV:9.9 ± 1.3%, NV:10.1 ± 1.4%; P:NS) achieved and complications like hypertension, angina, myocardial infarction, neuropathy and nephropathy were not significantly different (Chi square test) in the LV and NV groups.

This study brings out the prevalence of undernutrition and obesity in NIDDs. It describes the pre-consultation dietary patterns and the common diets prescribed. It shows that metabolic control and complications are not different in LV and NV group thus requiring equal efforts to improve control and minimise complications in both groups.

1509

W-3 POLYUNSATURATED FATTY ACIDS IN TREATMENT AND PROPHYLAXIS OF DIABETIC ANGIOPATHIES

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Dietary supplementation with fish oil,a source of highly long chain marine polyunsaturated fatty acids has been proposed as an antithrombotic and antiatherosclerotic therapy.High dietary intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is of special interest in the possibility of influencing the metabolism of lipids and synthesis of eicosanoids.The aim of this study was to assess the long term effect of EPA and DHA on the activities of protein-kinase C (PK-C), Na^+,K^+-ATPase, Ca^{2+},Mg^{2+}- ATPase, levels of phospholipids and fatty acids in the membranes of erythrocytes and the levels of the ^{125}I-6-ketoprostaglandin F_{1a} (6-ketoPGF$_{1a}$) and ^{125}I-thromboxane B_2(TXB$_2$) in the blood plasma, activities of hexokinase (HK), pyruvatkinase (PK), lactatedehydrohenase (LDH) glucose-6-phosphatedehydrohenase (G-6-PDH) in the RBC in NIDDM patients. 47 patients (5♂+6♀, ages 25m-48m) were allocated into two treatment groups. The 1st group (n=25) was receiving capsules of fish oil (1,5 g EPA 1,5 g DHA and 0,1% a-tocopherol acetate) and the 2nd group (n=21) was receiving placebo capsules of olive oil. All patients were on the same diet.Dietary adherence (7-day dietary history was good). After 2 months of treatment there was a decrease in TXB$_2$ level (136,5±19,8 pg/ml,p<0,001),activities of HK,LDH,G-6-PDH with simultaneous increases in EPA level, EPA/arachidonic acid (AA) ratio (from 0,4 to 0,7), activities of PK-C (from 13,2±3,41 to 22,49±3,13 pmol ^{32}P/mg protein per 1 min, p<0,001), Na^+,K^+-ATPase (from 0,04±0,002 to 0,07±0,003 mMol Pi/ mg protein per 1 hour,p<0,001), Ca^{2+},Mg^{2+}-ATPase and the concentration of 6-ketoPGF$_{1a}$ in the first group.Increases in the level of EPA and EPA/AA ratio in the membrane lipids and the activities of membrane-bound enzymes of the blood cells lead to an increase in their deformability and a decrease in the ability to aggregate.This effect is conditioned also by increase prostacyclin I_2 production as well as by inhibition of platelet activity Therefore it seems that a 3,5 g fish oil treatment during following 2 months result the tendency of normalising the state of prostacyclin I_2-thromboxane A_2 system,activeness of membrane-bound enzymes in NIDDM patients.In conclusion, EPA and DHA at moderate doses may exert antithrombic effects and may be used for prophylaxis and treatment of diabetic angiopathies.

1510

INSULIN RESISTANCE AND N-3 FATTY ACID (FISH OIL) SUPPLEMENT ATION.

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It has been observed that supplementation with n-3 fatty acids (fish oil) may, by reducing insulin sensitivity, increase blood glucose. Abnormalities of glucose homeostasis might reflect impaired hepatic lipogenesis or increased proteolysis which provide substrate for gluconeogenesis. This study was designed to examine this. We studied serial (0, 2, 4 and 6h) fasting glucose, lipids and insulin and whole body protein (WBP) kinetics, measured by continuous L-(1-^{13}C) leucine infusion, in 10 normolipidaemic non-diabetic males (age 32 ± 6 years; weight 71 ± 10 kg; body mass index 23 ± 2 kg/m^2) given n-3 fatty acid (Maxepa 10g/d) for one month. Numbers are mean ± SD. Statistics by paired t-test. Supplementation reduced fasting glucose (4.9 ± 0.4 versus 4.8 ± 0.3 mmol/l; p< 0.05), insulin (19 ± 20 versus 10 ± 11 pmol/l; p< 0.05) and triglyceride (1.0 ± 0.3 versus 0.8 ± 0.2 mmol/l; p< 0.001) but there was no effect on WBPbreakdown (108 ± 8 versus 108 ± 8 μmol/kg/h), synthesis (90 ± 8 versus 87 ± 9 μmol/kg/h) or balance (-19 ± 6 versus -21 ± 6 μmol/kg/h). Body weight and fat free mass remained unchanged. These findings imply n-3 fatty acids promoted rather than inhibited insulin action on carbohydrate and lipid but appeared not to influence whole body protein metabolism.

1511

DIETARY HABITS OF INDIVIDUALS WITH NIDDM

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The aim of this study is to describe the dietary habits of individuals with NIDDM who are admitted in our hospital or are followed in the outpatient clinic. This report is a preliminary part of a detailed analysis of eating habits performed by the dietetic dept. Material and Methods: They were 182/389 men and 207/389 women, with mean age 65,4±20 years, BMI 27.59±9 and mean daily caloric intake 1400 kcal. Results: Written dietary instructions had got 199 of them and 63 had attended dietary education. The number of daily meals was six for 28 of the individuals, five for 126, four for 42 and three for 193. Constant meal time had got 203 of our diabetic persons. Forty nine of them used special "diabetic food". Sugar was used by 84 of them and was avoided by the rest 304. Milk and dairy products were used in the skimmed form by 35, in the semiskimmed form by 91 and with total fat by 263 of them. Salad was eaten daily by 315, seldom by 35 and was not eaten at all by 49. Legumes were used daily by 238, seldom by 105 and were not used at all by 46. Sixtythree of our diabetics ate peanuts every day, 196 of them often ate and 130 never ate peanuts. Alcoholic beverages drank 105 persons daily, 77 persons rarely and 207 of them never drank. Finally, 343 of them had had breakfast daily and 46 had not breakfast. Conclusions: The reported numbers are almost similar with those mentioned in other studies elsewhere. Unfortunately, most people with NIDDM do not follow the dietary instructions. Most of them have got incomplete dietary education or lack it completely.

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DIETARY HABITS IN NORMALS AND DIABETICS IN THE MEDITERRANEAN BASIN.

The Mediterranean Group for the Study of Diabetes (MGSD) Multinational Nutrition Study

The Mediterranean Diet is considered by many as the Golden Standard of Healthy Nutrition and most of the dietetic recommendations for diabetics are based on it, although its composition is poorly defined, mainly from data gathered several decades ago. The MGSD Nutrition Study Group in order to compare the dietary habits of populations of different ethnic origin (non-diabetics and diabetics) living in the Mediterranean basin organized a multicenter study in 6 Countries of this region. Randomly selected normals and diabetics were studied. The protocol required anthropometric measurements, fasting blood samples and completion of a dietary Questionnaire comprising 74 questions. Serum lipid measurements and the analysis of the dietary questionnaires were done in one Center. The validity of the dietary questionnaire was assessed by comparison to the 3-days diet diary in 119 subjects and found very satisfactory. In total 3680 subjects were studied, i.e 1746 normals, 1934 diabetics. In normals, daily energy intake varied significantly among Centers i.e. Italy-1 1702, Greece 2340, Egypt 2430, Italy-2 2509, Algeria 2668, Bulgaria 2865, Kcal/day, p<.001, or expressed as Kcal/Kg BW, 24, 30, 31, 36, 38 and 38 respectively. The carbohydrate(CHO) contribution to the energy intake was for Bulgaria 43.2%, Algeria 46.9%, Greece 47.5%, Italy-2 50.4%, Italy-1 50.9% and Egypt 58.2%, p<.001, while the Fat contribution was Egypt 26.4%, Italy-1 31.2%, Italy-2 33.0% Algeria 35.1%, Greece 38.8% and Bulgaria 40.3%, p<.001. In all Centers energy intake, Kcal/Kg BW, was significantly lower in diabetics by 7-12 Kcal/Kg BW, p<.001. CHO contribution to total energy was significantly lower in diabetics by 2-7 percent units i.e. 48vs50% the lower in Italy-1 and 41vs48% the higher in Greece. Fat contribution was not different in 3 Centers (Italy-1, Italy-2, and Bulgaria), while it was higher in the others, Algeria 37vs35%, Egypt 29vs26% and Greece 40vs37%, p<.001. **Conclusions: a)** Total energy intake and diet composition in normals differ among Mediterranean countries, making thus the definition of "The Mediterranean Diet " difficult **b)** Diabetics in general, compared to normals, consume less energy and less CHO, while in some regions more fat.

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Low Glycemic Index Foods and Management of Diabetes

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The latest local survey indicated a diabetes prevalence rate of 4.8% or roughly a total of 3,130,000 cases. With the crude birth rate of 3%, about 36,000 new cases will be added to existing cases annually. Foods with low glycemic index (GI) have been shown to be beneficial in the management of diabetes. The objective of the study is to determine the glycemic effects of locally available foods using normal and NIDDM subjects. Local food studied include legumes, mixed meals and foods incorporated with rice bran and seaweed extracts which were fed to the subjects after an overnight fast. Blood samples were collected at regular intervals and analyzed for glucose using the oxidase method. Results indicated that these foods have significantly lower ($p<.05$) glycemic responses compared with the control samples. It is concluded that these foods may be beneficial not only in the dietary management of NIDDM but for the general population as well.

1514

EFFECTS OF INHIBITION OF B-OXIDATION ON HEPATIC AND PERIPHERAL INSULIN RESISTANCE IN HIGH-FAT-FED RATS

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To elucidate mechanisms of hepatic and peripheral insulin resistance induced by excess dietary fat we studied conscious, chronically high-fat-fed (HFF) and control rats during euglycemic-hyperinsulinemic clamps. In HFF rats hyperinsulinemia (560pM) significantly suppressed circulating free fatty acids but had no effect on the 3-4 fold higher levels of long chain fatty acyl CoA esters in skeletal muscle. Acute blockade of B-oxidation using etomoxir increased insulin-stimulated glucose uptake in muscle from HFF rats via a selective increase in glycolysis but etomoxir did not reverse the defect in glycogen synthesis or restore the decreased glycogen synthase activity observed in HFF rats (HFF 3.1±0.1 vs CON 3.9±0.1 and HFF 6.6±0.3 vs CON 8.8±0.3nmol/min/mg protein at 0.1mM and 10mMG6P respectively). Etomoxir did not significantly alter the elevated hepatic glucose production (HGP) seen in clamped HFF rats but induced substantial depletion of hepatic glycogen content. This implies that the other source of glucose for HGP (gluconeogenesis) had been reduced by inhibition of hepatic fatty acid oxidation and that factors independent of increased gluconeogenesis are involved in the elevated HGP in HFF rats. This independent mechanism may involve the reduction in hepatic glucokinase (GK) activity seen in HFF rats and the inability of insulin to acutely lower glucose-6-phosphatase (G6Pase) activity in HFF rats during the clamp. Overall a 76% increase in the activity ratio G6Pase/GK (HFF-clamp 3.0±0.5; Con-clamp 1.7±0.2, $p<0.05$) was observed which would favour net hepatic glucose release in HFF rats. In conclusion B-oxidation is responsible for some but not all of the components of insulin resistance induced by chronic high-fat-feeding.

1515

THE EFFICIENCY OF DIET THERAPY BY THE COMBINATION OF VERY LOW CALORIE DIET(VLCD) AND LOW CALORIE DIET(LCD) IN OBESE ADOLESCENTS

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Thirteen adolescents of simple obesity ($\bar{x}$ age 13.4 years, $\bar{x}$ wt 79.6kg) were hospitarized and placed on diet therapy combining VLCD and LCD for 30 days (group A: GA) or 10 days (group B: GB). They took 420 kcal of 'Optifast 70' every day and meals containing carbohydrate, protein and lipid in the ratio of 55: 20: 25(%). Total calorie/day was changed every 6 days in GA and every 2 days in GB which was 1200, 820, 420, 820 and 1200 kcal, succesively.

Following parameters monitored for change on a daily bases showed greater decrease in GB than those in GA: body weight (BW), %IBW; GPT, GOT, γ-GTP; triglycelide, atherogenic index; plasma glucose (PG) score based on the diabetes standard of children by the Japanese Ministry of Health and Welfare, ΣPG during oral glucose tolerance test; systolic and diastolic blood pressure.

Conversely, total cholesterol, ΣIRI, ΣCPR showed greater improvement in GA.

%FAT measured by impedance-fatmeter showed no change in either group during hospitalization, but markedly decreased from 36.0% to 27.3% in pts. whose BW either decreased or remained stable at 6 months after discharge. Two of 6 and all of 7 pts. in GA and in GB, respectively, could be followed up on an outpatient basis after discharge. None of 6 pts. in GA and 3 of 7 in GB desired to undergo the diet therapy with hospitaliztion again.

We conclude that the diet therapy by short-term hospitarization might be better method for children in terms of much improvement of the daily value of each parameter, less stress, higher compliance and acceptability.

1516

The effect of n -3 fatty acids on peripheric nerve in diabetic rats: a morphologic study.

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Forty Wistar Albino rats were included into the study. 20 of them were injected streptozotocin to make them diabetic. 10 of the diabetic rats were in group 1 which were given MaxEPA [Eikosapentonoik acid (EPA) + Dekosaheksanoik acid (DHA)] , 10 mg EPA and 7 mg DHA per day)]. The other 10 diabetic rats were in group 2 which were not on MaxEPA therapy. 20 rats were selected as the control group. 10 of these rats were in the group 3 which were on MaxEPA. The other 10 rats of the control group were not given MaxEPA (group 4). After 4 months , their sciatica nerve examined with electronmicroscopy. The structure of peripheric nerve was normal in the control group (group 3-4). In the group 2 , there was dissociation and fragmentation in myelin level in places , in addition to the normal appearance of myelin sheath . Some findings of leukodistrophic degeneration were also seen. We found diffused degeneration findings in diabetic rats using maxEPA in the group 1 .

In conclusion, the damage seen in the peripheric nerves was more serious in the diabetic rats using n-3 fatty acids than the diabetic rats .

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PREVENTION OF DIABETIC ANGIOPATHY BY TREATMENT WITH INSULIN AND DIETARY FIBER

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Aim : We evaluated histological changes in the eye and kidneys in diabetic rats that were given a high concentration of cholesterol (Ch) and treated with insulin (Ins) or Glucomannan (Gm), a soluble dietary fiber. Methods : STZ(50 mg/kg, iv) was administered to SD male rats, and diabetic rats showing an FBS of 150mg or more after 4 weeks were divided into 4 groups. Group I received no treatment. Group II was treated with Ultralente Ins (15 u/kg/day). Group III was given 1.5% Ch food. GroupIVwas given 1.5% Ch and 15% Gm food. Group V consited of normal controls. After 12-week maintenance, blood was collected during fasting. The eye and kidneys were removed, and observed by light and electron microscopy. Results: The blood glucose levels decreased after administration of Ins or Gm. The Ch level increased in Group III (322±106mg/dl), but markedly decreased after Gm administration in Group IV(96±12mg/dl). Histologically, Group III given a high concentration of Ch showed dilatation of retinal blood vessels, atherom of A.chorioidea and A.iridis suggesting diabetic rubeosis and basement membrane thickening of glomeruli. These changes were marked in Group III, but slight in Group IV treated with Gm. Slightly thickening of basement membrane was found in Group I and II. Conclusion : After administration of a high concentration of Ch to diabetic rats , i.e. , in the presence of impairment in both glucose and lipid metabolism , diabetic complications tended to occur easily , and dietary therapy seems to be important.

1518

The effect of the ethanol on the fasted- and prandial glucose level in conscious dogs.

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We investigated the effect of the ethanol on the glucose level (GL) before and after the glucose load. Subjects were conscious over-fasted dogs with a gastric tube and the catheter inserted into the femoral artery. After a 15-min. basal period, a 120-min. experimental period followed in which the ethanol(15%,5ml/kg) (E,n=5),the glucose(2.5g/kg) (G n=5) or the mixture of ethanol and glucose(E+G,n=5) was given via the gastric tube at 0 min. In the E,G and E+G group, respectively,the mean ± se of the fasting GL was 81±5 ,84±3,95±6mg/dl and the maximum GL was 85± 4 at 75 min.、209±24 at 60 min. and 308±17mg/dl at 45min.、AUC above the basal level was 7185,12173 and 16421min. · mg/dl. The glucose level of the E+G group increased more acutely and higher significantly than that of the G group between 30 and 75min. after the glucose load. In summary, the ethanol couldn't change the GL alone, but enhanced the increase of the prandial GL by interacting with the glucose.

1519

COMPUTER AIDED INTERACTIVE NUTRITION ASSISTANT - A UNIQUE TOOL FOR IMPROVING DIABETES NUTRITION MANAGEMENT

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Nutrition management remains an ineffective tool due to inadequate knowledge, both, amongst patients and health care professionals. Services of nutritionists are not readily available and there is a crying need for a cost effective, comprehensive, customizable and interactive tool for diabetes nutrition management. We have developed a computer based interactive nutrition assistant to meet this need. The program is modular. The first module records anthropometeric and medical data. Using internationally accepted nomograms and based on height, weight, waist, hip, skin fold measurementsas well as age, sex and activity, the program records BMI, WHR, BMR, % Body Fat, Risk indication and energy requirements. The program records diagnosis and associated conditions such as hypertension, cardiac, renal, pregnancy, obesity, malnutrition etc. requiring special nutritional assistance in respect to energy, salt and protein intake as well as, medications including creating a graphic visual representation of the insulin algorithm. The second module records a 24 h dietary recall, using simple pull down screens and lists (can be customized by the user to suit local and regional dietary habits), provides household measures help and necessary prompts for an effective recall. The recall is brokenup into Calories, CHO, Protein, Fat (Sat and Unsat), Na^+ K^+, fibre etc and is also presented as bar diagram plotted against time to allow visual comparison between insulin algorithm and 24 caloric spread. The third module is nutritional advice. Based on calculated energy requirements program suggests energy intake which can be modified to accommodate changes based on clinical needs. Thus a patient with renal failure on protein restriction will get options of protein restricted diets only. Based on current dietary recommendations an exchange based spreadsheet is generated which can be modified to suit individual patient and generate a daily meal plan. The daily plan can be modified by simply replacing one food item with another in the same calorie exchange to provide variety on different days. Additional features include flexibility to customize database, language preference, screen specific help, easy to use screen design and graphics. Also a diet query where nutritive value of a given food item from the data base can be accessed as well as the feature allowing nutritive value of a known recipe to be calculated. We and others who have used the program believe that it will be useful not only in day to day work but also as a research tool.

1520

REGULATION OF FATTY ACID SYNTHASE ACTIVITY : EFFECTS OF DIETARY N3 AND N6 FATTY ACIDS (PUFA) IN INSULIN RESISTANT RATS

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To elucidate the mechanisms implicated in decreased plasma triglycerides by dietary n-3 PUFA (fish oil) compared to n-3 PUFA (sunflower), fatty acid synthase (FAS)activity was evaluated in the liver and adipose tissue of insulin resistant rats. Thirty two male Sprague Dawley rats were randomized into 4 groups. Three groups were submitted to a diet containing (w/w) : 57% sucrose and 14% lipids as either Fish Oil (FO), Sunflower Oil (SO) or a mixture of Standard Oils (STO). The control group was fed a diet containing 57% starch and 14% standard oils (C) After 3 week diet , body weight was comparable in the four groups, but adipose tissue weight was decreased in the FO and SO-fed rats (STO=0.9±0.1, SO= 0.7±0.02, FO= 0.6±0.03 g/100g B.W $p<0.01$). The sucrose fed rats showed high glycemia compared to the control group ($P<0.05$) Fish oil corrected the sucrose- induced hyper insulinemia (ANOVA,$p< 0.01$) but had no effect on plasma glucose. Plasma lipids were lower in the FO fed rats than those fed SO or S/STO diet (triglycerols $p<0,0005$, free fatty acids, $p<0.01$). In the liver, FAS activity increased in the STO fed rats as compared to C. Fish oil decreased this activity by 80% and sunflower oil by 33% (C= 0.98±0.13, S/STO= 1.74±0.16, S/FO=0.37±0.06 nmol/min/g of tissue). FAS activity in adipocytes was weaker in STO than in C. This activity increased by FO diet, but unaffected by the SO (C= 0.36±0.06, STO=0.2±0.02, SO=0.12±0.02, FO= 0.43± 0.09, $p<0.05$). In conclusion , 3 weeks of a n-3 PUFA rich diet given to sucrose-fed rats : 1) decreased hyperinsulinemia and hypertriacylglycerolemia, 2) prevented the increase in FAS activity in the liver, and prevented any decrease in this activity in the adipose tissue. N-3 PUFA decreases plasma lipids by an effect on FAS activity, and its effect was superior to that of n-6 PUFA .

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METABOLIC CHANGES AFTER INGESTION OF DIFFERENT KINDS OF FAT (PRELIMINARY DATA).

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It was investigated if there is any difference in the postprandial metabolic changes observed in diabetic subjects following the ingestion of different kinds of fat. Two test meals were given on separate days to 7 type-2 diabetic patients (diet alone) after a 12-hr fast. The meals contained 100g of white bread with either 42g of butter on day 1 (mainly saturated fat, SAFA) or olive oil (35g, mainly monounsaturated fat, MUFA) on day 2. Plasma glucose, triglycerides, insulin and C-peptide were measured at 30 minute time intervals for a total of 6 hours after meal ingestion. Areas under triglyceride curves were significantly lower after the olive oil-meal (514±110mmol.min) as compared to the butter-meal (617±192, $P<0.05$). There was also a trend for lower glucose values after olive oil intake (3668±978 vs 4165±933 mmol.min). No significant differences were observed in the insulin and C-peptide values. Thus, in type-2 diabetic persons the ingestion of MUFAs is associated with a lower prostprandial triglyceridemia as compared to SAFAs.

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METABOLIC EFFECTS OF ALTERATIONS IN MEAL FREQUENCY IN NON-INSULIN-DEPENDENT DIABETES

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Thirteen free-living men and women with non-insulin-dependent diabetes participated in a randomised crossover study to determine the extent to which meal frequency influences measures of glucose and lipid metabolism. During experimental periods, each of 4 weeks duration, participants consumed their daily food intake as either 3 or 9 meals per day. Nutrient intake was assessed and fasting plasma lipids and lipoproteins, glycated haemoglobin and glucose, insulin and triglyceride responses to a 75 g oral glucose load were measured during the experimental periods. Nutrient intakes and all laboratory measurements were virtually identical on the 3 and 9 meal regimens. Thus the results of this longer term controlled study could not confirm the findings of acute experiments in people with diabetes and comparable 4 week studies in non-diabetic individuals which suggest possible benefits of increased meal frequency. However, as there were no adverse effects of consuming 9 meals each day it would seem appropriate that meal frequency in those with non-insulin-dependent diabetes should be left to personal choice provided energy balance is maintained.

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THE EFFECT OF A NOVEL DIETARY FIBRE ON POST-PRANDIAL GLUCOSE AND INSULIN RESPONSES IN HEALTHY INDIVIDUALS

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Although dietary fibre may play an important role in the prevention and management of non-insulin dependent diabetes (NIDDM), high fibre diets are not well accepted. The effect of a novel dietary fibre (NF, patent pending) on post-prandial glucose and insulin responses was therefore examined. NF extracted from cereal grains was incorporated into white bread loaves at 5% and 10% dry weight. NF breads closely resembled white bread in appearance and were highly palatable. Three isocaloric breakfast meals with either white or NF bread were given to ten healthy individuals (4 males, 6 females). Each meal comprised 75g of available carbohydrate (50g starch, 25g sugars), 10g protein and 14g fat. Plasma glucose and insulin levels were monitored for 120 minutes postprandially. Fasting glucose and insulin levels did not differ significantly before each of the three breakfast meals. (Data: mean ± SEM)

Bread type	White	5% NF	10% NF	*p* ANOVA
Peak glucose (mmol/l)	7.22±0.33	6.35±0.42	5.83±0.33*	0.003
Peak insulin (µU/ml) #	54.8±1.2	52.7±1.1	36.8±1.2	0.072
Incremental glucose area	100.0±20.4	76.7±16.5	63.0±14.9*	0.022
Incremental insulin area #	3715±2	2981±2	2253±2*	0.009

geometric mean ± SEM, *$p<0.05$ vs white bread (*t* test with Bonferroni correction)

Consumption of NF reduced peak glucose levels and incremental glucose and insulin areas in a dose-dependent fashion, although only the 10% NF bread produced a statistically significant reduction compared to white bread. There was a trend towards a reduction in peak insulin response following NF consumption. Given the high palatability and acceptability of NF breads, there is potential for incorporation of NF into a wide range of cereal products which could be used to improve metabolic control in people with NIDDM.

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EFFECTS OF FRUCTOOLIGOSACCHARIDES ON LIPID AND GLUCOSE METABOLISM IN INSULIN RESISTANT RATS

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Fructooligosaccharides (FOS) is a relatively new bulking and sweetener agent used by food industry. It is a nondigestible fermentable product, having thus some properties of dietary fibers. Therefore, we aimed at studying the effects of FOS on glucose and lipid metabolism in insulin-resistant rats. Twenty four Sprague Dawley rats, 5 weeks old, were randomized into three groups fed either a 57% sucrose rich diet. (S) ,the same sucrose diet supplemented with 10% FOS (S/FOS), or a standard diet (control group :C) : After 3 weeks of such diets there was no difference in body weight between groups. The S fed rats had heavier liver and retroperitoneal adipose tissue and higher plasma triglyceride levels than the control group. **Effects on lipid metabolism:**The addition of FOS for 3 weeks to the S diet resulted in decreased liver weight gain by -11% ($P<0.05$) and retroperitoneal adipose tissue weight gain by -25% (NS). Plasma triglycerides, chylomicrons + VLDL, and IDL concentrations were also lower by 22%, 15%, and 45% respectively, but NS. The addition of FOS lowered plasma free fatty acids by 28% ($P<0.001$) as compared to S fed rats. In the liver the fatty acid synthase (FAS) activity was higher in S fed rats than that of controls. The FOS supplementation prevented this increase. In the adipose tissue, however, this activity was low in the sucrose-fed rats compared to controls and increased after the FOS diet. **Effects on glucose metabolism:** The presence of FOS decreased postprandial plasma glucose (S= 1.41± 0.05, S/FOS= 1.28± 0.05 g/l, $p<0.05$) and fasted plasma insulin (S= 82± 5, S/FOS=47± 5 µU/ml, $p< 0,0005$). Maximal insulin-stimulated glucose transport showed a nonsignificant increase in the adipocytes of S/FOS fed rats. The quantity of both GLUT 4 proteins and their mARN were increased after S/FOS diet in adipocytes but not in the muscle. Plasma acetate was 26% higher in S/FOS than the S fed rats. Propionate and butyrate were not different. **In conclusion:** Three week addition of 10% FOS to insulin resistant rats 1) prevented some lipid disorders observed in the sucrose fed rats and showed a tendency to normalize FAS activity., 2) restored the quantity of GLUT 4 protein and their mARN. The use of FOS may be of interest from a preventive and therapeutic point of view for the pathology related to insulin resistant status and hyperlipoproteinemia.

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EFFECT OF A SHORT TIME OF DIET THERAPY ON SERUM LIPID IN NIDDM PATIENTS. Y. Higuchi and K. Noda. Harasanshin Hospital, Japan.

To evaluate effect of diet therapy on serum lipid, we investigated change in TCH, HDL-ch and TG for 2 weeks of educational hospitalization in 91 NIDDM patients. In this period, patients were given diabetic diet (appropriate Calorie, cholesterol < 250 mg/day, P/S > 1.0). Change in these parameters during 2 weeks were evaluated using principal component analysis (PCA), a statistical method that can be used to reveal the general properties of multiple variables. All three parameters were significantly decreased during 2 weeks ($p < 0.001$, decrease rate: TCH 15.9 %, HDL-ch 12.6 %, TG 43.8 %). These decreases were not correlated with improvement in blood glucose control. PCA indicated an index increasing when levels of TCH and TG were high and level of HDL-ch was low. The principal component scores in 75 patients were reduced after 2 weeks of educational hospitalization. In conclusion, Three parameters were significantly decreased with a short time of diet therapy. PCA suggested that the diet therapy led our patients to a preferable pattern of serum lipid.

1526

EFFECTS OF LONG-TERM HIGH VS LOW GLYCAEMIC INDEX STARCHY DIET IN NORMAL AND DIABETIC RATS. M. Kabir, S. W. Rizkalla, J. Luo, G. Slama, Department of diabetes, INSERM U341, Hôtel-Dieu hospital, Paris, FRANCE.
We previously found that short periods (3-5 weeks) of a high vs low glycaemic index starchy diet led to deleterious effects on lipid metabolism. We aimed to study the effects of the same diets after a longer period of time (14 weeks) on glucose and lipid metabolism. Sixteen normal and 16 diabetic (postnatal Streptozotocin-n2) male Sprague Dawley rats were submitted to a diet containing 57% starch as either waxy corn starch in the high glycaemic index diet (HGI) or Mung bean starch in the low glycaemic index diet (LGI). After the 14 week diet, body weight was comparable with no change in either adipose tissue or liver weights in the LGI vs HGI. Variation in the type of starch had no effect on plasma glucose levels in either normal or diabetic rats. The plasma insulin to glucose ratio was also nonsignificantly different.There was no change in plasma triglycerides. Plasma free fatty acids, however, were lower after the LGI diet especially in normal rats (normal rat: 0.22±0.02 vs 0.38±0.05, diabetic rat: 0.31±0.04 vs 0.38±0.06 mmol/L, LGI vs HGI, ANOVA $p<0.01$). Fatty acid synthase activity tended to be decreased after the LGI diet in normal (35±7 vs 54±9 mU/mg protein) but not in diabetic rats. There was no detected modification in the activity of this enzyme in the adipose tissue. At this stage, we can only conclude that a long term low glycaemic index diet led to only slight modifications: in normal rats to decreased plasma free fatty acids that was associated with a trend to decrease fatty acid synthase activity in the liver.

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ALCOHOL INTAKE DOES NOT INFLUENCE SHORT TERM METABOLIC CONTROL IN TYPE-I-DIABETIC PATIENTS
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Background: Epidemiological evidence suggests that increased alcohol intake may impair metabolic control in diabetic patients due to insulin resistance and increased hypoglycaemia unawareness. We tried to find correlations between measures of metabolic control and alcohol intake. Research design and methods: We investigated 91 randomly selected type-I-diabetic out-patients (mean age 39 ± 10 ys., mean duration of diabetes 19 ± 8 ys., mean HbA1c 7.6 ± 1.1%; x ± SD). Alcohol intake was estimated with an interview, the concentration of carbohydrate deficient transferrin (CDT), mean corpuscular erythrocyte volume (MCV), and the activity of γ-glutamyltransferase (γ-GT) as well. Metabolic control was characterized by self-reported hypoglycaemic episodes in the last 4 weeks before the investigation and the HbA1c concentration. Results: Reported daily alcohol consumption varied from 0 to 187 gr. and correlated with CDT ($r=0.51$, $p<0.0001$) but not with MCV and γ-GT-activity. There were no correlations between alcohol intake (or surrogate variables of alkohol intake) and the number of hypoglycaemic episodes or the HbA1c concentration. Medical history did not give any indication of increased hypoglycaemia unawareness. Conclusion: Even excessive daily alcohol intake does not detoriate short-term metabolic control in otherwise healthy, well educated type-I-diabetic patients treated with an intensified conventional insulin therapy in a diabetes centre.

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THE EFFECT OF LOW GLYCAEMIC INDEX DIET ON INSULIN SENSITIVITY IN WOMEN WITH A FAMILY HISTORY OF HEART DISEASE.
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Decreased insulin sensitivity (IS) is a feature of both non insulin dependent diabetes (NIDDM) and coronary heart disease (CHD). Non diabetic children with a NIDDM parent have decreased IS. We investigate whether children with a parent with CHD also have decreased IS, and if so, whether this is influenced by diet. Twenty-two women (12 with a family history of CHD (HHD) and 10 without (NHHD)) were recruited and studied at least two weeks prior to elective tubal-surgery. In-vivo IS was assessed twice using the short insulin tolerance test (SITT) on day 14 and one day prior to surgery. Subjects were then randomised to a high (HGI) or low (LGI) glycaemic index diet after the initial SITT. In-vitro IS was assessed by insulin stimulated glucose uptake in subcutaneous (SC) and omental (OM) fat cells obtained at surgery. The two groups were similar for age, BMI, waist:hip ratio, fasting lipids. Results are expressed as medians comparisons made by Mann-Whitney U Test. A increase in in-vivo IS occurred in 7/11 women on a LGI diet verses 3/11 women on the HGI diet. Comparison of in-vitro glucose uptake in OM and SC adipocytes in the group with, verses those, without a family history of CHD was significantly less following the HGI diet, however with a LGI diet in-vitro IS was similar in both groups. These results demonstrate that women at risk of CHD have decreased IS in both peripheral and visceral adipose tissue, and that this can be favourably modified by a LGI diet.

	NHHD				HHD			
	HGI		LGI		HGI		LGI	
	B	S	B	S	B	S	B	S
OM Fat	160	300	74	146	55	68	183*	256*
SC Fat	51	56	52	56	35	34	55*	68*

* significant difference between LGI v HGI diet $P<0.05$
B = basal glucose uptake S = 1nM insulin stimulated glucose uptake

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GLUCOSE AND INSULIN RESPONSES TO INGESTED CARBOHYDRATE AND FAT COMBINATIONS AS TWO MEALS IN NIDDM PATIENTS

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Plasma glucose and insulin responses to various nutrient combinations are different between diabetic and normal individuals.In normal subjects, ingestion of butter with potato results in considerably lower blood glucose levels but similar or higher insulin consentrations compared with those observed after patato ingestion alone,while in subjects with NIDDM there is no change in glucose levels despite a greater insulin response.We studied the effects of various patato-butter combinations given as two meals in NIDDM patients. Eight untreated NIDDM patients (6 female ,2 male ,mean age 62.2±10) enrolled in the study.Subjects ingested two meals consisting of potatoes containing 50 gr carbohydrate either alone or with 50gr fat as butter,in four combinations on four different days. Meals were ingested at 8:00 AM and noon.Plasma glucose, glucagon, and C-peptide, serum insulin, triglyceride and NEFA concetrations were determined over an eight hour period. Mean fasting plasma glucose was 7.59±0.02 mmol /l. Peak glucose level was reached at 60 minutes after potato ingestion in the first meal,while patato plus fat ingestion caused a delayed glucose peak level at 120 minute. A potato -fat combination in the first meal caused a delayed glucose peak at 120 minute after the second meal consisting of only potatoes. Plasma glucose area responses were significantly lower after the first meal ccmparing potato-butter with only potato (7.99±0.85 vs 11.68±1.25 mmol.h/l, $p<0.05$). This was not observed after the second meal. Plasma insulin and C-peptide responses were correlated ($r=0.59, p<0.0001$),but not different for the first and second meals. Plasma NEFA levels following the four combinations were not different either. In summary, fat added to carbohydrate causes a delayed plasma glucose peak after both meals and a lower plasma area response only after the first meal in NIDDM patients. As there are no differences between insulin area responses, this effect of added fat may be due to delayed gastric emptying time.

1530

DIABETES AND STARCHES : EVALUATION OF THE USE OF CARBOHYDRATES EXCHANGES AND COMPARISON BETWEEN IDDM, IRDM (INSULINO REQUERANT) AND NIDDM PATIENTS.

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Dietetic education messages about starches are: to know quantities, to make carbohydrates exchanges, and to eat starches in each meal. Aim of the study: check whether diabetic patients know and use these 3 notions. 163 diabetics (57 IDDM, 42 IRDM 64 NIDDM ,82 BMI <25, 81 BMI ≥ 25 Kg/m^2) answered a questionnaire and served themselves different portions of starches: starches often eaten (Of), starches rarely eaten (Ra), mixed starches (Mi) (ex: pizza). Then they were asked to select an equivalent quantity of bread (Br). Results : 1/ Questionnaire: a) Starches are eaten at each meal in 100%, 95% and 79% of IDDM, IRDM and NIDDM, respectively ($p<0.0003$); b) 61% IDDM, 59% IRDM and 34% NIDDM know the recommended quantities ($P< 0.0047$); c) 61% IDDM, 59% IRDM and 44% NIDDM think they know how to evaluate the quantities (NS); d) 30% IDDM, 21% IRDM and 11% NIDDM weigh starches ($p<0.03$). 2/Behaviour: a) 45% IDDM, 40% IRDM and 37% NIDDM serve theirselves a correct quantity of (Of) (NS); b) 54% IDDM, 62% IRDM and 40% NIDDM make a correct exchange with (Ra) (NS); c) 51% IDDM, 66% IRDM and 39% NIDDM make a correct exchange with (Mi) (NS); d) 44% IDDM, 57% IRDM and 53% NIDDM make a correct exchange with bread (Br) (NS). conclusion: IDDM and IRDM patients eat more starches, evaluate quantities better than NIDDM patients.

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THE EFFECTS OF SOME FOOODS ON POSTPRANDIAL BLOOD GLUCOSE AND INSULINE LEVELS

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It has been demonstrated that carbohydrate-rich foods result in different plasma glucose responses when eaten alone by normal subjects and patients with non-insulin-dependent diabetes mellitus. This study was designed to determine and compare the glycemic and insulinemic responses of 14 different foods in non-diabetic subjects and to examine the relationship between the indices and food constituents. Fifty-gram carbohydrate portions of the foods or glucose were fed to volunteers after an overnight fast in randomized order. The resultant plasma glucose and insulin responses were determined and compared with glucose load. The calculated glycemic indices were highest for banana (77±3) and potato (79±8) and lowest for white bean (28±4) and pear (29±2). The insulinemic indices ranged from 20±4 for apple to 137±26 for macaroni. Insulinemic index values didn't have the same order with glycemic index values. The main effect on the glycemic index values was obtained by soluble dietary fiber expressed as g/100g dry weight of food which explained about %39 of the variability of glycemic indices ($p<0.05$). A significant negative correlation ($p<0.05$) was obtained between the uronic acid content in soluble dietary fiber and insulinemic index values.

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T_3 COMPENSATES FOR CHRONIC INSULIN DEFICIENCY IN LEAN NIDDM UNDER LOW-CALORIE AND HIGH-CARBOHYDRATE

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The aim of this study was to find a role of T_3 for lean NIDDM since T_3 was reported to induce GLUT4. **Methods**:10 lean NIDDM (51yr,BMI:21.8 kg/m^2) were included in a **Diet Crossover Study**: First, they were given control diet(C)(2,012 kcal/d;CHO 299g) for more than 3 days and then C was substituted by low-calorie and low-CHO diet(L-CHO)(1,156 kcal/d: CHO 139g) for 2 wk. L-CHO was subsitituted by low-calorie and high-CHO diet (H-CHO) (1,154 kcal/d: CHO 176g) for 2 wk and H-CHO was subsitituted by 2nd L-CHO for 2 wk. Statistical analysis was performed by ANOVA and Fisher's test. **Results**: **1**) Under C, glucose intolerance, impaired IRI- and impaired T_3-response (ΣdT_3) were found. **2**) Effects of **CROSSOVER** upon 75gOGTT parameters: (1) ΣGlucose (mmol/l) decreased from 134.7±8.7(C) to 95.9±6.3 after L-CHO and it reached the lowest level (83.1±7.3) after H-CHO but it increased to 84.7±7.4 after 2nd L-CHO ($F=10.40, p<0.0001$). (2) ΣIRI(pmol/l) remained unchanged($F=0.99$,NS). (3) ΣdT_3(nmol/l) increased from -0.18±0.16(C) to 0.12±0.21 after L-CHO and it reached the highest level (0.92±0.19) after H-CHO but it decreased to 0.36±0.21 after 2nd L-CHO ($F=5.92$, $p<0.002$). T_3 played a compensatory role for chronic insulin deficiency in lean NIDDM under high-carbohydrate.

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PHYSIOLOGICAL AND PSYCHOLOGICAL EFFECTS OF ACUTE CAFFEINE WITHDRAWAL.

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Acute ingestion of caffeine can augment both the perception of and physiological responses to hypoglycaemia in healthy volunteers and patients with IDDM. These effects of caffeine relate to direct actions on blood glucose supply (cerebral blood flow) and glucose utilisation by the brain. The aim of this study was to investigate the effect of acute caffeine withdrawal on aspects of brain and peripheral function. 12 healthy volunteers (9 female, aged 20-50years) who regularly consumed caffeine (145-790mg/day)were studied. On their normal diets, baseline measurements were made of cognitive function (4-choice reaction time), middle cerebral artery velocity (MCAV- an index of cerebral blood flow), blood pressure and heart rate, and mood (UWIST mood adjective checklist). Following this, measurements were repeated 28 and 52 hours after complete caffeine abstention and 2 hours after re-introduction of caffeine (250 mg). Measurements were also made 72 hours after re-commencing their normal caffeine diet. Acute caffeine withdrawal was associated with a rise in MCAV (+7% [3.6-9.6]%, mean [95% CI], $p<0.02$), and a fall in diastolic pressure (-3%[1-6%], $p<0.005$). Heart rate was unchanged. 2 hrs after acute caffeine ingestion, MCAV fell 14 % ([-7to-21%], $p<0.001$) below the level seen during the withdrawal period, associated with a rise in diastolic pressure (+6% [2-11%], $p<0.002$). 72 hours after returning to their normal caffeine intake, MCAV and diastolic pressure were indistinguishable from baseline. Feelings of energy fell markedly during caffeine withdrawal (-7 [4-10], $p<0.005$ vs baseline), without any change in reaction time. Following re-introduction of caffeine, this mood change was reversed (+10[7-13], $p<0.005$) and was associated with positive feelings of hedonism (+6[2-10],$p<0.02$).In conclusion, acute caffeine withdrawal is associated with a rise in brain blood flow, a fall in blood pressure and negative mood changes. On restarting caffeine, the results show that this substance has a stimulant effect on mood but not cognitive performance.

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PROTEIN RESTRICTION AND MICROALBUMINURIA IN NIDDM

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Protein restriction preserves renal function in nondiabetic renal disease and in patients with IDDM; is it feasible and effective in NIDDM? NIDDM patients (n=90) were selected with: a. albuminuria > 6.5 $mg.l^{-1}$ (=detection level) in two 24hr urines or albuminuria (ALB) >20 $mg.24hr^{-1}$ in at least one, but mean ALB $\leq$300$mg.24h^{-1}$, or b. diabetes duration $\geq$5y. Only patients were included with protein intake >0.80 $g.kg^{-1}$ according to urinary urea and without leucocyturia or nitrite-positive urine. All subjects received dietary guidance for 12 months on fat intake, a randomly selected subgroup (E) also on protein restriction. Baseline data for E (32♂, 13♀) and control group (C) (25♂, 20♀), respectively, are (means): age 66 and 64 y, diastolic blood pressure (DBP) 80 and 79 mmHg, systolic (SBP) 140 and 138 mmHg, HbA1c 7.6 and 7.6 %, protein intake 1.16 and 1.11 $g.kg^{-1}$, (medians:) ALB 16.6 and 18.7 $mg.24hr^{-1}$, albumin-creatinine ratio (ACR) 1.39 and 1.46 $mg.mmol^{-1}$, fractional albumin clearance (FAC) 3.0 and 3.9 $.10^{-6}$, and creatinine clearance (mean, while blocking renal-tubular creatinine secretion by cimitedine, CrCl) 80 and 80 ml $min^{-1}.1.73m^{-2}$. The following *changes* in E and C, respectively, were observed (means): protein intake -0.06 and +0.01 $g.kg^{-1}$ ($p<0.10$), DBP -4 and -1 mmHg (NS), SBP -6 and -2 mmHg (NS), CrCl -6 and +2 $ml.min^{-1}.1.73m^{-2}$ ($p<0.10$), body weight -0.7 and +1.0 kg ($p\leq0.005$)and (medians:), ALB -1.84 and +0.18 $mg.24hr^{-1}$ (NS), ACR -0.03 and 0.02 $mg.mmol^{-1}$ (NS), and FAC -0.16 and +0.26 $.10^{-6}$ (NS). When combining both study groups, no significant correlations between individual protein intake and change in albuminuria were found. From the only small contrast so far in our population we conclude that protein restriction is difficult to put into practice, the effect of a more substantial restriction to be assessed by intention-to-treat analysis remaining largely unknown. The dose-response analysis, however, based on non-randomly selected subgroups, does not suggest an effect of protein restriction.

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THE SEVERITY OF PARBOILING INFLUENCES THE GLYCAEMIC RESPONSE TO RICE IN NIDDM SUBJECTS.

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Rice is the staple food for almost half of the world's population, and approx. 20% of the rice is parboiled. Conflicting results have been found regarding the influence of parboiling on the glycaemic and insulinaemic responses to rice. We hypothesize that the discrepant findings are related to the use of different parboiling methods. *Aims:* to investigate the influence of parboiling and the severity of the parboiling method on the glycaemic and insulinaemic responses to rice in NIDDM patients. *Study design:* twelve Bangladeshi NIDDM patients participated (6M, 6F): age: 52±2 years (mean±SEM); BMI. 23.4±0.9 kg/m^2; HbA_{1c} 7.4±0 3% The subjects ingested 4 test meals: white bread (*a*) and 3 meals of cooked, polished rice of the same variety being non-parboiled (*b*), mildly parboiled by the method traditionally used in Asia (*c*) and pressure parboiled as used in industrialized countries (*d*). All test meals contained 50 g available carbohydrates The rice meals were cooked to the same degree of gelatinization. Plasma glucose and serum insulin concentrations (ELISA method, C.V.:1.7%) were measured fasting and during 180 minutes after ingestion of the meal. Repeated measurements, a multivariate-analysis of variance, followed by paired t-tests were used for statistical analyses. *Results.* All rice samples gave lower glycaemic responses (*b*.417±39; *c*:426±56; *d*:300±29 mmol/L*180 min) compared to white bread (*a*:631±42 mmol/L*180 min; $p<0.001$). Within the rice samples, the pressure parboiled (*d*) gave lower glycaemic response than *b* and *c* ($p<0.03$). The glycaemic indices for *b*,*c* and *d* were 67±6, 69±9 and 49±5, respectively. The insulin responses reflected the observed glycaemic responses. *Conclusions*: in NIDDM the influence of parboiling on the glycaemic and insulinaemic responses to rice depended on the severity of the parboiling method All rice samples studied were low-glycaemic - the pressure parboiled sample being most pronounced - and may be considered useful in a low-glycaemic diet.

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DIETARY-HABITS IN DIABETICS WITH CENTRAL DISTRIBUTION OF BODY FAT

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Central distribution of body fat characterised by waist-to-hip ratio (WHR) above 0.8 for women and 0.95 for men is linked to a greater risk of NIDDM and complications of diabetes. The aim of this study was to analyse dietary habits of newly diagnosed diabetics, to relate them to WHR, and to determine necessary dietary interventions. On the first visit in the outpatient clinic 55 diabetics (33 m, 22 f) completed the questionnaire about dietary history and food frequency for 38 items, characterising all food and beverage groups. According to WHR the patients were classified into "Normal WHR Group" (NWHR) (16 p, 12 m, 4 f) or "Increased WHR Group" (IWHR) (39 p, 21m, 18 f). The IWHR, though significantly heavier (91.3 ± 17.5 kg vs. 80.6 ± 13.4 respectively), consumed significantly less meals (3.23 ± 0.84 vs. 4.06 ± 0.77 respectively). It was due to skipped breakfast (35.9% patients) and supper (20.5% patients) which resulted in significantly longer time between evening and morning meal (14.34 ± 2.78 vs. 12.59 ± 2.18 hours). Some food frequencies expressed by Food use score (FUS) differed. The IWHR preferred whole to semi-skimmed milk, white to brown and wholemeal bread, hogs grease and butter to oil, butter to margarine, beef to fish. The consumption of other meats, meat products, offal, pasta, rice, legumes, fatty sweets, chocolate, biscuits, fruit and vegetables were similar. In contrast to NWHR which preferred mineral water, IWHR drank more carbonated soft drinks, pulpy juices, (sweetened) tea and compotes. Wrong eating patterns, selection of greasy, hidden sugar rich and dietary fibre depleted food and excessive weight in individuals with increased WHR ratio show that these patients need nutritional intervention, highlighting the most important mistakes in their diets and recommending the way of reducing their weight. For prevention of diabetes associated with central distribution of body fat, National nutritional guidelines and education programmes based on Healthy nutrition recommendations could be useful.

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SETTING DIABETES DIETARY STANDARDS -IS IT WORTH THE EFFORT?

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Diet is the 'cornerstone' of diabetes management. In 1992 The British Diabetic Association (BDA) set standards for the dietary intake of people with diabetes. The question is, are they achievable and what measurable outcomes can be evaluated against them? In Bournemouth, patients with newly diagnosed NIDDM attend a series of education sessions including staged dietary instuction. Basic advice is given at diagnosis followed by group education and then individual counselling by a specialist diabetes dietitian and diabetes nurse specialist. Patients are managed for the first 3 months according to pre-determind protocols without formal 'medical' input. This study aims to determine whether patients can achieve dietary standards and, if so, do they lead to positive clinical outcomes.

Method: 36 consecutive patients with NIDDM completed 7 day food and drink diaries at diagnosis and after individual counselling. The results of analysis were compared with BDA standards.

Mean % Energy Contribution

	Standard	Before Education	After Education
Fat	30-35	31.7	28.2 ***
Protein	15	18.8	19.5*
Carbohydrate	50-55	44.1	50.6***
Added Sucrose:	<25g	-	12.1g
Salt:	<6g	-	5.9g

[* p<0.05, ** p<0.01, *** p<0.001]

After 3 months, 70% (n=25) of patients remained on diet alone. Of those on diet alone ; HbA1c had dropped from 11.7 to 7.9; -3.7 [-4.9 to -2.6]%, p<0.001 (mean difference [95%CI]) associated with weight loss of -3.4 [-5.2 to -1.7]Kg, p<0.001.

Conclusion: National diabetes dietary standards are achievable and can lead to improved diabetes control without the need for formal medical intervention; They are worthwhile!

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SHORT TERM VITAMIN E TREATMENT NORMALIZES LDL OXIDATIVE SUSCEPTIBILITY IN DIABETIC SUBJECTS.

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Increased LDL oxidability might contribute to accelerated atherosclerosis in diabetes. It has been shown that Vit E supplementation can reduce LDL susceptibility to oxidation in diabetic subjects. It is not known, however, whether Vit. E treatment is able to restore normal LDL oxidability in these subjects. Aim of this study was to observe whether short term Vit E treatment of diabetic subjects could reduce LDL peroxidation state and LDL susceptibility to oxidation to values observed in non diabetic control subjects. To this end we studied 9 non smoker subjects with NIDDM (M/F=4/5, age 56.4±3.1yrs, BMI 28.1±2.3.Kg/m2, duration of diabetes 12±3yrs, FBS 8.4±0.9mmol/L, HbA1C 8.7±0.7%) and 13 non-smoker non diabetic subjects matched for gender, age and BMI. Baseline blood samples were obtained in all subjects for determination of plasma Vit. E concentration and LDL Vit E content (HPLC), LDL susceptibility to oxidation (lag time before propagation of oxidation reaction with Cu++ [LAG]) and native LDL oxidative state (LDL content of Fluorescent Lipoperoxidation Products [FLP]). Diabetic subjects were then given a 600 mg/day Vit. E supplement for 3 weeks and measurements were repeated As compared to controls, at baseline diabetic subjects exhibited shorter LAG (59.5±3.8 vs 76.2±3.2 min, p< .0003) increased FLP (13.9±1.5 vs 7.4±0.6 urf/LDL-C, p< .0001), decreased plasma Vit. E (30.1±1.8 vs 40.9±3.1 µmol/l, p< .006) and no change in LDL Vit E content (4.1±0.4 vs 4.5±0.2 nmol/mg LDL-C). Vit. E supplementation determined an increase in LAG (84.0±6.6 min, p< .0007 vs baseline), a decrease in LDL-FLP, and an increase in plasma Vit E and LDL Vit E content (5.6±0.6 urf/LDL-C, 60.3±5.7 µmol/l and 6.2±0 6 nmol/mg LDL-C, p< .002, p<.0001 and p< .0004 vs basal values respectively) so that no difference between diabetics and controls was observed after Vit. E in any of the parameters evaluated. We conclude that in NIDDM subjects short term Vit. E treatment is able to normalize LDL susceptibility to oxidation and LDL oxidation state.

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DIETARY ASSESSMENT OF AN INDUSTRIAL POPULATION SAMPLE IN NORTH INDIA

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Background: High prevalence of diabetes and risk factors for coronary heart disease have been reported from an industrial population in North India. Proximate principals of diet are considered as an important modifiable risk factor.

Aim: to assess the consumption of proximate principals of diet in an industrial population, and to determine the proportion of these contributed by the subsidised industrial canteen.

Design: Cross sectional survey of a sample of industrial population (22 Females, 25 Males) for estimation of proximate principals of diet by a detailed 24 hour recall method. Estimates of consumption from the industrial canteen were made by dividing total consumption by number of consumers.

Results: Female (40 ± 4 years) and male (42 ± 5) employees consumed 1879 ± 39 and 2519 ± 72 kcal/day. This included 260 ± 52 and 378 ± 14 g of carbohydrates per day (69% and 72% of total calories); 69 ± 22 and 78 ± 23 g fat (33% and 28% of total calories); and, 53 ± 16 and 75 ± 24 g of protein per day. Employees of the industrial unit consume one major meal (of three) and one minor (of two) per day in the industrial canteen. This contributes 28% and 33% of total daily calories consumption and 12% and 11% of total daily fat consumption.

Conclusions: Coronary heart disease risk factor modification in this industrial population requires dietary intervention strategies targeting both home and (grossly subsidised) industrial canteen consumption patterns. It is proposed to extend this study to a larger sample and subsequently evaluate intervention strategies.

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METABOLIC RESPONSE TO MEALS VARYING IN CHO AND STARCH CONTENT IN SUBJECTS WITH NIDDM

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A high carbohydrate (CHO)-high starch diet has been recommended for people with diabetes, and the population in general. Therefore, we decided to determine the metabolic response to three identical meals ingested at 0800, 1200, and 1700 hr, with a snack at 2100 hr, in six male subjects with untreated non-insulin-dependent diabetes mellitus (NIDDM). Three diets differing in type and content of CHO were tested. On separate occasions, subjects ingested meals composed of 55% CHO, 15% protein, 30% fat (high CHO-high starch); 40% CHO, 20% protein, 40% fat (usual CHO and starch content, typical American); and 43% CHO, 22% protein, 35% fat (usual CHO, low starch). They were ingested in random order. Total energy was ~ 2100 Kcal/day. Blood was obtained at 15 and 30 minutes after each meal, and hourly thereafter from 0800 on the day of the test until 0800 the following morning for determination of plasma glucose, insulin, C-peptide, glucagon, and triglycerides. The 24 hour integrated values above the initial overnight fasting values were determined. The plasma glucose area response was 31 mM·hr, 23 mM·hr, and 1 mM·hr, respectively for the three diets (high CHO-starch, usual CHO, low starch). The insulin area responses were ~6000 pM·hr, 6660 pM·hr, and 3570 pM·hr, respectively. The C-peptide data confirm the insulin data. The glucagon area responses were 336 ng·hr/l, 1243 ng·hr/l and 1178 ng·hr/l, respectively for the three diets (high CHO-starch, usual CHO, low starch). The triglyceride area responses were 15 mM·hr, 16 mM·hr and 22 mM·hr, respectively. **Conclusion**: A diet in which fruits, non-starch vegetables, and dairy products are emphasized results in a much smaller plasma glucose and insulin rise, but a modestly greater glucagon and triglyceride increase compared to a high CHO, high starch diet. A low-starch, moderate CHO diet could be useful for people with NIDDM.

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SOME IMPORTANT REVELATIONS IN A DIET SURVEY OF SOUTH INDIAN IDDM SUBJECTS WITH AND WITHOUT COMPLICATIONS.

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Self reported dietary intake was estimated by 24-hours-dietary recall questionnaire, completed by type-I diabetes subjects attending V.H.S. Diabetes Department. 51 Randomly selected patients had received individual diet counselling. They were classified for gender, economic status, physical activity, duration of diabetes and treatment. The macronutrients were assessed based on the National Institute of Nutrition (India), recommendations (Carbohydrate-60 to 70% : Protein - 10 to 12.5%: Fat - 18 to 25% of the total energy). Patients were grouped according to the proportion of energy from macronutrients. 33 (65%) subjects were taking optimal carbohydrate intake (270 gms per day), 24 (47%) were taking higher protein (62 gms per day) and 24 (47%) were taking higher fat (57 gms per day), in their diet. 75% of the subjects derived fat from vegetarian sources. Mean crude fibre intake at 3.5 gms per day was very low. Persons with low income and those with sedantary habits were consuming high carbohydrate. Intake of protein and fat increased with increased income and activity. Contrary to our long held belief, the prevalence of BGDR and ECG changes was found to be higher among people consuming low fat rather than high fat, more in the vegetarian than the non-vegetarian diet. Nephropathy was associated with higher protein intake; lower HbA1C levels were noted with higher carbohydrate in the diet. Preliminary analysis of the reasons for **higher microvascular complications associated with lower fat and vegetarian diet revealed that the type of oil used by these subjects could have a bearing on the vasculopathy.**

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ENERGY INTAKE DURING AN ISO-CALORIC LOW PROTEIN DIET IN IDDM PATIENTS WITH DIABETIC NEPHROPATHY (DN).

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The aim of our study was to measure compliance during a 4 weeks period of protein restriction with a recommended iso-caloric diet in IDDM patients with DN. In a 8 weeks prospective, randomized, controlled trial, we compared changes in energy-, carbohydrate-, fat- and alcohol intake during low protein diet (LPD, 0,6 g/kg/24 h) and normal protein diet (NPD, 1,0 g/kg/24 h) in 29 IDDM with DN. At baseline the patients were randomized to either NPD (n=15) or LPD (n=14) for 4 weeks. Between week 4 to 8, all patients received NPD. Energy and protein intake were measured by means of a three-days dietary record; at baseline, after 4 and 8 weeks. Patients randomized to LPD were advised by a dietitian in an isocaloric, low protein diet. All LPD food for lunch and dinner were supplied. At baseline patients in the LPD and the NPD groups were comparable regarding age, energy-, protein-, fat-, carbohydrate- and alcohol intake. Changes in protein (intake), weight, energy- and carbohydrate- (cho) intake from baseline to 4 weeks and from 4 to 8 weeks, were compared between the LPD and the NPD groups (median (range)):

	Baseline to 4 weeks			4 to 8 weeks		
Changes in	LPD	NPD	p	LPD	NPD	p
protein (g/kg/24 h)	- 0,6 (-1,3 to -0,1)	- 0,1 (-0,6 to 0,9)	<0,001	0,4 (-0,1 to 0,9)	-0,1 (-1,0 to 0,4)	<0,001
weight (kg)	-1,5 (-4,4 to 0,8)	-0,6 (-3,8 to 1,8)	<0,05	1,1 (-1,0 to 2,6)	0,3 (-1,3 to 3,2)	NS
energy (MJ)	-0,6 (-3,0 to 2,1)	0,2 (-2,3 to 5,4)	<0,05	0,2 (-7,3 to 3,5)	-0,3 (-3,8 to 1,7)	NS
cho (E%)	12 (-1 to 22)	2 (-5 to 11)	<0,005	-12 (-22 to 5)	-2 (-14 to 11)	<0,005

No changes in HbA_{1c}, fat- or alcohol intake were seen between the two groups. In conclusion LPD was associated with a decrease in energy intake, despite a recommended iso-caloric diet. Increased carbohydrate intake during LPD,did not affect metabolic control.

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HIGH PLASMA VALUES OF N6 FATTY ACIDS AND N6/N3 RATIO;A RISK FACTOR FOR NIDDM AND DYSLIPIDEMIAS IN URBAN ASIAN INDIANS.

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Increased susceptibility of urban Asian Indians to coronary heart disease is often associated with NIDDM and dyslipidemias such as high plasma triglycerides (TG), low HDL-cholesterol (HDL-c) and elevated total cholesterol (Tc) / HDL-c ratio. High values of plasma n6 fatty acids and n6/ n3 ratio resulting from increased consumption of n6 fat may be a common risk factor for the above metabolic disorders. To test this hypothesis, we measured various n6 and n3 fatty acids in plasma by gas liquid chromatography in 59 unselected diabetic subjects (D) with NIDDM (M=24, F=35) age 51.41 ± 7.9 yr. and compared with 32 controls (C) non diabetic subjects (M=10, F=22) age 50.32 ± 8.34 yr. Plasma lipid values (mg/dl) measured by enzymatic methods showed no difference between the D and C groups in Tc [192.4 (50.28) vs 184.67 (26.51), P:N.S.] but D had higher TG [176.15 (98.48) vs 110.5 (31.31), P < 0.0004], Tc/HDL-c [4.43 (1.29) vs 3.52 (0.78), P < 0.001] and lower HDL-c [44.89 (10.92) vs 53.95 (9.72), P < 0.0002]. D also had higher values (mg/dl) than C of linoleic acid [97.91 (45.33) vs 75.5 (28.89), P < 0.02],arachidonic acid [24.73 (13.48) vs 17.21 (4.22), P < 0.002], total n6 [122.66 (52.73) vs 92.71 (29.04), P < 0.004] and n6/n3 ratio [100.38 (45.96) vs 78.38 (45.75), P < 0.03] but lower values of alpha linolenic acid [0.54 (0.37) vs 0.78 (0.49), P < 0.01]. Regular intake of fish oil by > 50% of D was reflected in improved values in D of eicosapentaenoic acid [0.43 (0.17) vs 0.34 (0.25), P : NS], docosahexaenoic acid [0.35 (0.21) vs 0.35 (0.25), P : NS] and total n3 fatty acid [1.31 (0.5) vs 1.46 (0.65), P : NS]. Results suggest adverse impact of high plasma n6 fatty acids and markedly elevated n6/n3 ratio may not be corrected by fish oil supplements alone and imply that increased consumption of n6 fat may be a common risk factor for above disorders in Asian Indians.

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EATING ATTITUDES IN PATIENTS WITH NIDDM.

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The present study is aimed at the identification of clinical and psychopathological features related to eating attitudes and behavior in NIDDM. A consecutive series of 450 patients (223 F, 227 M) with NIDDM aged 30-70 years (m±sd 58.4±8.7), with a duration of NIDDM of 10.0±8.2 yrs, a Body Mass Index (BMI) of 27.8±4.6 kg/m^2, and a HbA_1c of 7.4±1.7%, was studied; 84 were treated with diet only, 237 with oral hypoglycemic agents, 76 with insulin, and 53 with combinations of insulin and oral hypoglycemic agents. A structured psychiatric interview (SCID) was performed, using DSM-IV criteria for the diagnosis of eating disorders. Eating attitudes were further examined with the BITE questionnaire. Anxiety (STAI), depression (Ham-D), and diabetes-related quality of life were also applied. 26 of the patients (20 F, 9.0%; 6 M, 2.6%) were found to be affected by Binge Eating Disorder, and 55 (40 F, 17.9%; 15 M, 6.6%) showed subclinical alterations of eating behavior. BITE scores were found to correlate significantly with BMI (r=0.29, p<0.01), HbA_1c (r=0.27, p<0.01), DQOL scores (r=0.40, p<0.01), STAI-1 (state anxiety) scores (r=0.33, p<0.01), STAI-2 (trait anxiety) scores (r=0.32, p<0.01), and Ham-D scores (r=0.38, p<0.01), but not with age and duration of diabetes. These results were confirmed at multivariate analysis. No significant difference was observed between the different groups of treatment. In conclusion, disturbances of eating behavior are common among NIDDM females, and interfere significantly with metabolic control and quality of life. For this reason, eating attitudes should always be evaluated, at least in overweight NIDDM patients. Eating disorders in NIDDM are associated with high levels of anxiety and depression.

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Endothelial Function in Vitro and in Vivo

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MONITORING OF THROMBOMODULIN LEVEL AND RENAL FUNCTION IN DIABETIC PATIENTS DURING AFTER ANTIPLATELETS ADMINISTRATION

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In order to evaluate the effect of antiplatelets (AP), we examined the correlation between serum thrombomodulin (TM) levels and renal function in diabetic patients during one year administration of AP. TM levels were determined in 34 patients. They were divided into group A who received AP (n=14) and group B who did not received AP (n=20). 14 patients in group A were further divided into group A-I who received veraprost (n=8), and group A-II who received cilostazol (n=6). There was a significant increase of the TM levels in group B after the follow-up (4.0±2.1FU/ml) compared with those before the follow-up (3.7±1.8) (P<0.01) without affecting renal functions. A significant decrease was noted in group A from the pre-treatment (5.9 ±2.4) to one month (5.4±2.2) and three months (5.0±2.1) after the AP administration. Though there was a slight decrease of TM levels (5.6 ±2.5) in group A, serum creatinine (Cr) values increased from the pre-treatment (142±62) to the post-treatment (186±177 μ mol/L). We noted a significant positive correlation (r=0.75, p<0.01) between the TM levels and Cr values before the AP administration. TM levels in the group A-I (6.9±2.7) and group A-II (4.6±1.0) decreased to (6.7±2.7) and (4.1±1.3), after AP administration respectively, although the Cr values showed a slight increase in group A-I. These results suggest that AP administration is beneficial for inhibiting the progression of vascular endothelial cell injuries in DM when they do not show the worsening of renal dysfunction.

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MATERNAL DIABETES TOGETHER WITH A HIGH SATURATED FAT DIET AFFECTS VASCULAR FUNCTION OF MOTHER AND OFFSPRING

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Maternal nutrition and diabetes may affect cardiovascular development in the offspring. Vascular function was determined in maternal and offspring arteries from STZ diabetic rats fed a breeding or a high saturated fat diet (30% w/w, 10 days before mating to 16 days post partum). Maternal (day 16 post partum) mesenteric arteries and offspring (15 days) femoral arteries were mounted on a small vessel myograph.

Maximum constrictor responses to noradrenaline were increased in maternal and offspring arteries from high fat fed diabetic rats (DF) (mothers: 147±40 % of K-induced contraction, n=4 v diabetics on breeding diet (DC) 102±3%, n=9, p<0.05; offspring: 122 ±14% v 76 ±6%, p<0.01) Neither diabetes alone nor the high fat diet alone was associated with a constrictor defect. Similarly, endothelium-dependent relaxation to acetylcholine was impaired in the DF animals (mothers: 70±7% reduction of NA-induced constriction v DC 94±2%, p<0.001; offspring: 41 ±10% v 83 ±4%, p<0.001). Endothelium dependent relaxation was also impaired in the offspring of non-diabetic mothers fed a high fat diet.

We conclude that a high saturated fat diet in the presence of diabetes has a deleterious effect on vascular function in mother and offspring.

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The Relationship between Serum sVCAM-1 and Plasma von Willebrand Factor (vWF) or Thrombomodulin (TM) in Diabetics

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We have previously demonstrated that circulating soluble vascular cell adhesion molecule-1 (sVCAM-1) levels are increased in diabetics compared with healthy volunteers (in 3rd IDF-WPRC, Hong Kong). However, the mechanisms of increased serum sVCAM-1 levels in diabetics are unknown. There are several possible mechanisms, including endothlial activation or dysfunction. To investigate whether elevated serum sVCAM-1 levels in diabetics may simply reflect endothlial dysfunction, we evaluated the relationship serum sVCAM-1 levels and the markers of endothelial damage (vWF and TM).Fourteen diabetic patients without hypertension, hyperlipidemia, and any diabetic vascular complications were selected, and serum sVCAM-1 levels were measured by sVCAM-1 ELISA Kit (R&D Systems,Ltd). Although serum sVCAM-1 levels (mean± SE) were elevated in diabetics compared to healthy volunteers (1085.7± 74.9 vs 632.1 ± 118.6 ng/ml, p < 0.01), plasma levels of both vWF and TM in diabetics were within normal limits (vWF;70-140%,TM; 1.3-2.1FU/ml). Serum sVCAM-1 levels neither correlated to plasma vWF nor TM levels (sVCAM-1 vs vWF : r=0.24, p=0.41 ; sVCAM-1 vs TM : r=0.31, p=0.28). These results suggests elevated circulating sVCAM-1 may not simply be derived from endothelial damage.

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Effects of Shear Stress on Glucose Transport in Vascular Endothelial Cells

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(Purpose) Hemodynamic shear stress plays an important role in atherogenesis. It was postulated that vascular endothelium sheared in vitro closely mimic those in vivo. Hyperglycemia has been implicated in the pathogenesis of vascular complications in diabetes. In this study, the effect of shear stress on glucose transport was examined in cultured endothelial cell (EC). **(Flow experiments)** Porcine ECs were seeded on a polyester sheet and cultured until cells reached confluence. Flow experiments were performed by the methods of Levesque et al. A confluent monolayer of EC on the polyester sheet was placed in a parallel plate flow chamber and subjected to steady shear stress of 30 dyn/cm^2 for 24 h. Control cells were grown on polyester sheets in the same experimental medium (DMEM+10%FCS+5.5mM glucose)and maintained in the incubator. **(2DG uptake)** Immediately after exposure to shear stress, the cells on polyester sheets were placed in plastic dishes. For the uptake studies, the cells were rinsed 5 times with PBS and incubated for 5 min with 10 ml of PBS containing 5-10 μCi[^{3}H] 2DG. Cell-associated radioactivity was measured after extensive washes and solubilization of cells. **(Results)** A comparison of transport activity in the two types of endothelial cells at 5.5mM glucose concentration showed that the transport rate in sheared cell was about 1.5 fold higher than that of control cell. (cont. us sheared:19.50±1.20 us 29.78±4.51 dpm/μg protein , P<0.05)**(Conclusion)**It was postulated that shear stress increased glucose transport in cultured EC.

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PLASMA vWF LEVELS CORRELATE WITH SOLUBLE INTERLEUKIN-2 RECEPTOR (sIL-2R) LEVELS IN TYPE 2 DIABETICS WITH RETINOPATHY.

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The endothelial injury and/or dysfunction in diabetes may be associated with activation of monocyte-macrophage system and T lymphocytes. Plasma sIL-2R level reflects mononuclear cell activation. The aim of this study was to investigate the possible relationship between sIL-2R and vWF levels, since the latter has been reported to reflect endothelial injury/dysfunction in diabetic patients with microvascular complications. Twenty-four type 2 diabetic patients [14 females and 10 males, median age 56 years (range 29-68), median duration of diabetes 5 years (range 0-18)] were included. Nine patients had background retinopathy (R+), while 15 had no retinopathy (R-). Eighteen healthy subjects [7 females and 11 males, median age 43 (range 27-64)] were also included for comparison. Plasma levels of sIL-2R and vWF were measured by EIA. Both median sIL-2R [57 pM (12-101) vs 42 pM (0-109)] and median vWF levels [118% (96-149) vs 107%(62-140)] were comparable in R+ and R- groups. R+ patients had higher vWF levels than healthy controls [104% (59-140)] (p=0.027). No significant differences were found in sIL-2R levels of patients and controls [62 pM (11-168)]. vWF levels correlated positively with sIL-2R levels only in the R+ group (r= 0.8, p=0.008). These findings suggest that mononuclear cells might be involved in the pathogenesis of microvascular injury in diabetic retinopathy. Further studies are needed to clarify the reason why sIL-2R levels do not show any significant difference in our R+ and R- diabetic patients.

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THE EFFECT OF IMPAIRED GLUCOSE TOLERANCE AND NIDDM ON THE FOREARM MICROCIRCULATION

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The natural history of the endothelial dysfunction in diabetes is not known. We have examined the effect of impaired glucose tolerance (IGT) and recently diagnosed NIDDM on the microcirculation. We studied three groups matched for age and sex: 14 healthy controls (C) (3 males, mean age 45 years), 13 subjects with IGT (4 M, age 48) and 11 recently diagnosed NIDDM patients (DM) (7 M, age 53, mean DM duration 2.3 years). Significant differences existed in the body mass index [DM 32.4 ± 4.8 (mean ± sd), IGT 36. 9 ± 7.9, C 26.4 ± 4.6, p < 0.001], the fasting plasma glucose levels [DM 174 ± 69 mg/dl, IGT 108 ± 10, C 96 ± 7, p<0.001, ANOVA test], the HbA1c levels [DM 8.2 ± 2.0%, IGT 5.6 ± 0.4, C 5.7 ± 0.4, p<0.001], and the fasting insulin levels [DM 15.8 ± 9. 3μU/ml, IGT 21.0 ± 12.5, C 5.4 ± 1.4,p <0.01]. All subjects had total cholesterol <300 mg/dl, triglycerides< 300 mg/dl, were non-smokers and normotensive or mildly hypertensive (HTN) without anti-HTN medications. Microalbuminuria and retinopathy were present in 3 (27%) DM subjects, and neuropathy in none. We employed single point laser Doppler and laser Doppler imaging scanner to measure vasodilatation at the forearm in response to heating to 44 °C, and to iontophoresis of 1% acetylcholine (endothelium-dependent) and 1% sodium nitroprusside (endothelium-independent). The response to heat was reduced in the DM (825 ± 390, % of increase over the baseline) compared to IGT (1368 ± 671), and C (1561 ±, 695), (p <0.05). Using the laser scanner, the response to acetylcholine, which stimulates the production of Nitric Oxide (NO), was different among the three groups [DM (64 ± 33 % of increase over the baseline), IGT (98 ± 54), C (118 ± 57), p <0.05]. The response to sodium nitroprusside (a NO donor) showed a similar trend [DM (64 ± 29), IGT (80 ± 34), C (96 ± 41)], but failed to reach statistical significance (p = 0.09). The above data indicate that endothelial dysfunction is present in early stages of NIDDM and may even precede its development during the IGT phase.

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Modulation by High Glucose of Thrombin Stimulated Protein Kinase C in Cultured Human Endothelial Cells

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Hyperglycemia is a major factor in the development of cardiovascular complications in diabetic patients and has been reported to activate Protein Kinase C in cells derived from vascular tissue. This study was consequently designed to evaluate the effect of long term incubation (16±1 days) in 30mM vs 5mM glucose on ligand induced activation of PKC in paired cultures of individual isolates of human umbilical vein endothelial cells (HUVECs). Cells from these isolates were stimulated with increasing concentrations of thrombin for 30 seconds and analyzed by immunoblotting for PKC-isoforms α, β_{II}, and ε. An increase in intensity of the isoform specific band in the membrane fraction was assumed to reflect PKC activation. Thereby, longterm incubation with 30mM vs 5mM glucose caused no change of total and membrane bound PKCα, PKCε, or $PKC\beta_{II}$. Interestingly, $PKC\beta_{II}$, was only found in the membrane fraction, even in resting cells. Stimulation by thrombin (0.01, 0.1, 1, 10 and 100nM) of confluent cultures displayed a concentration dependent rise in membrane bound PKCα and ε. In that context thrombin induced activation of PKCα remained unaffected by high ambient glucose, while translocation of PKCε in cells grown in 30mM glucose was reduced at high thrombin concentrations (AUC: 90.4±7% of control cells; p < 0.008; n=6) versus control cultures kept in 5mM glucose. Cells cultured in 30mM mannitol did not show reduced translocation of PKCε. These data suggest modulation by 30mM glucose of ligand induced PKC activation in an isoform specific manner, whereas basal expression of PKC isoforms remained unaffected suggesting interference by hyperglycemia with dynamic endothelial function.

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EFFECT OF C-PEPTIDE ON FOREARM BLOOD FLOW IN IDDM PATIENTS SEEM NOT TO BE ENDOTHELIUM - MEDIATED

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Recent studies suggest that C-peptide increases blood flow in both exercising and resting forearm skeletal muscle in patients with IDDM. To find out whether this effect is dependent on endothelial function non-invasive measurements of endothelium-mediated arterial response were undertaken using a high-resolution ultrasound doppler technique. Ten IDDM patients, age 30±2 yrs with a diabetes duration of 17±2 yrs, were studied twice within one week. They were treated with insulin s.c. four times per day. Their mean insulin dosage and HbA1c were 0.70±0.05 U/kg/24 hrs and 7.6±0.3%, respectively, prior to the study. No clinical sign of neuropathy, nephropathy and retinopathy were found. They recived i.v. insulin the night before and during the study so regulated that euglycemia (5-6mmol/l) was achieved. Blood flow and the diameter of the brachial artery were measured in basal state, one min after reactive hyperemia (4 min distal arterial occlusion) and 10 min later. Subsequently, C-peptide (6 pmol/kg/min) or saline was given i.v. in a randomized double blind order. Repeated measurements were performed during 60 min of infusion as well as after administration of sublingual glyceryl trinitrate. Close to the blood flow measurements both before and during the infusion period left ventricular function was measured by echocardiography. All patients showed a blunted brachial arterial dilatation (2.0±0.5%) after reactive hyperemia in comparison to healthy controls (9.3±0.3%), indicating a disturbed endothelial function. C-peptide infusion compared to saline resulted in increasead basal blood flow (+36±8%,p<0.001) and augmented left ventricular ejection fraction (+5±2%, p<0.05) and a tendency to increased dilatation (+3±1%,ns) of the brachial-artery. However, the vascular response to reactive hyperemia (endothelium-mediated) and glyceryl trinitrate did not differ between the periods. It is concluded that C-peptide at physiological levels (1.5 nmol/l) increases resting forearm blood flow in IDDM patients, but the effect is probably not endothelium-mediated.

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GLYCATION OF LOW DENSITY LIPOPROTEINS DISTURBS THE PRODUCTION OF FIBRINOLYTIC REGULATORS IN VASCULAR ENDOTHELIAL CELLS

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Attenuated fibrinolytic activity and increased incidence of intravascular thrombosis are found in diabetic patients. The present study investigated the effect of glycated low density lipoproteins (LDL) on the production of plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA) in human umbilical vein endothelial cells (HUVEC). Native LDL moderately increased the production of PAI-1 and reduced the generation of tPA in HUVEC. Glycation further increased the production of PAI-1 induced by LDL at both mRNA and protein levels. The synthesis and secretion of tPA from the cells treated with glycated LDL were significantly lower than that from native LDL-treated cultures. The production of PAI-1 and tPA was not affected by glycated albumin modified at the equivalent condition. Aminoguanidine, an inhibitor of the formation of advanced glycation end products (AGEs), effectively inhibited the glycation of LDL. Aminoguanidine treatments of LDL during the glycation inhibited glycated LDL-induced overproduction of PAI-1 and the reduction in tPA synthesis in HUVEC. The results indicate that glycation enhances LDL-induced imbalance of the production of PAI-1 and tPA in HUVEC. AGEs may be involved in the disturbed production of fibrinolytic regulators in vascular endothelial cells induced by glycated LDL, that may contribute to the reduction of fibrinolytic activity and the increase in thrombotic vascular complications in diabetic patients (supported by grant provided by Canadian Diabetes Association in memory of the late Archibald Mitchell).

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ARTERIAL NOREPINEPHRINE IS DECREASED IN NON-COMPLICATED IDDM

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Microangiopathy in IDDM is preceded by a state of generalized capillary hyperperfusion. These observations have led to the hypothesis that changes in regional haemodynamics contribute to microvascular complications. Up to now the pathogenesis of the increased blood flow is not completely understood. Studies using microneurography showed reduced sympathetic nerve activity (SNA) in non-complicated IDDM. A decrease in SNA is accompanied by a decrease in basal vascular tone. Therefore we performed a study in which we investigated basal forearm blood flow (FBF) and arterial norepinephrine (NE) concentration reflecting total body sympathetic activity in 50 non-complicated type I diabetic patients (DP) and 50 healthy controls (C). Forearm blood flow was measured by plethysmography. Arterial blood samples for NE and epinephrine (E) were drawn 45 minutes after cannulation of the brachial artery in the supine position. Blood pressure (BP) was measured intra-arterially. All subjects were in a fasting state. Plasma glucose concentration was not corrected to avoid confounding by the vasodilator effect of insulin. Data were analyzed by unpaired Student-*t*-test. Results are expressed as means$\pm$SE. Basal FBF was increased in DP compared to C (2.9 ± 0.1 and 2.0 ± 0.1 ml/dl/min, $p<0.01$). Arterial NE concentration was decreased in DP (NE concentration 0.58 ± 0.03 in DP and 0.81 ± 0.05 nmol/l in C, $P<0.01$). Epinephrine concentrations did not differ between groups (E concentration 0.21 ± 0.03 in DP and 0.21 ± 0.02 nmol/l in C, NS). Systolic / diastolic blood pressure was 116.2 ± 1.2 / 61.7 ± 0.8 in DP and 115.6 ± 1.2 / 63.2 ± 0.9 mmHg in C. Heart rate was not statistically different in both groups (66.5 ± 1.3 in DP and 63.3 ± 1.7 b/min in C, NS). It is concluded that forearm blood flow is increased in non-complicated IDDM. Arterial NE concentrations are decreased in non-complicated IDDM reflecting a decrease in sympathetic activity. A decrease in SNA could probably play a role in the pathophysiology of the hyperperfusion in non-complicated IDDM.

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OXIDATIVE STRESS AND ACE ACTIVITY IN IDDM PATIENTS

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Angiotensin converting-enzyme (ACE) is synthesized by endothelial cells and play a role in vascular tone, modulating anion superoxide and vasodilating kinins production. Elevated plasma levels of ACE have been demonstrated in IDDM patients with microangiopathy. In addition, oxidative stress is increased in IDDM patients and correlates with vascular diseases. We evaluated the relationship between ACE plasma activity and markers of oxidative stress such as lipid hydroperoxides (ROOHs), conjugated dienes (CD) and plasma anti-oxidant capacity. 70 IDDM patients (mean age 34±9 yrs; duration of disease 13.5±8.8 yrs; mean HbA1c 7.4), without microvascular complications, and 30 controls matched for age and sex were studied. Plasma antioxidant capacity was evaluated by total peroxyl radical-trapping capacity (TRAP), ROOHs by colorimetric assay, CD in *cis-trans* and in *trans-trans* conformation, by second derivative spectroscopy and ACE activity by spectrophotometric assay. TRAP was significantly reduced(663±131 vs 951±105 µmol/L;p<0,0001), while ROOHs (7.2±2.0 vs 2.1±0.7 µmol/L;p<0,0001) and total CD (0,0368±0,0027 vs 0.0328±0.0028 AU; p<0,0001) were significantly augmented in IDDM. CD in *trans-trans* conformation were significantly elevated (0.0340±0.0025 vs 0.0259± 0.0035 µmol/L;p<0,0001), the ratio of *cis-trans/trans-trans* CD isomers (0.088±0.038 vs 0.310±0.148;p<0,0001), a marker of altered plasma redox status, was significantly reduced in IDDM. ACE activity was significantly augmented in IDDM (28±6 vs 20±5 UI/L;p<0,05). Significant direct correlations were found in IDDM between ACE activity with total CD(r^2=0.127;p<0,002) and with *trans-trans* C D isomers(r^2=0.138; p<0,002). Neither markers of oxidative stress nor ACE activity were correlated with metabolic control or diabetes duration. Increased plasma ACE activity, possibly due to an elevated oxidative stress, as evidenced by augmented plasma CD, may represents an early marker of vascular injury in IDDM, without complications. The lack of correlation of these markers with metabolic control and the duration of disease may suggest that diabetes per sè is a condition of altered redox status and endothelial damage.

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CAPILLARY PERMEABILITY TO ALBUMIN AND ERYTHROCYTE DEFORMABILITY ABNORMALITIES IN DIABETIC NEUROPATHY.

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Capillary permeability to albumin (CPA) is often higher in diabetic subjects, particularly in those with peripheral neuropathy. An abnormality in the microcirculation could contribute to an increase in CPA. The aim here was to study how erythrocyte deformability affects the increase in CPA in diabetic subjects with peripheral neuropathy. Thirty-eight IDD's and NIDD's with peripheral neuropathy confirmed by electrophysiology were included in the study. An isotopic CPA test, according to the Landis method, was performed, consisting of an IV of 99m-technetium-labelled albumin, venous compression applied to an arm for 12 minutes, and the analysis of the decrease in radioactivity after withdrawal of venous compression. Interstitial albumin retention was measured 10 minutes after compression withdrawal. A lymphatic index (LF/HF) was obtained by analysing the decreasing radioactivity curve by Fast Fourier Transform. The erythrocyte rigidity index was determined by the Hanss hemorheometer. Excessive albumin retention ($\geq$ 8%) and an abnormality in the lymphatic function of interstitial albumin recuperation (LF/HF $\geq$ 1%) were found in 18 and 17 patients respectively. The erythrocyte rigidity index correlated positively with the LF/HF index (r = 0.442, p = 0.005). This study confirms the frequency of an abnormality in capillary permeability to albumin in diabetic patients with peripheral neuropathy and suggests that the increase in capillary pressure linked to a rheologic abnormality may contribute to albumin hyperfiltration and saturation of the lymphatic function.

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HIGH GLUCOSE INDUCED GROWTH FACTOR RESISTANCE IN HUMAN FIBROBLASTS CAN BE REVERSED BY ANTIOXIDANTS AND PROTEIN KINASE C INHIBITORS

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Insulin resistance is clinically significant both in insulin - and non-insulin-dependent diabetes mellitus. Hyperglycemia on its own induces insulin resistance by decreasing the numbers of glucose transporters, but also by affecting the insulin receptor. Hyperglycemia is correlated with a decreased kinase activity in the insulin receptor due to a phosporylation of the insulin receptor by protein kinase C (PKC) and / or an increased activity of cytosolic protein tyrosine phosphatase (PTPase). Chronic diabetic foot ulcers show deficient granulation and epithelisation. We have shown previously that fibroblasts from chronic diabetic and non-diabetic wounds have decreased proliferation compared to those from uninjured skin. The aim of this study was to investigate the effects of high glucose on human fibroblast proliferation, influence of growth factors, and the effects of antioxidants, PKC inhibitors and troglitazone. Normal human dermal fibroblasts were obtained from biopsies taken from five patients undergoing mammary reduction plastic surgery. Fibroblasts in 12-well plates were serum starved for 24 hours, whereafter experiments were conducted. 1. D- and L-glucose in various concentrations were added to the cells. 2. Serum free medium, newborn calf serum, insulin, IGF-I, EGF in high (25.5 mM) and low (11.5 mM) D-glucose. 3. Ascorbic acid, selenite, alpha-tokopherol, Q10, N,N-dimethyltiourea, beta-carotene, troglitazone, H-7, isoH-7 and bisindolylmaleimide IX were added to high and low D-glucose. DNA measurement with a fluorometric method was performed 48 hours after the addition of glucose in all three experiments. D-glucose but not L-glucose in a concentration >15.5 mM inhibited fibroblast proliferation ($p<0,05$). The cells showed resistance against growth factors and insulin in high glucose. Antioxidants, PKC inhibitors and troglitazone could reverse the high glucose induced inhibition of fibroblast proliferation. We postulate that these findings may be of importance for the understanding and future treatment of chronic diabetic foot ulcers.

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EFFECTS OF CYCLOOXYGENASE INHIBITION ON THE ENDOTHELIUM DEPENDENT RELAXATION IN DIABETIC FEMORAL AND CORONARY ARTERIES.

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Interaction between arachidonic acid metabolism and endothelium dependent relaxation in the femoral and coronary arterial bed in diabetes was studied. Healthy (n=10) and alloxan-diabetic (560µmol/kg alloxan-tetrahydrate iv., Sigma, n=10) young mongrel dogs of both sexes, weighing 19-25 kg were used. Femoral (n=5-5) and left anterior descending (LAD) coronary (n=5-5) arteries were dissected under pentobarbital anaesthesia (133µmol/kg Nembutal, Ceva), freed from fat and connective tissues, cut into 3-4 mm rings and suspended in organ chambers containing 5 ml Krebs buffer (pH 7.4, 37°C) areated constantly with a mixture of 95% O_2 and 5% CO_2. Changes in isometric tension were registered by a microdynamometer (F-30, Hugo Sachs). Relaxation elicited by acetylcholine (Ach, 3nmol/l-10µmol/l, added in a cumulative manner) - compared to the effect of 1 µmol/l sodium nitroprusside as 100% - was studied in vascular rings precontracted with $PGF_{2\alpha}$. Ach induced relaxation did not differ significantly between diabetic and healthy femoral vascular rings, characterized by the maximal relaxing effect of Ach and Ach sensitivity (pIC_{50}), after incubation in Krebs buffer containing 3µmol/l indomethacin (INDO) either. In LAD rings the maximal relaxing effect of Ach and Ach sensitivity was lower ($p<0.05$) in the diabetic (86±2%, 6.53±0.07) than in the healthy group (92±2%, 6.80±0.14), respectively. After INDO incubation pIC_{50} decreased in both groups, but the maximal relaxing effect of Ach decreased ($p<0.05$) in the diabetic group (75±5%) only, and Ach sensitivity of the diabetic coronary arteries (6.25±0.11) was lower ($p<0.05$) than that of healthy vessels (6.54±0.10). These results suggest that arachidonic acid metabolism is involved in the alteration of the vascular reactivity in diabetes. In coronary arteries the impaired endothelium dependent relaxation is worsened by the inhibition of the synthesis of vasodilator prostanoids.

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CYTOCHROME P450 2E1 ACTIVITY IN DIABETIC AND OBESE PATIENTS

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Cytochrome P450 2E1 (CYP2E1) is a key-enzyme for detoxification, induced in particular after chronic alcohol consumption. It is also involved in carcinogen activation (benzene, N-nitrosamines...) and the production of highly reactive free radicals. Obesity and diabetes seem also to be induction factors for CYP2EE1 in the rat. The aim of this study was to measure CYP2E1 activity in nonalcoholic subjects : 19 patients with NIDDM and 7 with IDDM, with poor glycemic control (HbA1c = 9.1 ± 0.6 %, fructosamine = 385 ± 20 mol/l), 19 obese patients (BMI > 29 kg/m^2) and 42 controls, by measuring the level of 6-hydroxylation of chlorzoxazone, a myorelaxing drug, according to a method we have previously described. CYP2E1 did not differ significantly in the diabetic patients and controls (0.30 ± 0.02 vs 0.29 ± 0.02) and was slightly higher in the patients with NIDDM than in those with IDDM (0.33 ± 0.03 vs 0.21 ± 0.03, $p < 0.05$). In the obese patients CYP2E1 activity was increased by 30 % compared to the controls (0.38 ± 0.04, $p < 0.05$). These results show that CYP2E1 induction by diabetes or obesity is relatively weak in man unlike that in rats with experimentally-induced diabetes (level 3 to 8 times higher) and that in chronic alcoholism in man (level 2 to 5 times higher). This suggests that CYP2E1 does not play an important part in the complications of diabetes or obesity.

This study was carried out thanks to a PHRC grant (Brest, 1994).

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ALTERED ENDOTHELIAL FUNCTION IN DIABETIC CORONARY ARTERIES

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Diabetic basal coronary blood flow is known by our previous data not to be different from that of the healthy. However, its sensitivity to exogenous and endogenous vasodilators is markedly diminished.To study the mechanisms underlying the impaired endothelial dependent relaxation (EDR), effect of acethylcholine (Ach) (2.25-4.5-9-18-36 pmol/kg) was measured on coronary blood flow in 6 metabolically healthy and 6 alloxan-diabetic dogs. Additionally the influence of Ach (0.1-1-10 µmol/l) and L-arginine (A:1 mmol/l) on the release of cGMP by isolated coronary rings as parameter for the generation of NO was determined. The increase of coronary blood flow due to Ach was significantly ($b_o \neq b_\bullet$; $t=4.68$; $p<0.01$) depressed in diabetes. The basal release of cGMP by diabetic coronaries was also less (1.3±0.3 fmol/mg) than by healthy ones (2.2±0.4 fmol/mg). Ach stimulated the release of cGMP dose-dependently, however the amount of cGMP maximally released was reduced ($p<0.05$) in diabetes (C: 3.54±0.5 ; D:1.86±0.2 fmol/mg) . Accordingly, the effect of acethylcholine inducing cGMP release is higher ($p<0.05$) in healthy vessels ($y=0.05 \log x+3.21$; $r=0.9245$) then in the diabetic coronaries ($y=0.015\log x+1.69$; $r=0.8990$). L-arginine induced cGMP elevation only in the controls (179±10%), without any effect ($p<0.05$) in the diabetic arteries (97±40%). These results are suggesting the diminished vasodilation in diabetes to be developing due to the insufficiency of endothelial nitric oxide production.

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ARE SOLUBLE ADHESION GLYCOPROTEINS A MARKER FOR CORONARY LESIONS IN THE DIABETIC PATIENT ?

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The circulating level of certain soluble adhesion glycoproteins has been found to be high in diabetic patients and could be involved in atherogenesis. The aim here was to compare the level of 5 soluble adhesion glycoproteins : Intercellular Adhesion Molecule-1 (ICAM-1), Vascular Cell Adhesion Molecule-1 (VCAM-1), selectins (Sel) E, P and L in NIDD's with no inflammatory disease, but with a symptomatic coronary disease (group 1, n = 11), silent myocardial ischemia with significant coronary stenoses on angiography (group 2, n = 11), or who were free from ischemic heart disease after an exercise test, a thallium dipyridamole myocardial scintigraphy and a continuous 48-hour ECG (group 3, n = 11). Group 4 was made up of 16 poorly controlled NIDD's receiving a maximal oral antidiabetic treatment. Results were compared with a group of 23 controls ICAM-1 and Sel P were not different between the diabetic patients and the controls or between the groups of diabetics. In the 4 diabetic groups VCAM-1 was significantly higher than in the controls but without a significant difference between the 4 groups. In group 4, Sel E was significantly higher than in the controls ($p < 0.01$), and after 14 days of continuous subcutaneous insulin treatment sel E and VCAM-1 decreased significantly. Sel L was lower in groups 1 and 2 than in group 3 ($p = 0.01$ and $p = 0.07$) and the controls ($p = 0.003$ and $p = 0.01$). If only the poorly controlled diabetic patients were compared (HbA1c > 8 %), sel L was still significantly lower in groups 1 and 2 than in group 3 ($p = 0.03$ and $p = 0.01$). These findings suggest that in NIDD's without inflammatory disease 1) the high level of VCAM-1 and Sel E is related to poor glycemic control ; 2) the low level of Sel L, linked to a reduction in its release which could favour leukocyte aggregation and accumulation, could be a marker for coronary disease.

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ABNORMALITIES IN CORONARY VASOMOTRICITY IN DIABETIC PATIENTS AND SUPEROXIDE RADICALS.

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We have already shown than an intracoronary injection of acetylcholine induces coronary vasoconstriction in diabetics, which suggests that coronary dilatation mediated by the EDRF is abnormal. In order to determine the mechanism for this abnormal response, we studied the flow-dependent and cold pressor test responses in 15 normotensive diabetic patients with no hypercholesterolemia and with normal coronary arteries on angiography. In 7 patients the measurements were carried out before and after an i.v perfusion of L-arginine (L-arg), a precursor of EDRF, and in the other 8 patients before and after an i.v. perfusion of deferoxamine (DF), an agent preventing the formation of superoxide radicals. Quantitative angiography was used to determine the size of the proximal interventricular artery (pIVA) at the basal state, at the end of the cold pressor test and after an injection of 10 mg papaverine (PAP) in the distal IVA given in order to increase coronary flow in the pIVA. At the basal state the diameter of the pIVA decreased during the cold pressor test ($p < 0.01$) and did not change after PAP. Administration of L-arg did not change the response to these two tests. After administering DF the pIVA dilated significantly during the cold pressor test ($p < 0.01$) and after the PAP injection ($p < 0.001$). The isosorbide dinitrate injection induced a comparable coronary dilatation in both groups. In conclusion 1) the blood-flow-dependent and cold-induced coronary dilatation is abnormal in diabetic patients ; 2) the absence of an L-arg effect on these abnormal responses suggests that there is no deficit in the substrates necessary for EDRF synthesis ; 3) the improvement in the responses to the two stimuli by deferoxamine suggests that inactivation of nitrogen monoxide by superoxide radicals could be responsible for the subclinical abnormalities in coronary vasomotricity observed in diabetic patients.

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ADRENOMEDULLIN PLASMA LEVELS IN PATIENTS WITH INSULIN DEPENDENT DIABETES MELLITUS (IDDM)

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Adrenomedullin (AM) is a new vasodilatador and hypotensive peptide, originally isolated from human pheocromocitoma. AM is widely distributed in peripheral tissues including vascular endothelial cells. AM may have an important role in the regulation of cardiovascular system. We measured plasma levels of AM by radioimmunoassay (Peninsula Laboratories kit, after Sep Pack C18 extraction) in 135 patients with IDDM classified according to the presence of complications (91 patients without nephropathy -A-; 28 with microalbuminuria and serum creatinine concentration <1,3 mg/dl -B-; and 16 with serum creatinine concentration >1,3 mg/dl -C-), and 54 healthy subjects. Results are shown as mean±SD. Kruskal-Wallis and Mann-Whitney tests were used for comparisons between groups. Spearman test was used to study correlation.

	CONTROL	A	B	C
Adrenomedullin (pg/tube)	7,4±3,8	8,1±3,3	8,8±3,6	30,1±32,6

AM was not significantly higher when we compared all patients (A+B+C) with controls (p=0,053); AM was higher in C group than in other groups. There were not differences between A, B, and control groups. AM concentration was also higher in the patients with hypertension (15±7,8) than in the patients with normal levels of arterial pressure (10,8±14,5). AM levels correlated significantly with serum creatinine and HbA1c levels, and with duration of the disease. These data suggest that AM may be released from damaged vascular tissue as a compensatory mechanism.

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AUTOANTIBODY AGAINST OXIDIZED LDL AND PARTICLE SIZE: ASSOCIATIONS TO CORONARY REACTIVITY IN YOUNG MEN.

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Endothelial dysfunction is an early event in coronary atherosclerosis, and it leads to lowered coronary flow reserve (defined as the ratio of maximal to basal coronary blood flow). According to experimental studies oxidized LDL particles are injurious to endothelium impairing its normal vasodilator function. Small dense LDL particles may be especially susceptible to oxidative modification. We studied the importance of oxidized LDL and LDL particle size to coronary reactivity in healthy men (age 34±3 years). Coronary flow reserve was assessed with positron emission tomography imaging and oxygen-15-labelled water with intravenous dipyridamole infusion. Autoantibodies against oxidatively modified lipoproteins were measured with ELISA method using native LDL and copper-oxidized LDL as antigens. The antibody titer was calculated as the ratio of antibodies to native LDL against modified LDL. LDL particle size distribution was determined by nondenaturing polyacrylamide gradient gel electrophoresis. Mean myocardial blood flow was 0.8 $mL \cdot g^{-1} \cdot min^{-1}$ at rest, and 4.1 $mL \cdot g^{-1} \cdot min^{-1}$ after dipyridamole infusion. The mean coronary flow reserve was 4.9 (range 1.3 to 8.4). The titer of autoantibodies to oxidized LDL was inversely correlated with coronary flow reserve ($r=-0.42$, $P=0.02$). LDL particle diameter was not significantly correlated with coronary flow reserve ($r=0.16$, $P=0.40$) or with the titer of autoantibodies to oxidized LDL ($r=-0.08$, $P=0.83$). In conclusion, these data provide evidence for the role of oxidatively modified LDL in affecting coronary vasoreactivity in vivo.

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Effects of AGE on Glycosaminoglycan synthesis in Sheared Endothelial Cells.

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(**Purpose**)Glycosaminoglycans(GAGs) present on the surface of endothelial cells (ECs) has long been known to be function as a inhibitors for blood coagulation.It was postulated that vascular endothelium sheared in vitro closely mimic those in vivo. In this study, the effect of advanced glycosylation end product (AGE) on GAGs synthesis was examined in sheared or static ECs. (**Methods**) A confluent monolayer of ECs on the polyester sheet was placed in a parallel plate flow chamber and subjected to steady shear stress of 30 dyn/cm^2 for 24 h. Control cells were grown on polyester sheets in the same experimental medium and maintained in the incubator. Each cells were exposed with bovine serum albumin(BSA) or AGE which was prepared with BSA. DNA synthesis was measured with [^{3}H] thymidine and GAG synthesis was measured [^{35}S] sulfate.(**Results**)In the static condition with AGE, the amounts of newly synthesized GAGs was significantly decreased with dose dependent manner.Furthermore,this decrease was in proportion to that of DNA synthesis with time dependent manner.In the sheared condition,these effect by AGE has not been seen.(**Conclusion**)These protective effects of relative high shear stress on GAGs synthesized by ECs under the influence of AGE may be function as an antiatherogenic factors in vascular wall.

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ENDOTHELIAL FUNCTION IN COMPETITIVE ATHLETES: RELATIONSHIP TO ANTIOXIDANT STATUS AND LDL CONCENTRATION, SIZE AND OXIDABILITY

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Physical exercise induced increases in oxygen uptake is accompanied by increased free radical generation, which may exceed the capacity of antioxidant defenses. The functional consequences of such changes are unknown. We determined forearm vasodilatory responses to intra-arterial infusions of endothelium-dependent (acetylcholine, ACh) and -independent (sodium nitroprusside, SNP) vasodilatators, and levels of antioxidants, lipoproteins, LDL size and oxidation (diene formation, antibodies) in 8 competitive athletes (VO_2max 69±2 ml/kg·min, age 24±2 yrs; BMI 21±1 kg/m^2, n=8) and 13 normal men (50±2 ml/kg·min, p<0.001; 26±1 yrs; 22±1 kg/m^2, n=13). Athletes had lower plasma protein thiol (SH-group) (307±31 vs 368±14 mmol/l, p<0.05) but not ascorbate or uric acid concentrations. Thiol correlated with total plasma radical trapping capacity (r=0.49, p=0.025). Athletes had higher serum apoAI (141±12 vs 123±3 mg/dl, p<0.05) and HDL (1.6±0.1 vs 1.3±0.04 mmol/l), p<0.05), while LDL cholesterol (2.5±0.3 vs 2.6±0.1 mmol/l), autoantibodies against oxidized/native LDL (1.2±0.2 vs 1.0±0.1), LDL size (27.0±0.2 vs 26.8±0.2 nm) and oxidized LDL lag time (149±8 vs 158±10 min) were comparable. VO_2max was inversely related to endothelium-dependent/-independent vasodilatation (r=-0.45, p<0.05). In multiple linear regression analysis (multiple r=0.61, p<0.02), both VO_2max (p=0.008) and LDL cholesterol (p=0.043) were independently associated with impaired endothelium-dependent vasodilatation. We conclude that a high aerobic capacity in competitive athletes may impair endothelial function, possibly by impairing antioxidant defenses.

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ENDOTHELIAL INTERLEUKIN 6 SECRETION IS DOWN-REGULATED BY SHEAR STRESS AND POSITIVELY DEVIATED BY GLUCOSE

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We investigated the effect of shear stress and glucose concentration on interleukin 6 (IL6) secretion in cultured human umbilical vein endothelial cells (HUVEC) to clarify the mechanism of elevated plasma fibrinogen and IL6 concentration in diabetic patients. HUVEC at 3rd passage were subjected to laminar shear stress up to 7.3dynes/cm^2 loaded by static parallel plate flow chambers of 40cm^2 in cell attaching area with MCDB131 media containing 5mmol/l or 20mmol/l glucose in humidified 37℃ 5% CO_2 95% air. IL6 concentration of media samples obtained before and after 24h stress load was measured with ELISA. The rate of IL6 secretion by HUVEC was then calculated. Statically incubated HUVEC and media were used as control. The rate of IL6 secretion by HUVEC was reduced in accordance with increase of shear stress in media containing 5mmol/l glucose (18.8 pg/cm^2/24h in statical incubation vs 12.2pg/cm^2/24h in 7.3dynes /cm^2). The rate was elevated as 28.8pg/cm^2/24h in HUVEC statically incubated in media containing 20mmol/l glucose.

These results indicate that endothelial IL6 secretion is down-regulated by shear stress and positively deviated by glucose. We have reported previously that IL6 secretion is increased and IL6 mRNA levels are elevated also in human monocytes incubated in high glucose concentration media. Thus we suspect: 1)the negative feed back regulation system of plasma fibrinogen concentration through IL6 secretion by vascular endothelial cells, 2)the positive deviation by glucose concentration in this system. This deviation may be relevant to the development of vascular complications in diabetes.

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HIGH ICAM-1 IN CHILDREN WITH DIABETES MELLITUS: A POSSIBLE ROLE OF WHITE CELLS IN ENDOTHELIAL DYSFUNCTION IN DIABETES?

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Endothelial dysfunction was recognised recently to represent at early stage of diabetic angiopathy. Children and adolescents with diabetes generally do not show clinical evidence of such complications, and this is usually delayed to late adolescence or early adulthood. Hyperglycaemica and advanced glycation endproducts can cause endothelial dysfunction and this might trigger expression of cell adhesion molecules (CAM) via the cytokines tumour necrosis factor alpha (TNFα) and interleukin-1 (IL-1). Of CAMs, ICAM-1 represents a marker of endothelium/white cell interaction and trans-endothelial migration of white cells. We have found high levels of ICAM in 51 children, adolescents and young adults with insulin dependent diabetics (22 males and 29 females), mean age ± SD 14.7 ± 3.4 years, duration of diabetes 6.6 ± 4.6 years, and HbA1c of 8.6 ± 1.5% (normal < 5.8%), compared with 22 healthy age and sex matched normal controls (9 males and 13 females) age 15.6 ± 4.1 years. None of the diabetic patients had any clinical evidence of microvascular disease and all had urinary microalbumin within the normal range. The levels of ICAM-1 in the diabetic cohort were 286.5 ± 56.78 ng/ml vs 247.65 ± 71.38 ng/ml in the controls, reaching statistical significance of p < 0.02 ANOVA. ICAM-1 didn't correlated with duration of diabetes of glycated haemoglobin. These results support the hypothesis that microvascular disease starts early in the course of childhood diabetes. Furthermore elevated ICAM-1 delineates possible contribution of white cells in the pathogenesis of endothelial dysfunction in diabetes.

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HIGH GLUCOSE-INDUCED COLLAGEN IV UPREGULATION IS MODULATED BY ANTISENSE OLIGONUCLEOTIDES

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Increased synthesis of collagen IV is associated with the development of basement membrane thickening--a characteristic lesion of diabetic microangiopathy. Because antisense technology offers a unique opportunity to down regulate specific gene expression, we investigated the effect of antisense phosphorothioate oligonucleotides (PS oligos) directed against collagen IV mRNA on collagen IV synthesis and proliferation in a cell line derived from rat microvascular endothelium and cultured in high (30mM) glucose. The rat endothelial cells grown in high glucose for 5.6±1.3 days exhibited increased cell proliferation compared to control cells grown in 5mM glucose (169% of control, P=0.038). Collagen IV mRNA level (determined by Reverse Transcription-Polymerase Chain Reaction) was increased to 172% of control. However, when cells grown in high glucose were transfected with 0.4 μM collagen IV-antisense PS oligos in the presence of cationic liposomes, the cell number and collagen IV mRNA level decreased compared to cells grown in high glucose to 61% and 115% of control, respectively. Actin expression, used as control, was not affected by PS oligos. This study indicates that antisense PS oligos directed against collagen IV mRNA specifically reduce collagen IV mRNA in rat endothelial cells and decrease proliferative effects of high glucose. Since vascular endothelial cells overexpress collagen IV in diabetes such an intervention may provide a means to arrest collagen IV overexpression and clarify its role in the pathogenesis of diabetic microangiopathy.

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THE EXPRESSION OF ENDOTHELIAL NITRIC OXIDE SYNTHETASE IS REDUCED IN DIABETIC PATIENTS WITH PERIPHERAL NEUROPATHY

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Reduced production of nitric oxide has been proposed as an important factor in the development of peripheral diabetic neuropathy. We have studied the expression of nitric oxide synthetase activity in the skin of diabetic neuropathic patients and healthy subjects. Five control (2/3 M/F) and 5 diabetic subjects [3/2 M/F, 2 IDDM, DM duration 17 (range 6-33) years] matched for age [53 (26-76) (mean, range) years vs 58 (48-71), sex and body mass index [31.1 ± 5.0 (mean± sd) vs 28.6 ± 7.6] were included. Nerve function measurements were impaired in the diabetic group, including Neuropathy Disability Score (0 ± 0 vs 21.8 ± 5.5, $p < 0.001$), Vibration Perception Threshold (15 ± 10 Volts vs 50 ± 1.3, $p < 0.001$) and peroneal motor conduction velocity which was measurable in 1 patient. The transcutaneous oxygen tension was similar in both groups (69 ± 7 mmHg vs 58 ± 17, p=NS) and clinical peripheral vascular disease was not present in any participant. Full thickness skin biopsies from the dorsum of the foot were obtained and were immunostained with antiserum to human endothelial nitric oxide synthetase, glucose transporter I (GLUT I) and von Willebrand factor, an anatomical marker of the endothelium. The staining intensity was evaluated by a pathologist, who was blinded to the diabetes status of each subject, as normal or reduced. No differences were found between the two groups in the staining intensity of von Willebrand factor [reduced in 1 (20%) control vs 2 (40%) diabetic patients (p=NS)] and GLUT I [reduced in 1 control (20%) vs none (0%) diabetic patients p=NS]. In contrast, the staining intensity of nitric oxide synthetase was reduced in 1 (20%) control and in all 5 (100%) diabetic patients ($p < 0.01$). We conclude that in diabetic neuropathic patients the endothelial nitric oxide synthetase activity is reduced despite the fact that the endothelium is anatomically present indicating that endothelial functional changes may be related to the development of neuropathy.

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ORGANS' VASCULARISATION IN OFFSPRING OF PREGNANT RATS FED AN ISOCALORIC PROTEIN RESTRICTED DIET

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Functional and structural changes are induced in the fetal endocrine pancreas by derangements in maternal metabolism or nutrition such as in low protein diet. Brain and kidney manifest also alterations which may remain till adulthood. Vascularisation of organs have not been analyzed and may also be affected. The aim of the study is to investigate the blood vessel density (BVD) by morphometric analysis and blood flow (BF) by non radioactive microspheres injection in organs of offspring receiving 20 % protein (C) or 8 % protein (LP) during fetal life till adulthood. A recuperation group (R) received 8 % protein only during fetal life and a normal diet after birth. BVD of the islets was analysed on semithin sections. BVD of the brain and the duodenum was analysed on 30 μm sections in animals injected with nuclear track emulsion. Islet BVD decreased from 7.74 ± 0.47% in C to 2.79 ± 0.34% in LP newborns ($P<0.01$) and from 3.34 ± 0.28 % in C to 1.96 ± 0.11% in LP adults ($P<0.01$). Islet BF is reduced from 29 ± 5 μl/min/g in C to 10.3 ± 1.9 μl/min/g in LP adults ($P<0.05$). Islet BVD and BF were normalized in R adults. In the duodenum, BVD was decreased by 24 % ($P<0.05$) in LP while it was normal in R adults. In the latter BF was increased by 60 % ($P<0.05$). In contrast, BVD in brain of LP newborns showed a reduction of 70% ($P<0,01$). At adulthood, it remained decreased respectively by 35% and 30% in LP and R group. These results show that in the neonates, islet and brain vascularisation is decreased when a LP diet is given during fetal life. With a low protein diet given after birth till adulthood, the reduction of the vascularisation of the endocrine pancreas, the duodenum and the brain remains. With a normal diet given after birth, vascularisation in the adult duodenum and islet is recovered but reduced brain vascularisation still prevails.

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ENDOTHELIUM-INDEPENDENT CYCLIC GMP FORMATION IN CAROTID ARTERIES OF DIABETIC RATS

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The development of vascular complications in diabetes mellitus is often associated with endothelial dysfunction. Previously, we showed that cyclic 3'-5'-guanosine monophosphate (cGMP) production was reduced in denuded carotid artery segments of diabetic rats, compared to denuded segments of control rats, during stimulation with the NO-donor sodium-nitroprusside (SNP, 10^{-6} M).The present study was performed to investigate whether this reduced cGMP production was due to a decrease in sensitivity, or whether the maximal response was reduced. Furthermore, another NO-donor, S-nitroso-N-acetyl-penicillamine (SNAP), was examined. Diabetes was induced in Wistar Rp rats by streptozotocin (70 mg/kg, D). Control rats (C) received vehicle only. After 3 months, carotid arteries were isolated, mechanically denuded of endothelium and divided into 6 segments. Segments were pre-incubated for 60 min in Krebs-Henseleit buffer (glucose 7 mM for C and 20 mM for D), and then exposed for 10 min to 3-isobutyl-1-methylxanthine with addition of SNP (n=8-10) or SNAP (n=3) in 3 concentrations (10^{-6}, 10^{-5} and 10^{-4}M) during the last 2 min. cGMP was determined by RIA. SNP-stimulated cGMP levels (fmol/mm vessel ± SEM) were significantly lower in diabetic arteries than in control vessels (10^{-6}M: 2117 ±302 vs 3491 ±571; 10^{-5} M: 2480 ±438 vs 4734±611; 10^{-4} M: 3207± 474 vs 4828 ±590 fmol/mm, $P<0.05$). SNAP-induced cGMP production was comparable in arteries of diabetic and control rats (10^{-6} M: 2545±381 vs 4161± 755; 10^{-5} M: 3784±652 vs 3923±531; 10^{-4} M: 4390± 860 vs 4525±214 fmol/ mm). We conclude that in de-endothelialized carotid arteries of diabetic rats the maximal stimulation of cGMP production that can be induced by SNP, is reduced. There seems to be no general defect in endothelium-independent stimulation of cGMP since preliminary results indicate that SNAP-induced cGMP production is normal.

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MORPHOHISTOMETRIC INVESTIGATIONS OF PLACENTAS OF DIABETIC PATIENTS, USING A DIGITAL ANALYSER

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OBJECTIVE: We performed histomorphologic evaluation of placentas from pregnant women with diabetes mellitus and compared them with placentas from normal women.
STUDY DESIGN: 53 pregnant women with diabetes participated in the study treated according to SDM Program, 42 patients with with diabetes prior to pregnancy (PGDM) and divided into classes acc. to White, and 11 patients with gestational diabetes (GDM), 16 women with physiological pregnancy (N), after 37th week of gestation were used as a control. For digital image analysis an IBAS 2000 analyser (Kontron) was used with a Chalnicon B/W camera and a custom-made image analysing program. In each analysed field the program computed the following histomorphological parameters of selected vascular profiles:
- total area of selected profiles [FAREA],
- total circumference of the selected profiles [FPERIM],
- in dividual area of each profile [AREA],
- individual circumference of each profile [PERIM],
- diameter of the circle inscribed in each profile [DMIN],
- the number of complete vascular profiles in the microscopic field [COUNT].

RESULTS: **1.** Statistically significant differences were found in the diameters of vascular profiles [DMIN] between normal placentas and placentas of diabetic mothers. **2.** Placental vessels of diabetic mothers are wider than those of normal placentas, except RF diabetes, acc. to White. **3.** A significantly lager total area of selected profiles [FAREA] was found in the placentas of diabetic patients vs. normal pregnancy.
CONCLUSIONS: Statistically significance results was found between histometric parameters (AREA, PERIM, DMIN), in studied groups [acc. to White (B, C, D)] compared to a normal group.

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ABNORMAL RESPONSE OF NITRIC OXIDE METABOLITIES TO ACUTE HYPERGLYCEMIA AND IV FUROSEMIDE IN IDDM PATIENTS.

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Nitric oxide (NO) plays an important role in kidney function control influencing the renal vascular tone and sodium handling. The aim of the study was to evaluate the urinary NO_2^- /NO_3^- (NO_x) a) under basal conditions and after stimulation with iv furosemide (0.5 $mg.kg^{-1}$) and b) during glycemic clamp-induced normo- and hyperglycemia (5 and 12 mmol/l) in 19 patients with insulin-dependent diabetes mellitus (IDDM) without microalbuminuria compared to 12 weight-, age- and sex-matched healthy controls. Sodium excretion and renal hemodynamics using the clearances of inulin (C_{in}) and para-amino-hippuric acid (C_{PAH}) were measured during examinations in both groups. Despite comparable baseline urinary NO_x excretion in diabetics and controls, the different response of NO_x was found after furosemide administration: no changes in diabetics (228±50 vs 146±38 $nmol.min^{-1}$) and increase NO_x excretion in controls (196±54 vs 681±146$nmol.min^{-1}$; $p<0.001$), although the furosemide-induced increases in natriuresis were comparable. During clamp-induced euglycemia, NO_x excretion was comparable in diabetic and control subjects and significantly increased during hyperglycemia in controls (425±57 vs 560±57 $nmol.min^{-1}$; $p<0.01$), while it did not change in diabetics (352±56 vs 323±39 $nmol.min^{-1}$). Fractional excretion of sodium (FE_{Na}) was comparable and declined significantly during hyperglycemia in both groups ($p<0.05$). There were no significant relationships between NO_x excretion and FE_{Na} or renal hemodynamics. We conclude that 1) IDDM without alterations in renal hemodynamics is associated with blunted renal generation of NO in response to hyperglycemia and furosemide administration, and 2) the antinatriuretic effect of hyperglycemia is not mediated by increased renal NO production.

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SERUM VASCULAR ENDOTHELIAL GROWTH FACTOR LEVELS ARE ELEVATED IN DIABETIC PATIENTS WITH HYPERGLYCEMIA

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Diabetic complications (ex.proliferative retinopathy) are often angiogenic, so we studied the relation between vascular endothelial growth factor (VEGF) levels and plasma glucose (PG) levels in diabetic patients. We already reported VEGF level in aqueous humor of diabetic patients with rubeotic glaucoma is markedly elevated (Diabetes Care 19: 1996). We enrolled 108 diabetic patients (age was 59±12 years, man/woman rate was 67:41) and measured serum VEGF, PG and HbA1c. Serum VEGF levels were measured by our newly developed sensitive ELISA using the rabbit anti-human VEGF polyclonal antibody. We checked age, sex, type of DM and the degree of diabetic retinopathy, nephropathy and neuropathy. We divided patients into 4 groups with PG (A: PG<200, B: 200≤PG<250, C: 250≤PG<300, D: PG≥300 (mg/dl)) and into 4 groups with HbA1c (a: HbA1c<10, b: 10≤HbA1c<11, c: 11≤HbA1c<12, d: HbA1c≥12 (%)). In 108 patients, their mean VEGF was 67.2±48.0 (mean±SD) pg/ml, PG was 178±81 mg/dl, HbA1c was 8.9±2.1%, respectively. In group A (n=71)/ B (20)/ C (7)/ D (10), their VEGF levels were 58.8±37.8/ 76.9±45.8/ 63.9±35.1/ 110.6±90.2, respectively. VEGF level in group D was significantly higher than in group A (p=0.014). In group a (n=80)/ b (13)/ c (9)/ d (6), their VEGF levels were 60.6±39.3/ 58.5±40.4/ 88.8±38.8/ 142.3±100.9, respectively. VEGF level in group d was significantly higher than in group a (p=0.0006) and in group b (p=0.003). These results suggest that hyperglycemia (PG ≥ 300 mg/dl or HbA1c ≥ 12%) may up-regulate VEGF levels in vivo and influence diabetic complications.

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ENDOTHELIAL DYSFUNCTION IN IDDM IS ASSOCIATED WITH LOW CONCENTRATION OF ACTIVE TRANSFORMING GROWTH FACTOR-β.

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Low levels of active transforming growth factor-β (active TGF-β) have been observed in non-diabetic patients with advanced atherosclerosis and it has been proposed that active TGF-β might be a key inhibitor of atherogenesis. Since endothelial dysfunction is an early event in atherosclerosis, we hypothesised that low levels of active TGF-β might be associated with increased endothelial dysfunction in patients with IDDM. To test this hypothesis, we measured responses of the brachial artery to flow-mediated dilation (FMD) and to 400 µg sublingual glyceryl trinitrate (causing endothelium-dependent and endothelium-independent dilation respectively) using a high-resolution ultrasound technique in 31 patients [17 males, 14 females; age 38.5 ± 2.1 yr (mean ± SEM); duration of diabetes 27.6 ± 1.5 yr; HbA1 10.1 ± 0.4 %; total cholesterol 5.3 ± 0.2 mmol l^{-1}; triglyceride 1.8 ± 0.2 mmol l^{-1}; HDL-cholesterol 1.3 ± 0.1 mmol l^{-1}, urine albumin/creatinine ratio 25.5 ± 7.0 µmol l^{-1}. Active TGF-β was measured by ELISA. FMD response was 103 ± 0.6 %, GTN response was 116.8 ± 1.8 %. Active TGF-β concentration was 4.61 ± 0.53 $ng.ml^{-1}$ There was a positive correlation between active TGF-β concentration and endothelium-dependent flow-mediated dilation (FMD = 101 + 0.417.TGF-β, $r = 0.36$, $p < 0.05$) but not endothelium-independent dilation ($p = 0.27$) nor other biochemical parameters. In conclusion, these preliminary data suggest that low levels of active TGF-β are associated with impaired endothelial response in Type 1 diabetes.

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STUDIES OF PLASMA ECMR-III(CD44) AND PECAM-1(CD31) LEVELS IN DIABETIC PATIENTS

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In cases of chronic diabetic complication, highly organ-specific lesions involving endothelial cells are frequently formed. It has been demonstrated that vascular endothelium has various function as shown by the participation in the blood coagulation-fibrinolysis system, lipid metabolism, vasodilation, vasoconstriction and the immune system. As the cause of diabetic microangiopathy, adhesion molecules derived from vascular endothelial cells is drawing attention. In this study, plasma ECMR-III (CD44) and PECAM-1 (CD31) levels in diabetic patients were measured using ELISA kits, and the relation of these factors with microangiopathy was examined. Plasma CD44 level (Mean±SD, ng/ml) in 69 diabetic patients was 1.92±0.72 which was significantly higher than 1.22±0.18 in healthy subjects ($P<0.05$). Plasma CD31 level in diabetic patients was 6.04±1.73, which was not significantly different from 6.80±1.02 in healthy subjects. Plasma CD44 level in the microangiopathy group of diadetics was 1.92± 0.66. It was 1.84±0.76 in diabetics without microangiopathy. There was no significant difference between these two groups. However, plasma CD44 level in the macroalbuminuria group was 2.34±0.96, which was significantly elevated when compared with 1.70±0.31 in the normoalbuminuria group ($P<0.001$) and 1.80±0.33 in the microalbuminuria group ($P<0.05$). Plasma CD44 level in diabetic patients did not show any significant difference with the states of diabetic control and therapeutic methods. The level did not correlate with any of BMI, total cholesterol, HDL-C, Lp(a), and RLP-C. Involvement of CD44 in the development and progress of microangiopathy in diabetic patients was suggested.

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EVIDENCE FOR A DIRECT IN VIVO EFFECT OF INSULIN ON ENDOTHELIAL CONSTITUTIVE NITRIC OXIDE-SYNTHASE

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Insulin (I) augments endothelium(E)-dependent vasodilation induced by muscarinic (M) agonists, a finding interpreted as a proof that I stimulates endothelial constitutive nitric oxide (NO)-synthase (cNOS). However, I could do so also by acting at some point in the M-receptor-signal transduction pathway upstream to cNOS. We studied 7 (6 males, 1 female) young, healthy subjects with the forearm (F) perfusion technique and assessed the F blood flow (FBF, measured by Cardio-Green dilution) responses to graded intra-arterial (i.a.) doses of the M-agonist acetylcholine (ACH, 3, 9 and 30 µg/min/kg of F), of bradykinin (BK, 40, 120 and 400 ng/min/kg of F), which activates endothelial cNOS through a receptor and a transduction pathway completely independent of ACH, and of sodium nitroprusside (SNP, 1, 3, and 10 µg/min/kg of F), an NO donor, during the i.a. infusion of both saline (SLN) and I, the latter at a dose designed to raise local I concentrations to ~500 pmol/l with no systemic effects. Hyperinsulinemia enhanced both ACH (63.9±8.3, 115±19, 192±33 vs 52±11, 81.2±13, 171±21 ml/min/kg of F, $p<0.01$ by ANOVA, I vs SLN dose-response) and BK (81.3±14, 118±19, 159±13 vs 50±11, 99±20, 136±27 ml/min/kg of F, $p<0.05$ by ANOVA, I vs SLN dose-response), but not SNP (45.6±5.5, 65.9±5.5, 107±11 vs 42.9±7, 64.2±11, 93.6±10 ml/min/kg of F, p=n.s. by ANOVA, I vs SLN dose-response) effects on FBF. Identical patterns were observed when the dose-responses of FBF, expressed as % increases over baseline, and of F vascular resistance were examined. CONCLUSION: Since I improves the vasodilatory effects of both ACH and BK, which converge on endothelial cNOS through two completely independent transduction pathways, but not of SNP, which directly releases NO, these data are most consistent with a direct in vivo effect of I on endothelial cNOS.

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GLUCOSE-DEPENDENT RESPONSE OF RETINAL ENDOTHELIAL CELLS TO GROWTH FACTORS RM Knott, M Robertson, E Muckersie and JV Forrester. Department of Ophthalmology, University Medical Buildings, Foresterhill, Aberdeen, AB9 2ZD

Clinical studies have demonstrated that the onset and progression of diabetic retinopathy (DR) is influenced by the glucose control of the patient. DR is characterised by the co-existence of impaired cell growth and excessive cell proliferation and we wished to determine the effect that glucose has upon these parameters. Bovine retinal endothelial cells (BREC) were exposed to a range of glucose concentrations from 0 - 25 mmol/l. The level of DNA synthesis and cell number was then determined using pulse labelling with tritiated thymidine and a Coomasie blue dye based assay respectively. The level of DNA synthesis declined significantly as the concentration of glucose increased. This effect was shown to be dependent upon the presence of TGF-β and was mediated by a PKC dependent pathway. Furthermore, in the presence of glucose (5mmol/l), insulin failed to increase DNA synthesis in BREC while in its absence, insulin readily induced DNA synthesis. A similar loss of IGF-1 mediated DNA synthesis was observed in the presence of 5 mmol/l glucose. Thus, glucose impairs DNA synthesis via a TGF-β mediated mechanism and also renders BREC less responsive to the growth factors IGF-1 and insulin. This suggests that antagonistic effects of TGF-β and insulin/IGF-1 can be induced in BREC which is dependent upon the concentration of glucose that the cells are exposed to.

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IMPAIRED ENDOTHELIUM DEPENDENT DILATATION IN AFRICAN AMERICAN SUBJECTS MAY CONTRIBUTE TO INSULIN RESISTANCE

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Impairment of endothelium dependent NO mediated dilatation has been shown in insulin resistant states. It has been postulated that this defect might contribute to the decreased insulin sensitivity by altering the supply of insulin and substrate to the tissues. As the incidence of insulin resistance is high in the African American we have studied the dilatory response of the brachial artery following forearm ischemia, a response that is endothelium dependent and NO mediated, in a healthy subset of this population. Sixteen normotensive African Americans, age 20-47 years, with fasting blood glucose of 77 ± 10 mg/dl, total cholesterol 120.1 ± 31 mg/dl and twenty two age matched Caucasians were investigated. Ischemia was induced by inflating a cuff over the arm to a pressure of 40 mm of Hg over systolic for 5 minutes. Doppler ultrasonography (using Acuson 128XP10 ultrasonograph with 7.5 Mhz linear array transducer) was used to measure brachial artery diameter and blood flow velocity at 15 seconds and 45-60 seconds following deflation. Dilatation and hyperemia were defined as the percentage increase over basal diameter and percentage of basal flow respectively. Postischemic median dilatation was 0% (range 0-3.14%) in African Americans and 4.42% (range 4.42-8.70%) in Caucasians ($p<0.05$). Six out of 16 African Americans as compared to 4 out of 22 Caucasians did not dilate following ischemia (0%) ($x^2=4.39$, $p<0.04$). Hyperemic response was less than 300% in 5 out of 16 African Americans as compared to 1 out of the 22 Caucasians ($p<0.05$). We conclude that vasodilatation and hyperemic response to ischemia is decreased in a significant number of African Americans who are also likely to be more insulin resistant. An impairment in endothelium dependent NO generation may contribute to the insulin resistance, hypertension and vascular disease in this population.

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TROGLITAZONE INHIBITS PROLIFERATION OF MICROVASCULAR ENDOTHELIAL CELLS: IMPLICATIONS FOR DIABETIC RETINOPATHY

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We examined the effects of troglitazone, a novel member of the thiazolidinedione class of oral antidiabetic drugs, on microvascular endothelial cells from human and murine tissues. Thiazolidinediones have been shown in adipocytes to activate peroxisome proliferator-activated receptor γ (PPAR γ), a member of the nuclear receptor superfamily of ligand activated transcription factors believed to play a role in the regulation of lipid metabolism. Adipose tissue, one of the target tissues of troglitazone, is highly vascularized but endothelial cell sensitivity has not been examined previously. We discovered that PPAR γ is constitutively expressed in human and murine microvascular endothelial cells (MVEC), and that 20 μM troglitazone will upregulate the expression of PPAR γ. We sought to determine if there was a corresponding phenotypic change. Human MVEC treated with troglitazone *in vitro* were growth inhibited by 29.2-36.8% at 1 μM and 48.7-59% at 20 μM twenty four hours post treatment. By contrast, large vein human umbilical vein endothelial cells (HUVECs) and murine MVECs were not significantly growth inhibited with 1 μM troglitazone. There was, however, significant inhibition at higher concentrations (37% and 34% at 10 μM respectively). There was no decrease in cell viability at concentrations up to 20 μM, the highest dose tested. Based on the *in vitro* results, we performed experiments to determine if troglitazone would inhibit angiogenesis in a rat corneal angiogenesis wounding model. There was no significant change in angiogenesis at the doses tested (150 mg/kg and 500 mg/kg) in this rat model, but based on the proliferation results this model may not be predictive of effects on human vasculature. Troglitazone has a direct effect on human microvascular endothelial cells, both upregulating PPAR γ mRNA and inhibiting cell growth *in vitro*. These effects may possibly result in a decrease of pathological angiogenesis and a delay in the onset of proliferative retinopathy in diabetic patients.

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INSULIN-MEDIATED GLUCOSE UPTAKE IN NIDDM: METABOLIC PARAMETERS AND ENDOTHELIAL FUNCTION.

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Introduction. It is well known that insulin resistance is a typical feature of non insulin-dependent diabetes mellitus (NIDDM). Insulin resistance is worsened in microalbuminuric (>30μg/min) hypertensive (AER+) with respect to normoalbuminuric normotensive (AER-) NIDDM patients. However, if this phoenomenon is due to an endothelial dysfunction, i.e. an altered insulin transcapillary passage from the blood flow to the interstitial space, or to an impaired intracellular insulin action is not known. Aim and Subjects (i) to quantify insulin resistance parameters in 11 AER+ and 23 AER- well matched NIDDM patients (age 59±2 vs. 59±1yr; BMI 29±2 vs. 29±1 kg/m^2); (ii) to correlate insulin resistance with endothelial function evaluated by von Willebrandt factor (vWF). Methods. Insulin sensitivity (SI) was obtained by the minimal model method after an i.v. glucose test, and vWF by ELISA. Disposition index (DI) was calculated as the product between SI and the suprabasal area under the insulin curve during first 10 min. Results Basal insulin (Ib) was 66±12(SE) pM in AER- and 102±12 in AER+ ($p<0.02$). Basal glucose (Gb) was 7.4±0.7 mM in AER- and 11.8±0.9 in AER+ ($p<0.005$). SI was 0.25±0.07 10^{-4}min^{-1}/pM in AER- and 0.08±0.02 in AER+ ($p<0.032$). DI was 0.22±0.006 in AER- and 0.01±0.002 in AER+ ($p<0.04$). vWF was unchanged in the two groups (140±9 vs. 169±13%) and did not correlate with SI or DI ($p>0.2$). Conclusions (i) SI is impaired in all diabetic patients, and this impairment is more enhanced in AER+; (ii) the variability of endothelial status, in basal conditions, does not suggest that reduced insulin-mediated uptake of glucose is due to an impaired capillary permeability to insulin; (iii) a metabolic intracellular defect, more evident in AER+ than in AER-, can alternatively explain some components of insulin resistance in NIDDM.

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EFFECTS OF TROGLITAZONE ON EXPRESSION OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR γ IN ENDOTHELIAL CELLS

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We have examined the *in vitro* effects of troglitazone, a member of the thiazolidinedione class of oral antidiabetic drugs, on growth and gene expression in human and murine microvascular endothelial cells (HMVEC and MMVEC), human large vein umbilical endothelial cells (HUVEC) and murine heart endothelial cells (MHEC) to explore possible beneficial effects of troglitazone in microvascular complications of non-insulin-dependent diabetes mellitus (NIDDM). Troglitazone is being developed as a treatment for NIDDM, as it improves sensitivity to insulin and reduces plasma glucose and lipid levels. The antidiabetic effects of troglitazone correlate with effects upon the peroxisome proliferator-activated receptor γ (PPARγ), a member of the steroid/ retinoid/ thyroid hormone receptor family of ligand-activated transcription factors. We have obtained and characterized HMVEC, MMVEC, HUVEC and murine MHEC. Administration of 20 μM troglitazone for eight days results in a 2.1 +/- 0.3 fold induction of PPARγ mRNA in HMVEC (n=5), a 1.4 fold induction in MMVE cells, and a 3.7 fold induction in MHEC, but does not upregulate PPARγ expression in HUVEC. Induction of adipocyte binding protein 2 (aP2) mRNA, an intracellular lipid binding protein, was also examined, since it has been shown by other investigators that transcription of aP2 can be regulated by the PPARγ gene in adipocytes and fibroblasts. Following eight days of treatment with 20 μM troglitazone, aP2 is induced 5.8+/-2.5 fold in HMVEC and 4.9 fold in MHEC while no induction is observed in HUVEC or in MMVEC. Induction of aP2 mRNA is not observed in all endothelial cell types in which PPARγ mRNA is induced by troglitazone treatment, suggesting that transcriptional regulation of aP2 by PPARγ may be cell type specific, or that other factors influence expression of aP2. There may also be organ or tissue specific endothelial cell differences to the effects of troglitazone, since HMVEC and MMVEC both upregulate PPARγ, while HUVEC are not responsive.

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ACUTE HYPERGLYCEMIA REDUCES NITRIC OXIDE AVAILABILITY IN MAN.

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The aim of this study was to investigate whether the increase in vascular tone induced by acute hyperglycemia in man is related to reduced nitric oxide (NO) availability. Acute hyperglycemia (15 mmol/L) was induced in 12 healthy subjects. Systolic (8±3 mmHg, mean±SE) and diastolic (5±2 mmHg) blood pressure and heart rate (6±3 beats/min) showed significant ($p<0.05$-0.001) increments starting from 30 min of hyperglycemia; leg blood flow in the femoral artery decreased at 60 and 90 min (-91±23 ml/min, $p<0.05$). Platelet aggregation response to ADP (9±3%) and blood viscosity (0.7±0.2 centipois) also showed significant increments during acute hyperglycemia ($p<0.05$). The infusion of L-arginine (1 g/min), the natural precursor of NO, in the last 30 min of the hyperglycemic clamp completely reversed all hemodynamic and rheologic changes brought about by hyperglycemia. Neither the infusion of D-arginine (1 g/min), which is not used as substrate for nitric oxide synthase, nor L-lysine (1 g/min), which uses the same amino acid transport system as L-arginine does, changed the vascular responses to hyperglycemia. Acute hyperglycemia in man causes significant vascular changes which are reversed by L-arginine, but not D-arginine or L-lysine. Acute Hyperglycemia may reduce NO availability in man.

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PROTECTION OF ENDOTHELIUM BY BIMOCLOMOL (INCREASED EXPRESSION OF HEAT SHOCK, HSP-72 GENE)

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Bimoclomol (BRLP-42) is a vasoprotective agent under development by Biorex (Hungary) for the treatment of diabetic micro- and macroangiopathy (Phase II clinical trials). In the present study the effects of chronic Bimoclomol treatment on function and structural properties of the arterial wall and on the expression of heat shock protein (HSP-72) gene were investigated in insulin resistant rats. 3-month-old male spontaneously hypertensive rats (SHR) were treated with BRLP-42 (20 mg/kg/p.o.) for 1,2 and 3 months. The endothelium-dependent relaxation by Ach showed an age dependent decrease in preparations from SHRs. The maximal relaxing effect achieved with 10μM Ach in rings from 4-, 5- and 6-month-old SHR untreated animals were: 56,5±9,3; 42,9±10,4; 32,9±4,4%, resp. In contrast vasodilator responses were significantly improved in aortic rings of BRLP-42 treated rats (max. relaxation: 87,3±6,2; 79,9±4,6; 65,6±3,7%, $p<0.05$ for all). The endothelium of SHR aortas was severely damaged, some of the cells were necrotized or ulcerized. After BRLP-42 treatment the structure of the endothelium was intact, cells grew over the discontinuities. With ageing and deterioration of carbohydrate metabolism not only the endothelial dysfunction became more pronounced but also the expression of HSP-72 was less intensive. BRLP-42 treatment preserved the expression rate of HSP-72 gene ($p<0.05$) and restored the function and morphology of aortic endothelium.

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ENDOTHELIAL DYSFUNCTION IN NIDDM: ASSESSMENT BY L-ARGININE AND REVERSAL BY ANTIOXIDANTS.

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L-arginine (L-AR) is the natural precursor of nitric oxide (NO). In 20 healthy subjects (age range 20 to 37 yrs), mean blood pressure (MBP) fall to graded iv bolus (1 to 5 g) of L-AR was maximal with the 3 g dose (7.6±1.3 mmHg, m±SD). D-arginine, which is not used as substrate for NO synthase, did not change MBP or platelet activity; L-NMMA (6 mg/min), an inhibitor of NO synthase, reduced by 70% the MBP fall and the antiplatelt effect seen after L-AR. In the whole population of healthy subjects (n=52, 20 to 89 yrs), there was an inverse relation between age and MBP ($r=-0.88$) or platelet aggregation ($r=-0.69$, $p<0.001$) changes after L-AR. The MBP decrease after L-AR was lower in 20 NIDDM patients (47±7 yrs) than in 20 matched controls (-2.2±1.8 vs -4.8±1.2 mmHg, $p<0.01$). There was an inverse relation between hemoglobin A1c and both the MBP fall ($r=-0.40$) and the platelet aggregation response ($r=-0.52$, $p<0.01$) to L-AR. Treatment with vitamin E (600 mg/day per os) or glutathione (1200 mg/day im) for one month ameliorated the endothelial dysfunction seen in NIDDM patients. L-arginine test (3 g iv bolus) may be a simple tool to assess both the endothelial control of systemic vasodilation and platelet activity in human diabetes and the effect of interventions.

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EFFECTS OF GLUCAGON ADMINISTRATION ON MICROCIRCULATION IN DIABETES

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Microcirculation is very important for metabolism and gas exchange in tissue, and microvascular diseases are characteristic complications in patients with diabetes mellitus. It has been reported that some rheologic abnormalities are associated with diabetes. However it is difficult to realize the condition of microcirculation in men. We developed an intravital video-microscopic system (IVVMS) to observe microcirculation directly on the bulbar conjunctiva. Using this system, we investigated the effect of intravenous glucagon administration on microcirculatory parameters such as the internal diameter of venules, flow velocity and flow volumes in diabetic patients. Blood viscosity and rheology factors were also examined before and after glucagon administration. Plasma glucose, insulin, C-peptide and cyclic-AMP were increased after glucagon administration. The time required for erythrocytes to pass through pores of 5 μm diameter, as well as whole blood and plasma viscosity were decreased significantly after glucagon administration. Flow volumes of venules were increased significantly by glucagon. Leukocyte counts, platelet counts and fibrinogen did not change after glucagon administration. Changes of blood factors, such as improvement of deformability of red blood cell or decreased viscosity, were considered to cause increase in the blood flow. In conclusion, glucagon administration improved blood fluidity and modified the microcirculation in patients with diabetes mellitus.

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Insulin and Vasopressin Induce Vascular Endothelial Growth Factor on Human Vascular Smooth Muscle Cells

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Vascular endothelial growth factor (VEGF) is a secreted mitogen for vascular endothelial cells, and it promotes neovascularization. We investigated how low oxygentension, pancreatic hormones and vasoactivators could modulate the expression and production of VEGF in human aortic vascular smooth muscle cells (SMC). Human SMC were established from a piece of aorta obtained from non-atherogenic adult human. Hypoxic conditions were induced using the BBL Gas Pak Plus system at 37℃. RNAs were prepared by guanidine isothiocyanate solubilization and centrifugation over aCsCl cushion. Ten μg of total RNA was subjected to Northern blot analysis. Medium VEGF levels were measured by our newly developed high sensitive enzyme-linked immunosorbent assay (ELISA). Using rabbit anit-human VEGF/VPF polyclonal antibody for both capture (solid) and secondary (enzyme-labeled) antibodies. Hypoxia (0%O_2) and Cobalt (10^{-4}M) resulted in substantial induction of VEGF transcripts at 24 hr (each, × 2.4 fold). The VEGF production was significantly ($P< 0.05$) elevated (496±18~767±5 pg/ml) by 24h-exposure to insulin (10^{-6}M). But glucagon (10^{-6}M) did not elevate VEGF production. In addition, vasopressin (AVP) significantly stimulated the production of VEGF in a concentration- dependent manner (10^{-6}~10^{-10}M).

Our findings suggest that insulin and AVP may play a important role for vascular remodelling by up-regulating VEGF levels of SMC.

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Aortic supersensitivity to vasoconstrictor action of phenylephrine in Zucker diabetic fatty (ZDF) rats.

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The Zucker diabetic fatty (ZDF) male rat is a rodent model of NIDDM, which is characterized by early spontaneous development of hyperglycaemia, hyperinsulinemia and insulin resistance. In the present work, we studied the relationship between lipid profile, glucose level, microscopal vascular morphology and arterial reactivity in male ZDF rats and their lean normal male littermates at age 12 weeks. Arterial responsiveness to vasoactive agents was tested on isolated thoracic aortic ring segments. The ZDF rats were significantly obese at 12 wks of age and weighted 12% more than the lean control animals. The obese ZDF rats were also hyperglycaemic (33.03±1.2 vs 10.09±0.14 mM, $p<0.001$) and hyperlipidemic (triglycerides, 6.52±0.68 vs 1.38±0.09 mM, $p<0.001$; cholesterol, 4.65±0.08 vs 2.28±0.03 mM, $p<0.001$; HDL, 3.11±0.09 vs 1.71±0.06 mM, $p<0.001$; free fatty acids, 0.67±0.04 vs 0.4±0.02 mM, $p<0.001$, in ZDF and lean rats, respectively). No morphological alteration of the aortic wall in both lean and ZDF rats was observed. In isolated aortic rings, there was no difference in the acetylcholine induced endothelium-dependent vasodilation between ZDF and lean control rats. In contrast, the contractile capacity of the aorta in response to phenylephrine (PE) was increased: the concentration response curve was shifted to the left in diabetic animals (ED50: lean rats, 0.26 μM, n=6 versus ZDF rats 0.02 μM, n=7, $p<0.001$). The maximum response to PE was also enhanced in aortic rings from ZDF rats (28%±8.54, $p<0.001$). The overall results indicate that prolonged hyperglycaemia in insulin-resistant 12 wks old rats does not impair aortic endothelial-dependent dilation, but enhances vasoconstrictor responses to PE, exhibiting a change in both sensitivity (decrease in ED50) and responsiveness (increase in maximum response). The aortic supersensitivity to PE could precede endothelial dysfonction and vascular morphological alterations described in type 2 diabetes with macrovascular complications.

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MICROCIRCULATORY EFFECTS OF HYPERGLYCEMIC SPIKE IN HEALTHY SUBJECTS AND DIABETIC PATIENTS.

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The present work aimed to assess the direct and immediate effects of acute hyperglycemia on cutaneous blood flux and tissue oxygenation in 10 insulin dependent diabetic (IDDM) patients and 5 healthy control subjects. Investigations were performed by means of GCIIS Biostator allowing the control of blood glucose level. In fact, blood glucose level was first normalized (<1 10 g/l), then increased to a value within 2.5 g/l and 3.0 g/l and maintained at this value during 1 hour. Microcirculatory cutaneous blood flux and vasomotion amplitude and frequency were measured by a laser doppler fluxmeter (PF4 Perimed, Sweden) on the dorsum of the big toe at skin temperature before, during and after an arterial occlusion (200 mmHg during 3 minutes), during both normoglycemia and hyperglycemia. The transcutaneous oxygen pressure (TcPO2) was measured at the same time by an Oxymonitor SM 361 (Hellige, France) on the first intermetatarsal space on the dorsum of the foot heated at 44°C Acute hyperglycemia resulted in an increase in microcirculatory basal flux and vasomotion in IDD patients. The increase persisted despite normalization of the glycemia No change was observed in control subjects.

At normoglycemia, IDDM patients showed an increased duration of reactive hyperaemia, which was further increased as a result of the acute hyperglycemia. Furthermore, acute hyperglycemia was accompanied by a decrease in TcPO2 in IDDM patients. It is concluded that the acute hyperglycemia leads to functional microcirculatory alterations in IDDM patients.

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PREGNANCY INDUCED IMPROVEMENT IN VASCULAR FUNCTION PERSISTS POSTPARTUM BUT IS REVERSED BY A HIGH FAT DIET

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Diabetes and dyslipidaemia are both associated with vascular endothelial dysfunction while female sex hormones, particularly oestradiol, are considered protective of the endothelium. We have recently shown that pregnancy protects against vascular endothelial dysfunction in diabetic rats. We have now determined whether pregnancy induced changes persist in the puerperium and if they are compromised by a high saturated fat diet. Mesenteric small arteries were mounted on a small vessel myograph from the following five groups: control, STZ diabetics fed breeding diet and STZ diabetic rats fed high saturated fat diet (16 days post partum) and virgin diabetic or virgin control rats. Endothelial function, assessed by acetylcholine (Ach) induced relaxation, was abnormal in virgin diabetic rats compared with virgin controls (77±5% reduction of noradrenaline-induced constriction, n=6 v 90±3% n=13, $p<0.01$). In the diabetic rats on breeding diet postpartum the ACh induced relaxation (94±6%, n=7) was similar to both post partum controls (92±3%, n=12) and to virgin controls. However post partum diabetic on a high fat diet had similar ACh responses to virgin diabetic rats (70±7%, n=4). Thus, the protective effect of pregnancy persists post partum, but is abolished in animals on high fat diet.

PS 40

Haemostatic Abnormalities

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PLASMINOGEN ACTIVATOR INHIBITOR-1 ACTIVITY AND INSULIN RESISTANCE IN 70-YEAR OLD MEN

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Increased levels of plasminogen activator inhibitor-1 (PAI-1) has been discussed as a part of the insulin resistance syndrome and has been suggested to be the link between insulin resistance and cardiovascular disease. It is however not clear whether the relationship between PAI-1 and insulin resistance is independent or mediated by increased triglycerides levels. The aim of this study was to investigate if PAI-1 activity is associated with insulin sensitivity independent of serum triglycerides (sTG) and of potential confounders. 871 men aged around 70, participants of a cohort study undergoing extensive metabolic investigations, had blood samples taken for determination of PAI-1 activity. Insulin sensitivity was determined by the euglycemic hyperinsulinemic clamp procedure. In multivariate correlation and regression analyses, insulin sensitivity was a statistically significant determinant of PAI-1 activity (partial r=-0.14; p<0.001), independent of sTG, BMI, waist-hip ratio (WHR), systolic and diastolic blood pressure (BP), and treatment with lipid- and BP-lowering drugs. Levels of sTG was also independently related to PAI-1 activity (partial r=0.17; p<0.001). The relationships between PAI-1 and insulin sensitivity and sTG were not changed by fasting glucose levels. Men with IGT and NIDDM (prevalence: 13 and 15%, respectively) had higher PAI-1 activity than men with NGT, also after adjustment for insulin sensitivity, sTG, BMI, WHR, blood pressure, medications, and fasting glucose. Increasing number of risk factors associated with the insulin resistance syndrome increased the activity of PAI-1 in men with NGT. The odds ratio for having angina pectoris (prevalence: 14%) was 1.42 (p<0.001) for each increase of one standard deviation in PAI-1 activity. We conclude that PAI-1 activity is related to insulin sensitivity and serum triglycerides, independent of each other and of other potential confounders, and that incresed levels of PAI-1 should be regarded as a component of the insulin resistance syndrome.

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EVALUATION OF HYPERCOAGULABLE STATE IN DIABETIC SUBJECTS WITH AND WITHOUT RETINOPATHY

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The significance of haemostatic abnormalities in the pathogenesis of diabetic retinopathy is not clear. The aim of this study was to evaluate the potential hallmarks of hypercoagulable state in insulin dependent (IDDM) diabetics. The study was carried out in 50 well-controlled diabetics (aged 33.3±9.4 yrs) divided into: group A (22 patients with retinopathy; 12 M and 10 F; mean duration of diabetes 13.6±7.0 yrs) and group B (28 patients without any diabetic complications, 16 M and 12 F, mean duration of diabetes 5.4±1.3 yrs). Apart from that, a randomized group of 25 IDDM patients have been examined as a group C (aged 35.8±7.5, 7 M and 18 F, mean duration of diabetes 9.9 ± 5.0 yrs). The control group consisted of 22 well-matched healthy individuals (aged 32.3±5.2 yrs). All the subjects had the platelet count, activated partial thromboplastin time (APTT), prothrombin time, fibrinogen, euglobulin clot lysis time before and after venous occlusion, activity of antithrombin III (ATIII), the level of thrombin-antithrombin III complexes (TAT), D-dimers, fibrin(ogen) degradation products (FDP) plasma concentrations assessed. Results: There was no significant difference between the patients from group A or B. All diabetic patients (group A+B) showed significantly increased platelet count (353,800±112,100 vs 253,000±44,000/mm^3 in controls; p<0.001), poor fibrinolytic response to venous occlusion (114.3±60.5 vs 43.0±18.0 min in controls; p<0.001). Other haemostatic parameters in diabetic patients did not differ significantly from the controls. Patients from group C revealed significant elevation in plasma fibrinogen concentration compared to groups A and B, and the controls (333.4±103.4 vs 253.2±65.0 in groups A and B, and vs 263.0±32.0 mg/dl in controls; p<0.01). In conclusion, patients with well-controlled IDDM showed no symptoms of hypercoagulability, whether they had retinopathy or not. Therefore, it is possible that diabetic retinopathy is not always associated with hypercoagulable state. The results may encourage the revision of the views on the pathogenesis and treatment of diabetic retinopathy.

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NO IMPROVEMENT IN FIBRINOLYSIS AFTER STARTING INSULIN TREATMENT IN NIDDM PATIENTS WITH INSUFFICIENT GLYCAEMIC CONTROL.

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Fibrinolytic activity is reduced in non-insulin-dependent diabetes mellitus (NIDDM). Since disturbances in fibrinolysis are important in the pathogenesis of cardiovascular disease, this reduction might be responsible for the increased cardiovascular risk in NIDDM patients. The aim of our study was to assess fibrinolysis in NIDDM patients with poor glycaemic control and to investigate if a relation with improved control during insulin could be found. Seventeen NIDDM patients (10 F, 7 M, age 60.0 ± 11.2 y, diabetes duration 10.6 ± 6.6 y) with insufficient control during maximum sulphonylurea were treated with insulin. Variables of glycaemic control (HbA1c and fasting blood glucose) were collected before and after six months of insulin treatment, as were variables of fibrinolysis (plasminogen activator inhibitor 1 (PAI-1), tissue type plasminogen activator (t-PA) antigen, t-PA activity and Von Willebrand factor (VWF)). Fasting blood glucose decreased from 13.7 ± 2.0 (SEM) to 9.9 ± 0.8 mmol/l (p=0.001), HbA1c from 10.3 ± 0.3 to 8.6 ± 0.2% (p<0.0001). Body weight increased from 77.6 ± 4.6 to 82.0 ± 4.9 kg (p<0.0001), body mass index (BMI) from 27.4 ± 1.4 to 29.4 ± 1.4 kg/m^2 (p<0.0001). At baseline PAI-1 was positively correlated with body weight (r=0.39, p=0.04) and BMI (r=0.41, p=0.03); changes in these variables were not correlated with changes in PAI-1. PAI-1 was negatively correlated with tPA-activity at baseline (r=-0.71, p<0.0001) and after insulin (r=-0.75, p<0.001) and positively with t-PA antigen (r=0.46, p=0.01 and r= 0.57, p=0.02, respectively). Fibrinolysis variables did not improve: PAI-1 65.7 ± 9.4 to 55.7 ± 9.7 ng/ml, t-PA antigen 13.5 ± 1.4 to 13.1 ± 1.4 ng/ml and t-PA activity 0.58 ± 0.11 to 0.75 ± 0.12 IU/ml (all NS). VWF, however, increased from 148.2 ± 10.9 to 161.9 ± 13.4% (p=0.005).

Conclusion: Our results did not show an improvement in fibrinolysis during insulin treatment, despite a significant improvement in glycaemic control.

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FIBRINOGEN IN TYPE II DIABETES: CORRELATION WITH METABOLIC CONTROL. EFFECT OF BEZAFIBRATE.

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Different studies have shown a strong and independent relation between fibrinogen (F) levels and the incidence of arterial disease (AD), that is more severe and frequent in DM. This study was performed in order to measure F (immunoturbidimetric method) in patients with NIDDM (N = 104, AGE 54±8, 45 M and 59 F) and in controls (C) (N = 82, AGE 57±7, 35 M and 47 F), assess its relationship with metabolic control (MC) and other risk factors and the effect of Bezafibrate (BZ). ANOVA and multiple regression were used for statistical analysis. Significant differences were observed between C and NIDDM with and without AD (249.8 ± 39.5 vs. 319.4 ± 41.6 vs 285.7 ± 46.2 mgs/dl, p<0.01). Multivariate analysis with F as dependent variable showed that age, BMI, fructosamine, HbA1, LDL and TGC contributed to the regression, with a stronger relation for the group with AD (R^2 = 0.41) than without AD (R^2 = 0.27). BZ (N = 24) lowered F from 301.5 ± 37.8 to 255.4 ± 30.6 mgs (p<0.01) It is concluded that F increases in NIDDM, even more in the presence of AD, that it is influenced by MC and may decrease with BZ.

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INTERCELLULAR ADHESION MOLECULE -1 AS A PREDICTOR OF VASCULAR COMPLICATIONS IN DIABETES MELLITUS

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Diabetes mellitus has been described as a metabolic syndrome with vascular atherothrombosis being an important complication. Increased levels of soluble intercellular adhesion molecule - 1 (sICAM-1) have been described in diabetes mellitus (DM). We compared sICAM-1 levels in 36 patients (17 IDDM, 19 NIDDM; 20 males, 16 females) with 24 age matched controls (C). All diabetic patients were then followed up over a five year period for development of vascular complications (coronary, cerebral and peripheral) which occurred in 11 patients (B) (6 coronary, 3 cerebral, 2 peripheral vacular disease). sICAM-1 levels at baseline were significantly elevated in patients who later developed atherothrombosis (299 $\pm$ 32.9 ng/ml) compared to patients without manifest vacular disease (A) (243 $\pm$ 58.2 ng/ml) ($p<0.005$). sICAM-1 levels in A vs C was not significant. At 5 year follow-up the sICAM-1 levels were still significantly elevated in A vs B (361 $\pm$ 88.2 ng/ml vs 435 $\pm$ 92.7 ng/ml; $p=0.001$).There was no difference between diabetic groups for HbA1c and cholesterol and sICAM-1 levels between C and IDDM and NIDDM at baseline. . This study thus shows that diabetic patients with elevated sICAM-1 levels are more prone to develop vascular complications and possible future therapy could be targetted against sICAM-1.

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INCREASED FIBRINOGEN SYNTHESIS IN TYPE 2 DIABETES MELLITUS WITHOUT VASCULAR COMPLICATIONS.

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Whether fibrinogen synthesis is increased in non insulin-dependent diabetes (NIDDM), even without micro- and macrovascular complications, is not known. Therefore, we have measured fibrinogen fractional (FSR) and absolute (ASR) synthetic rates in 6 male NIDDM patients (age: 45 ± 5 yrs [mean $\pm$ SE]; duration of disease: 11 ± 3 yrs; HbA_1c: $10.1 \pm 1\%$) under spontaneous hyperglycemia after the overnight fast, and in 7 controls (C, 6M/1F), matched for age, physical activity, alcohol and smoking habits (all subjects non-smokers). FSR was determined using a precursor-product model with i.v. radioactive leucine infusion. ASR was calculated from FSR, measured plasma fibrinogen concentration and estimated plasma volume. Absence of micro- and macrovascular complications was assessed with clinical, instrumental and laboratory data. NIDDM subjects had slightly increased BMI, mean blood pressure (MBP) and triglycerides (TG) (BMI: 28.4 ± 2.2 vs 23.1 ± 0.4 kg/m^2, $p=0.03$; MBP: 105 ± 3 vs 97 ± 2 mm Hg, $p=0.04$; TG: 165 ± 31 vs 90 ± 14 mg/dl, $p=0.04$ vs C). Plasma glucose (10.3 ± 1.1 vs 5 ± 0.2 mM/liter, $p<0.01$), insulin (15 ± 2 vs 8 ± 1 μU/ml, $p<0.05$) and C-peptide (1.8 ± 0.3 vs 1.2 ± 0.1 ng/ml, $p<0.05$) were higher in NIDDM. Fibrinogen concentration was $\approx 40\%$ greater in NIDDM (270 ± 28 vs 197 ± 14 mg/dl, $p=0.034$), while FSR was comparable in the two groups (21.9 ± 3.6 vs $18.2 \pm 2.6\%$ per day, NS). In contrast, fibrinogen ASR was markedly increased in NIDDM, by $\approx 80\%$ (1847 ± 362 vs 1020 ± 153 mg per day, $p=0.048$). In summary, NIDDM patients without vascular complications have increased plasma fibrinogen levels and synthetic rates, in spite of normal FSR, suggesting that plasma levels may not accurately reflect the extent of fibrinogen altered turnover in NIDDM.

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LIPID PEROXIDATION OF LDL MODULATES PLATELET ADHESION AND PLATELET RELEASE REACTION IN TYPE 2 DIABETIC PATIENTS

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Nonenzymatic glycosylation of platelet membrane proteins and the interactions of platelets with nonenzymatically modified lipoproteins have been suggested to underlie the altered expression of platelet membrane receptors and platelet hypersensitivity in diabetic subjects. We monitored the associations between the expression of selected platelet membrane glycoproteins and the formation of reactive oxygen species in platelet membranes, the content of lipid peroxides in plasma LDL, membrane fluidity and the glycation of LDL and membrane proteins. We revealed that: 1/ the expressions of GPIIb-IIIa and GMP-140 were significantly enhanced in circulating platelets (resp., $p<0.035$ and $p< 0.01$ *vs.* platelets of control subjects), whereas that of GPIb was depressed (by 23%) in diabetic patients, 2/ these changes were paralleled by the significant increases of platelet aggregates and microparticles in diabetic individuals ($p< 0.025$ or less). Platelet membrane fluidity was reduced ($p< 0.02$), whereas the glycation of membrane proteins, and LDL apo-B, and the content of LDL lipid peroxides increased in diabetics ($p< 0.035$ *or less*). The generation of reactive oxygen species was increased in diabetic platelets ($p< 0.04$), especially in platelets treated with phorbol myristate acetate under *in vitro* conditions ($p< 0.005$). When control platelets were incubated in serologically-matched diabetic plasmas the expressions of platelet membrane receptors changed according to the impairments observed in diabetic circulating platelets, and such an activation of control platelets following their incubation in diabetic plasma was accompanied by their reduced membrane lipid fluidity ($p< 0.02$). The generation of reactive oxygen species in the incubated control platelets was slightly enhanced on average, and correlated with LDL peroxidation in diabetic plasma. We conclude that the glycation of membrane proteins and the interactions of plasma lipoproteins with blood platelets may coincide in affecting platelet function in diabetic patients. The altered structure of platelet membrane-associated components might result in the impaired metabolism of platelet arachidonate and lead to the augmented formation of reactive oxygen species in platelet membranes. Overall, the resulting augmented platelet activation and the accelerated release of platelet membrane microvesicles might underlie the reduced membrane lipid fluidity, and consequently the altered expression of platelet membrane receptors in diabetes mellitus.

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EFFECTS OF METABOLIC CONTROL ON FASTING AND POST-MEAL FIBRINOGEN FRACTIONAL SECRETION RATE (FSR) IN IDDM.

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Fibrinogen, a liver-synthetized, acute-phase protein which plays a key role in thrombus formation, is often increased in poorly controlled diabetes. Recently, isotope-determined fibrinogen FSR was found to be stimulated by acute insulin-deficiency in IDDM subjects, while it was decreased by insulin infusion in both IDDM and normal subjects. However, whether a good glycemic control can normalize fibrinogen FSR in IDDM also following a meal, is unknown. Therefore, we have studied fibrinogen FSR in five IDDM patients (age: 35 ± 6 yrs; BMI: 23 ± 1 Kg/m^2; sex: 4M/1F) infused overnight with insulin to maintain blood glucose at $\approx$euglycemic values (≈ 120 mg/dl) and in 6-10 controls (age: 32 ± 4; BMI: 24 ± 1 Kg/m^2; sex: 8M/2F). All subjects were free of clinical and laboratory signs of either micro- or marco-angiopathy, and were not smokers. The subjects were studied both in the postabsorptive state and following the continuous administration of a mixed meal (≈ 11 Kcal/Kg over ≈ 4 hours). FSR was calculated at steady-state using precursor-product relationships with i.v. radioactive phenylalanine tracers, and estimated intrahepatic phenylalanine SA as precursor pool. In the fasting state, fibrinogen FSR was comparable in IDDM (18.2 ± 1.2 percent per day) and controls ($n=10$) (18.4 ± 3.9). During the meal, the IDDM subjects were infused with stepwise i.v. insulin to maintain blood glucose below ≈ 170 mg/dl. Post-prandial fibrinogen FSR tended to increase in both groups ($p<0.05$ by ANOVA), but it was not different between IDDM (25.3 ± 4.5 percent per day) and controls ($n=5$) (36 ± 8.6). In conclusion, a near normal glycemic control achieved by i.v. insulin is capable to normalize fasting fibrinogen FSR in IDDM. Meal ingestion may be associated with increased fibrinogen FSR, which is maintained at normal values in IDDM by physiologic insulin infusion.

1600

INCREASED BLOOD LEVELS OF PLATELET-ACTIVATING FACTOR IN INSULIN-DEPENDENT DIABETIC PATIENTS WITH MICROALBUMINURIA.

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Platelet-activating factor (PAF) has potent vaso- and glomerular permeability properties, in experimental animals. In the present study we evaluated the intravascular levels of PAF and of its main catabolic enzyme, the PAF-specific plasma acetyl-hydrolase, in basal conditions and after exercise in normo- or microalbuminuric insulin-dependent diabetic (IDD) patients and in normal subjects. In addition, we studied the responsiveness of platelets in platelet-rich plasma to PAF and ADP. The results indicate that the concentration of PAF in whole blood was significantly increased in basal conditions, during and after the exercise in microalbuminuric, but not in normoalbuminuric IDD patients or in controls ($p=<0.05$). In microalbuminuric IDD patients, the levels of PAF correlated with the albumin excretion rate (pre-exercise $r=0.71$ $p=0.000$, exercise $r=0.64$ $p=0.001$, post-exercise $r=0.55$ $p=0.007$). The exercise induced an hyper-responsiveness of platelets to PAF and ADP in both normoalbuminuric IDD patients and controls. The hyper-responsiveness of platelets to ADP was abrogated by a PAF receptor antagonist, suggesting an endogenous synthesis of PAF within platelets. In contrast, an hypo-responsiveness of platelets to PAF was found in microalbuminuric IDD patients undergoing exercise, suggesting an "in vivo" desensitization of platelets to PAF. These results indicate that the increased production of PAF is associated with an enhanced glomerular permeability in microalbuminuric IDD patients.

1601

DETERMINANTS OF SERUM FIBRINOGEN LEVELS IN MALTESE PATIENTS WITH NIDDM

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Serum fibrinogen (FIB) is an independent cardiovascular (CVD) risk factor, and high FIB levels have been reported in NIDDM. The aim of the present study was to determine the FIB levels in 431 Maltese NIDDM patients, and the effect of the age-at-onset (ONS), the duration (DUR) of NIDDM and the glycaemic control (GLY) on FIB. Moreover, we wanted to find out the extent to which the variation in FIB levels in NIDDM patients are related to other CVD risk factors. The mean FIB was 363 µmol/l, the 90th percentile 470, and no sex difference in FIB was found. Overall, the following parameters contributed significantly to the variation in FIB: Age, total cholesterol (CHOL), DUR and waist circumference. In separate models by sex, CHOL was significant in women, and DUR in men. In patients with DUR<6 years only CHOL and serum uric acid were related to FIB, while in patients with ONS<40 years and in normotensive patients age and BMI were related to FIB. In hypertensive NIDDM patients treated with antihypertensives DUR, smoking and CHOL were the strongest predictors of FIB. In obese (BMI≤30) and well-controlled (usual blood glucose <8.2) patients DUR was the most important predictor of FIB. In conclusion, DUR is a major determinant of FIB in NIDDM. Obesity and CHOL are other important predictors, but there seems to be a marked variation in the relationship between FIB and CVD risk factors among various subgroups of NIDDM. These results also suggest that it might be possible keep FIB levels in NIDDM low by healthy life style.

1602

POSSIBLE INVOLVEMENT OF GENETIC FACTOR IN PLATELET RESPONSE ALTERATIONS IN PATIENTS WITH IDDM

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Flow cytomertic assay using anti CD41-PE, anti CD61-FITC and anti CD62-PE antibodies (*Becton Dickinson Procedure*) was applied to analyze the value of platelet parameters in 36 IDDM families (45 patients, aged: 13.8±0.6; 65 parents, aged: 41.4±0.8; 17 healthy siblings, aged: 15.5±1.0) and 50 unrelated controls, aged: 10.7±1.0. The following platelet parameters were investigated: %activated platelets, %aggregates, %microparticles, medians of fluorescence intensity of anti CD41-PE, anti CD61-FITC and anti CD62-PE (before and after thrombin activation). We found the activation of platelets in IDDM patients to be elevated comparing to other studied groups. The %activated platelets were as follows: 7.535±2.314 in diabetics; 1.476±0.152 in parents; 1.297±0.204 in siblings and 1.349±0.145 in control subjects. We carried out stepwise discriminant analysis of obtained results. The highest discrepancy was found between IDDM patients and controls ($p=0.000001$) in comparison to IDDM children and their siblings ($p=0.001016$) as well as IDDM patients and their parents ($p=0.008479$). There was no difference between parents and siblings ($p=0.169830$). A control group representing unrelated subjects is particularly well separated from other groups what implies involvement of genetic factor(s) modifying platelet response. For further investigation of putative influence of hereditary factors on platelet features, 16 families with at least one affected and one not affected child (control group was excluded) were chosen for discriminant analysis. Obtained results showed highly marked similarity between IDDM children and parents ($p=0.657803$) while statistically significant separation ($p=0.015581$) between IDDM children and healthy siblings was found. It seems to suggest that similarities observed between IDDM children and their parents result from non-genetic factors (both parents and children as well as siblings share half genes) In conclusion, the data presented above suggest genetic factor(s) contribution in determinig some platelet properties. Age of patient could be the additional factor which strongly affect platelet features.

Supported by KBN grant 4 P05E053 08

1603

PLASMA FIBRINOGEN IN A SAMPLE OF INDUSTRIAL POPULATION OF NORTH INDIA

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Diabetes mellitus is an established risk factor for cardiovascular diseases and part of the increased risk in diabetic patients could be related to enhanced thrombogenic factors like fibrinogen. The aim of the present study was to compare the fibrinogen levels between diabetics and nondiabetics in a nested case control design from an industrial population survey. A total number of 92 subjects were sceened (47 diabetic and 45 non diabetic).

RESULTS: Mean fibrinogen levels was not significantly different in diabetics as compared to non diabetics (2.96 ± 0.463 g/l vs 2.86 ± 0.479g/l). A highly significant positive correlation was observed between fibrinogen and HBA1c levels (r = 0.35, 95% confidence limits : 0.16 < R < 0.52) though no correlation was found between fibrinogen and fasting and postload plasma glucose levels. None of the other variables studied correlated with fibrinogen levels. We did observe a higher fibrinogen levels in current smokers as against exsmokers (2.98 ± 0.48 vs 2.68 ± 0.36 g/l).

CONCLUSION : Plasma fibrinogen levels in an industrial population were studied; there was no difference between diabetic and non-diabetic samples. Fibrinogen levels correlated with HbA1, it did not correlate with the other risk factors for coronary heart disease.

1604

REVERSAL OF ERYTHROCYTE OSMOTIC FRAGILITY WITH VITAMIN E SUPPLEMENTATION IN DIABETICS WITH MICROANGIOPATHY

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Free radical mechanisms are increasingly being implicated in the pathogenesis of tissue damage in diabetes. Various sources of free radicals may modulate oxidative stress in diabetes, including nonenzymatic glycosylation of proteins and monosaccharide autooxidation, polyol pathway,indirect production of free radicals through cell damage from other causes and reduced antioxidant reserve. The present study was designed to examine the effect of antioxidant vitamin E on osmotic fragility in human diabetic red blood cells. Two groups of noninsulin dependent diabetic patients matched for duration of disease and metabolic control, received daily vitamin E supplementation of 900 mg and placebo, respectively, for 6 months. Fasting plasma glucose and hemoglobin A_1 (Hb A_1), postprandial plasma glucose and erythrocyte fragility tests in the basal state and after 6 months of treatment were measured. In the basal state diabetic patients without microangiopathic complications (n= 8) and control group consisting of healthy people (n= 8) showed no difference in the red blood cell osmotic fragility indices ($p > 0.05$). On the other hand diabetic group with microvascular complications (n= 21) displayed significantly higher ratios ($p < 0.05$) of osmotic fragility indices compared to the uncomplicated diabetics and healthy subjects. Glycemic indices did not show any significant changes during study, whereas erythrocyte osmotic fragility decreased significantly after 6 months in patients on vitamin E supplementation. The decline in osmotic fragility indices were especially prominent at sodium chloride concentrations greater than 0.03 % (p=0.001). This study shows that red blood cell osmotic fragility is increased in diabetic people with microangiopoathic complications and is reversible by antioxidant therapy with vitamin E.

1605

Effect of glycemic control on fibrinogen plasma levels in diabetic subjects. Relationship with β fibrinogen genotype.

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Recent studies demonstrate that in diabetic subjects an increase of plasma fibrinogen level is associated with a high risk of cardiovascular complications. Either environmental and genetic factors contribute to the plasma fibrinogen level. Several studies indicate a relationship between the polymorphism in the 5' region of the b fibrinogen gene and plasma protein levels. In this study, such kind of relationship was investigated in 25 NIDDM diabetic patients before and after three months of intensive insulin therapy. Hind III polymorphism was evaluated by a PCR-based technique. On the basis of the observed allelic combination, the patients were divided into three groups ($\alpha1\alpha1$: 11, $\alpha1\alpha2$: 9, $\alpha2\alpha2$: 5). After insulin therapy, the improvement of glicemic control was equivalent, in terms of HbA1c, in all the three groups ($\alpha1\alpha1$: from 8.7±0.5 to 6.3±0.4%; $\alpha1\alpha2$: from 9±0.4 to 6.6±0.3%; $\alpha2\alpha2$: from 9.2±0.5 to 6.4±0.3%; M±SD). In all the three groups a fibrinogen reduction was observed. However, this decrease was low in $\alpha1\alpha1$ group (from 327±25.4 to 293.6±21.4 mg/dl, -11%), intermediate in $\alpha1\alpha2$ group (from 377.2±22.4 to 315.2±15.2 mg/dl, -17%), and very evident in the $\alpha2\alpha2$ group (569.6±31.2 to 412.5±22.3 mg/dl, -28%; $p<0.01$ between groups). These results underline a strong relationship between fibrinogen genotypes and glycemic control in determining plasma fibrinogen levels in diabetic patients.

1606

VASCULAR COMPLICATIONS OF DIABETES MELLITUS: ROLE OF ANTIPHOSPHOLIPID ANTIBODIES ?

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Risk factors for micro and macro vascular disease in diabetes remain uncompletely elucidated. Antiphospholipid antibodies (APA) are thrombogenic and arterial thrombosis and microangiopathy are described in the APA syndrome. The purpose of this study was to determine the frequency of APA prevalence in diabetic patients according to the presence of micro or macro vascular complications. We studied 141 diabetic patients classified into 3 groups: group I: 53 uncomplicated diabetic patients, type 1 (n=32) or type 2 (n=21); group II: 37 diabetic patients with severe macroangiopathy (n=37); group III: 51 diabetic patients with microangiopathy, either isolated proliferative retinopathy (n=23) or clinical nephropathy (n=28); 22 non diabetic patients served as control. APA were considered present either if lupus anticoagulant was positive (PTTLA and Staclot LA, Diagnostica Stago, Asnieres, France), or if anticardiolipin antibodies IgG or IgM titers were above 15 or 12.5 units/ml respectively (ELISA, quantalite ACA, Inova Diagnostics, San Diego, USA). In group I, the prevalence of APA was comparable to that of control patients (type I diabetes: 9.4%; type 2 diabetes: 9.5%; control group: 4.6%; p=0.76). In group II, the prevalence of APA was significantly higher (32.4%, p=0.006 vs group I). In group III, the prevalence of APA was increased only in patients with clinical nephropathy (32.1%, p=0.015 vs group I), while patients with isolated retinopathy were comparable with group I (4.3%, p=0.66). In a logistic regression analysis, independant risk factor for macroangiopathy were age, HDL cholesterol, Lp(a) and presence of APA. We conclude that uncomplicated diabetes does not appear to be linked with a higher prevalence of APA. However, our results suggest that APA could play a role in the patients'susceptibility to complications and promote the occurence of diabetic macroangiopathy and/or nephropathy.

1607

The relationship of arterial blood pressure values with body mass index , viscosity, fibrinogen and microalbuminuria in children with IDDM.

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Arterial diastolic and systolic pressures were measured in a group of 46 type 1 diabetic patients -27 females, 19 males- whose mean age was 12±2.8 years (range:6-16 years), duration of diabetes 4±3 years (range:0.2-2years) , and glycosylated haemoglobin value was 7.35±1.4% .The results were compared to those of two groups comprised of 29 healthy children and 32 healthy obese children . Furthermore , the relationship of systolic and diastolic blood pressure levels in diabetic children with biochemical factors (blood , plasma and serum viscosities; total protein, albumin globulin, fibrinogen , haptoglobulin, triglyceride ,HDL,LDL,VLDL values) , urinary albumin excretion and body mass index (BMI) was studied.

1. In diabetic children , despite short duration of illness and good metabolic control , systolic (109±13 mmHg) and diastolic (74.3±9.5 mmHg) pressures of diabetic children were higher than systolic (97.9±10.3 mmHg) and diastolic (66±5.6 mmHg) pressures of healthy children irrespective of age , sex and disease duration ($p<0.05$ and $p<0.01$, respectively).
2. No significant difference was seen between the systolic and diastolic pressure values of diabetic and obese children.
3. In the diabetic group , a positive correlation was found between BMI , viscosity (blood , plasma and serum), total protein, albumin , and fibrinogen values and the systolic and diastolic blood pressures, while no such correlation was present in microalbuminuric levels.

In multiple regression analysis , increase in systolic and diastolic blood pressure levels was found to be most closely associated with BMI , plasma viscosity and fibrinogen.

Conclusion: The mechanism of hypertension in type 1 diabetic patients and obese children may be the same. High BMI , plasma viscosity and fibrinogen levels may be forewarning signs of developing hypertension in type 1 diabetic children.

1608

ANTITHROMBIN III ACTIVITY AND LEVEL OF THROMBINOGENESIS MARKERS WITH RESPECT TO THE DIABETIC RETINOPATHY

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The aim of the study was to measure the activity of antithrombin III (AT III) and concentrations of prothrombin fragments (F1+2), thrombin-antithrombin III complex (TAT) and D-Dimer in patients with IDDM and NIDDM, and varying severity of diabetic retinopathy (R). The study population consisted of 89 patients (47 IDDM, 27 NIDDM, 15 controls) AT III was determined by using Chromo Time System, whereas F1+2 and D-Dimer with the ELISA method. Ophthalmoscopy with a photograph of the eye fundus was performed, and classification of retinal changes was based upon angiography. In IDDM a significantly decreased AT III was seen in nonproliferative mild R as compared with preproliferative R (mean 101.19, SE 5.46% and mean 136.96, SE 8.10%; p<0 05). D-Dimer was significantly increased in preproliferative R as compared with nonproliferative R (mean 11.84, SE 1.06 ug/l and mean 10.65, SE 0.31 ug/l; p<0.05) and patients without retinopathy (mean 11.18, SE 0.54 ug/l; p<0.05). In NIDDM a significant increase was observed in F1+2 in proliferative R as compared with nonproliferative R (mean 3.41, SE 0.86 nmol/l and mean 1 18, SE 0.35 nmol/l; p<0.05). TAT was significantly increased in proliferative R as compared with nonproliferative R (mean 31 05, SE 16.28 ug/l and mean 6.17, SE 3.12 ug/l; p<0.05) and patients without retinopathy (mean 8.91, SE 6.42 ug/l, p<0.05). The present study demonstrates differences in the thrombophilia pattern in IDDM vs. NIDDM. In IDDM with nonproliferative R the decreased AT III may indicate thrombophilia as well as increased D-Dimer in IDDM with proliferative R. In NIDDM the markers of thrombophilia may be: increased F 1+2 and TAT.

1609

HAEMORHEOLOGIC DETERMINANTS OF BLOOD PRESSURE REGULATION IN OBESE NIDDM WITH HYPERINSULINAEMIA.

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Hyperinsulinaemia may be related to hypertension and obesity in NIDDM and might alter arterial blood pressure regulation by means of several haemorheological modifications. In 59 obese NIDDM(BMI>30 kg/m^2),systolic(SBP)and diastolic(DBP) blood pressure levels were determined together with fasting and post-prandial serum insulin(FSI and PPSI:RIA procedure), erythrocyte deformability(ED:filtration),blood viscosity(BV at 1,200,1/200 shear-rate x1/s,HAAKE Rotovisco CV100),fibrinogen(FG:nephelometry)and urinary albumin(UAER:nephelometry). Higher FSI(mean±SD:251±67 pmol/L),PPSI(1106±202 pmol/L),BV (19.7±2 mPas at SR 1x1/s and 4.38±0.15 mPas at 200x1/s)and FG(459±68 mg/dL),and lower ED(1.87± 1.21 mL/min)were found in obese NIDDM than in non-obese NIDDM(p<0.001)and in healthy subjects(p<0.001).Significant correlations among FSI, PPSI and haemorheologic parameters(p<0.01 vs BV,p<0.001 vs ED)were found in these patients.Moreover,increased BV and FG levels and reduced ED significantly correlated with DBP. A significant increase of UAER was found in obese NIDDM with blood rheology alterations(145±33 ug/min),in comparison with non-obese NIDDM(39±11 ug/min,p<0.001)and healthy subjects (6.5±3 ug/min,p<0.001). Hyperinsulinaemia may determine haemorheological disturbances linked to blood pressure alterations in obese NIDDM.The haemorheologic mechanism of hypertension in NIDDM obese can represent a high risk factor.

1610

A PROTHROMBOTIC STATE AS A RISK FACTOR PROMOTING VASCULAR DISEASE IN IDDM AND NIDDM PATIENTS.

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A disturbed balance between coagulation and fibrynolysis is a proposed risk factor for accelerated atherosclerosis and vascular complications in NIDDM patients. Its role in type I diabetes remains unclear. Therefore the aim of the study was to estimate selected parameters of hemostasis and fibrinolysis in both types of diabetes. The investigation was carried out in 23 IDDM patients aged 33.7 ± 11.5 ys, in 25 NIDDM patients aged 55.4 ± 8.3 ys and in 38 healthy subjects: 16 „young" - aged 32.5 ± 13.2 ys and 22 „old" - aged 56.2 ± 9.4 ys. In all persons fibrinogen, plasminogen activator inhibitor (PAI-1) activity, glycemia, HbA1c and microalbuminuria were determined. Plasma fibrinogen concentration was elevated in NIDDM subjects (390.33 ± 109.03 mg/100ml vs 331.04 ± 91.73 mg/100ml in „old" controls), and the highest levels were noted in overweight persons (BMI > 28 kg/m^2) - 403.06 ± 105.59 mg/100ml and in the group with diabetic neuropathy, retinopathy or arterial hypertension (435.92 ± 116.89 mg/100ml vs 336.45 ± 71.07 mg/100ml, p<0.05). Surprisingly, similar levels were found in IDDM subjects with nephropathy (412.0 ± 82.09 mg/100 ml, p<0.05) and coronary vascular disease (CVD) - 401.6 ± 130.79 mg/100ml vs 307.88 ± 69.19 mg/100ml, p<0.05. There were also positive correlations between fibrinogen level and systolic blood pressure (r=0.3413, p<0.02), diastolic blood pressure (r=0.3809, p<0.002) and microalbuminuria (r=0.3552, p<0.05). PAI-1 activity was slightly higher in IDDM patients than in the other groups (19.28 ± 11.9 Au/ml in IDDM and 17.34 ± 11.77 Au/ml in NIDDM vs 14.62 ± 13.2 Au/ml in „young" and 17.53 ± 13.46 Au/ml in „old" healthy population). This increase was mostly pronounced in patients with type I diabetes complicated with neuropathy (25.95 ± 7.21 Au/ml), nephropathy (32.5 ± 3.87 Au/ml, p<0.02) and CVD (26.0 ± 7.0 Au/ml). These changes were not so evident in NIDDM subjects. Surprisingly, the highest activity of PAI-1 was found in overweight controls (28.87 ± 6.24 Au/ml, p<0.002). It would seem that obesity and insulin dependent diabetes are the factors also connected with the risk of a prothrombotic state - accelerating atherosclerosis and triggering vascular complications.

1611

NID DIABETES ASSOCIATED TO HYPERTENSION DETERMINES THE RELATIONSHIP BETWEEN PAI-1 AND Lp(a) PLASMA LEVELS

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A reduced fibrinolytic activity has been found in diabetes mellitus and hypertension. In a previous work we demonstrated a negative correlation between two fibrinolytic inhibitors, plasminogen activator inhibitor-1 (PAI-1) and lipoprotein(a) (Lp(a)) plasma levels in a NIDDM population. Present work was aimed to investigate whether other risk factors, such as hypertension, obesity, dislipidemia and oxidative stress, may influence the observed correlation between PAI-1 and Lp(a). 108 NIDDM patients (age 65.1±9.5 yr.) and 49 non diabetic subjects (age 66.7±8.3 yr.) were studied. Blood concentrations of Lp(a), PAI-1 antigen and activity and the main parameters of lipo and glycometabolic balance were determined. Antioxidant defense was assayed as oxygen radical absorbance capacity ($ORAC_{OH}$). The subjects were divided into 4 groups based on the presence or absence of diabetes and essential hypertension. No statistical differences among groups were found as regards BMI and cholesterol, while, as expected, significant statistical differences between diabetic and non diabetic subjects were found as far as triglycerides and $ORAC_{OH}$ are concerned (p<0.01). Covariance analysis was performed in order to evaluate the entity of the relationship between PAI-1 and Lp(a) in the four groups, considering triglycerides and $ORAC_{OH}$ as confounding variables. We found the F test statistically significant in evaluating the Log PAI-1/Lp(a) plasma levels relationship among the four groups (p<0.02), while $ORAC_{OH}$ and triglycerides do not influence the above relationship. Regression analysis of Log PAI-1/Lp(a) showed a strong correlation only in hypertensive diabetic patients (r=-0.76, p=0.00001). These results suggest a close association between NID diabetes and hypertension in determining PAI-1 and Lp(a) relationship.

1612

COAGULATION PARAMETERS IN IDDM AND NIDDM PATIENTS WITH AND WITHOUT LATE COMPLICATIONS.

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Introduction: The role of coagulation factors in diabetes and specially in the pathogenesis of late comlications is still unclear. Therefore we measured fibrinogen,F XIII, F VIII,vWF,TAT,AT III,PAI-1,a2 Plasmin-Inhibitor in IDDM and NIDDM patients with and without late complications.
Subjects and methods: We investigated 11 IDDM patients without complications (8m,3f;27+/-6 years), 9 IDDM patients with diabetic microangiopathy (6m,3f;39+/-16), 47 IDDM adulescents (29m,18f;13+/-3) and 113 age matched controls (44m,69f;44+/-18) and 21 NIDDM with peripheral arterial occlusive disease (PAOD) stage IIb-IV (14m,7f; 72+/-6),10 NIDDM with microangiopathy (6m,4f;67+/-8), 9 NIDDM without complications (5m,4f;57+/-14), 62 ageadapted controls (20m,42f;62+/-8) and 9 non-diabetics with PAOD (7m,2f;62+/-7) using commercially available kits.
Results: There was a significant ($p<0.05$) elevation of fibrinogen and vWF in all patient groups, but independent from diabetes and complications. TAT was elevated only in the IDDM groups. In IDDM correlations between HbAlc and vWF ($r=0.27$; $p<0.03$) and a2-PI ($r=0.29$,$p<0.02$) were seen.
Conclusion: The role of coagulation parameters in diabetes and its complications remains unclear. The constant elevation of vWF and fibrinogen seen already at an early stage may indicate alterations (at the endothelial level?) long before of the clinical manifestation of complications.

1613

THE EFFECT OF ALPHA-LINOLENIC ACID ON COAGULATION IN JAPANESE PATIENTS WITH NON-INSULIN-DEPENDENT DIABETES MELLITUS

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We investigated the effects of supplemental dietary linseed oil, containing the omega-3 fatty acid, alpha-linolenic acid, on coagulation and fibrinolysis in 12 Japanese patients with non-insulin-dependent diabetes mellitus. Five grams of linseed oil per day containing 58% alpha-linolenic acid was administered to each patient for 2-4 weeks. Plasmin • $\alpha_2$plasmin inhibitor complex (PPI), plasminogen activator inhibitor-1 (PAI-1) and thrombin • antithrombin III (TAT) were measured before and after the supplementation period. PPI showed a significant decrease (0.62±0.18 vs. 0.48±0.1 μg/ml, $p<0.01$), while PAI-1 showed a decreasing trend (59.5±34.8 vs. 35.3±20.3 μg/ml, $p=0.059$). There was no significant change in TAT. These findings suggest that the change in the omega-3/omega-6 fatty acid ratio caused by supplementation with linseed oil affects on coagulation and fibrinolysis.

1614

REGIONAL ADIPOSITY AND HAEMOSTATIC PROFILE IN DIABETIC WOMEN AND WOMEN WITH NORMAL GLUCOSE TOLERANCE

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Women with non-insulin-dependent diabetes (NIDDM) have a greatly elevated risk of coronary heart disease (CHD), possibly through increased formation of occlusive thrombi. Android obesity in women appears to be an important determinant of increased CHD risk. We therefore examined the relationship between regional adiposity and haemostatic profile in women with well-controlled NIDDM (HbA_{1c} 7.3 ± 0.3%) compared to a group of normoglycaemic women matched for age and BMI. Body composition was assessed by dual-energy X-ray absorptiometry. Two regions of interest were delineated to depict body fat distribution: an android waist (AW) region and a gynoid leg (GL) region. Although there were no differences between women with NIDDM and controls in either total fat mass (30.9 ± 1.5 and 32.8 ± 1.5 kg, respectively) or lean tissue mass (39.5 ± 0.86 and 38.3 ± 0.63 kg, respectively), differences in regional adiposity were evident. (Data: mean ± SEM, PAI-1: geometric mean ± SEM)

	NIDDM (n=45)	**Control** (n=40)	***p* value** (*t* test)
age (years)	64 ± 1	62 ± 1	-
BMI (kg/m^2)	29 ± 1	29 ± 1	-
AW/GL fat ratio	1.07 ± 0.04	0.75 ± 0.03	< 0.001
PAI-1 antigen (ng/ml)	26.3 ± 1.2	12.7 ± 1.2	< 0.005
factor VII (% activity)	115 ± 3	110 ± 4	n.s.
fibrinogen (g/l)	2.92 ± 0.07	2.88 ± 0.07	n.s.

Women with NIDDM had more fat mass in the AW region and less fat mass in the GL region, thus giving a significantly higher AW/GL fat ratio. They also had higher levels of plasminogen activator inhibitor-1 (PAI-1) antigen, suggestive of reduced fibrinolytic activity. Elevations in fibrinogen or factor VII were not observed in NIDDM. A significant relationship ($r = 0.37$, $p = 0.001$, Pearson's correlation) was evident between PAI-1 antigen levels and the AW/GL fat ratio. Upper body obesity in women with NIDDM may contribute to increased CHD risk through impaired fibrinolysis, rather than increased tendency for thrombosis.

1615

THE EFFECT OF INSULIN AND PROINSULIN ON THE SECRETION OF PAI-1 BY INSOLIN-RESISTANT $HEPG_2$ CELL LINE

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Abstract: High plasma plasminogen activator inhibitor-1 (PAI-1) is associated with insulin resistance. The heptoma cell line $HEPG_2$ has been shown to synthesize PAI-1 in response to insulin and proinsulin. The secretion of PAI-1 by insulin-resistant HEPG2 cell were evaluated after insulin , proinsulin stimulation. The results showed that:① PAI-1 secretion levels from insulin-resistant $HEPG_2$ cells were not significantly different from those observed with control cells . ② The levels of PAI-1 by insulin-resistant $HEPG_2$ cells is higher than by control $HEPG_2$ cells after stimulation of insulin or proinsulin. In the present of 10^{-4}M metformin the over stimulation effect of insulin and proinsulin on insulin-resistant $HEPG_2$ cells were abolished. The results indicated that: In insulin-resistant $HEPG_2$ cells,PAI-1 plasma levels are largely increased after stimulation of insulin or proinsulin. Metformin inhibites the insulin and proinsulin mediated PAI-1 over synthesis.

1616

EFFECT OF TROGLITAZONE ON PLASMINOGEN ACTIVATOR INHIBITOR ON (PAI-1)

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NIDDM is associated with impaired fibrinolysis which may contribute to the increased incidence of cardiovascular disease. Plasma concentrations of plasminogen activator inhibitor (PAI-1) are elevated in patients with NIDDM and correlate with hyperinsulinemia and insulin resistance. We measured plasma PAI-1 antigen concentrations in two groups of patients participating in two clinical trials with the "insulin sensitizer" Troglitazone in doses of 200 to 600 mg daily. In one trial patients were treated with Troglitazone alone and in the other Troglitazone was added to previous treatment with insulin. In 8 patients on placebo (4 in each trial) the mean ± SD plasma PAI-1 was of 72.6 ± 39.3 ng/ml at the beginning of the study and did not change significantly by the end of the trial (54.9 ± 30.8 ng/ml, ns). In contrast, in 18 patients treated with Troglitazone (8 monotherapy, 10 with insulin) PAI-1 levels fell significantly from 68.8 ± 32.3 ng/ml to 40.4 ± 20.4 ng/ml ($p < 0.01$). The change in PAI-1 levels did not correlate with changes in glycemic control. We conclude that Troglitazone has a direct beneficial effect on the impaired fibrinolysis associated with NIDDM and may, therefore, protect patients from cardiovascular disease.

1617

BOTH ACUTE AND CHRONIC HYPERGLYCEMIA DECREASE PLASMA FIBRINOLYTIC ACTIVITY IN THE RAT

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Decreased plasma fibrinolytic activity has been observed in diabetes and this might contribute to accelerated atherothrombosis in this disease. It is unclear, however, whether hyperglycemia per se is directly responsible for this finding or whether hypofibrinolysis is a result more than a cause of diabetic vascular complications. Aim of this study was to determine the effect of both chronic and acute hyperglycemia on plasma fibrinolysis in the rat. To this end, we compared plasma fibrinolytic activity (lysis of fibrin plates) in 9 Sprague Dowley rats who had undergone a 90% pancreatectomy 5 weeks before (weight 235±14 g., blood glucose 301±9 mg/dl) and in 8 control rats (weight 208±5 g., blood glucose 119±4 mg/dl). In separate experiments we compared rat plasma fibrinolitic activity after 4 hrs of saline infusion (n=9, weight 285±4 g., blood glucose after saline infusion 106±4 mg/dl) or after a 4 hrs hyperglycemic clamp (n=6, weight 281±8 g., blood glucose clamped at 291±12 mg/dl while insulin concentration was kept constant by infusion of octareotide 100 ng/Kg/min and insulin 0.5mU/Kg/min). Plasma fibrinolytic activity was significantly reduced both in the pancreatectomized as compared to control animals (mean lysis areas on the fibrin plate = 163±23 vs 308±21 mm^2, $p< 0.001$) and after the hyperglycemic clamp as compared to saline infusion (mean lysis areas on the fibrin plate = 105±16 vs 373±40 mm^2, $p< 0.001$). This data show that both chronic and acute hyperglycemia can decrease plasma fibrinolytic activity and suggest that hyperglycemia could be the primary culprit of impaired fibrinolysis in patients with diabetes mellitus.

1618

CAN IDDM CAUSE ATHEROSCLEROSIS BY GENERATING ALTERATIONS IN A SET OF PLASMA PARAMETERS?

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The studies were performed to elucidate an impact of IDDM on fibrinolytic markers (t-PA, PAI-1, active PAI-1), thrombin/antithrombin III complex and blood lipids distribution (TG, LDL, HDL, total cholesterol) and lipoprotein (a). 35 families were studied including: 45 IDDM patients (aged 13.8±0.6), their parents (n=65, aged 41.4±0.8) and non-diabetic siblings (n=17, aged 15.5±1.0), as well as 50 unrelated controls (aged 10.7±1.0) without clinical symptoms of IDDM and no family history of the disease. Stepwise discriminant analysis and Tuckey honest significant differences test for unequal N were applied to assess the differences between groups of subjects. Obtained results did not reveal any statistically significant differences in concentration of fibrinolytic markers. However, decreasing tendency of the level of both inhibitors and activators of fibrinolysis in IDDM subjects was found. Elevated concentration of thrombin/antithrombin III complex in parents group (mean 4.2 mg/L) and IDDM children (mean 4.07 mg/L) was not statisical significant comparing to controls (mean 3.45 mg/L). We did not find any influence of IDDM onset age and values of HbA_{1c} as well as fructosamine concetration on the value of studied parameters in IDDM patients. Discriminant analysis showed altered distances between parents v. IDDM (p=0.0036), v. siblings (p=0.0091) and v. controls (p=0.000034). HDL (p=0.027) and total cholesterol (p=0.035) were found to strongly discriminate studied groups. Means of total cholesterol concentration in parents, IDDM children, siblings and controls were as follows: 209.9, 173.6, 174.0 and 168.1 mg/dL, respectively and means of HDL cholesterol: 49.1, 47.6, 53.9 and 39.0 mg/dL, respectively. Average values of lipoprotein (a) concentration in studied groups were as follows: 199.2, 209.3, 136.0 and 205.4 mg/dL, respectively, without any significant differences. Our observations enable us to suggest the similarity between IDDM children and their parents in recpect to plasma parameters distribution. It indicates that the age caused atherogenic changes in blood can be accelerated by hyperglycaemic state in IDDM.

Supported by KBN grant 4 P05E053 08

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OXIDATIVE STRESS AND FIBRINOLYSIS IN PATIENTS WITH INSULIN-DEPENDENT DIABETES MELLITUS.

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The development of diabetic complications may be related to diminished fibrinolytic activity, which could be associated with oxidative stress. We studied the relationship between oxidative stress measured by malondialdehyde levels (MDA) and plasma concentrations of Plasminogen Activator Inhibitor-1, antigen and activity (PAI-ag resp. PAI-ac), tissue type Plasminogen Activator, antigen and activity (t-PA-ag resp t-PA-ac). The study was conducted in 278 consecutive IDDM patients (153 men, 125 women; mean age 38.1 years (SD12.0), duration of diabetes 17.2 years (SD 10.2), HbA1c 8.2 % (SD 1.89)).

A significant correlation was found between age and respectively MDA-levels (r= 0.12), t-PA-ag (r= 0.13) and t-PA-ac (r= 0.40).

Duration of disease was related with PAI-ag (r= -0.14), PAI-ac (r= -0.14), and t-PA-ac (r=0 .21), but not with MDA-concentrations or t-PA-ag. Metabolic control, as assesses by HbA1c-levels, did not correlate with MDA-concentration nor with the parameters of fibrinolysis. No relationship could be found between oxidative stress and the fibrinolysis parameters.

These results suggest that oxidative stress, as measured by malondialdehyde concentrations, is not related with parameters of fibrinolysis, which does not support a role of oxdative stress in diminishing fibrinolysis.

1620

DAYTIME FLUCTUATIONS OF PLASMINOGEN ACTIVATOR INHIBITOR-1: IMPAIREMENTS IN NIDDM PATIENTS WITH CORONARY HEART DISEASE

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It has been previously shown that increased plasminogen activator inhibitor-1 (PAI-1) levels could be an important risk factor for coronary heart disease (CHD). Also, diurnal fluctuations in PAI-1 levels have been demonstrated in healthy subjects with the highest value in the morning and the nadir in the late afternoon. However, the relations between the daytime fluctuation pattern of PAI-1 levels and the appearance of CHD in NIDDM patients have not yet been clarified. The aim of this study was to analyse the pattern of diurnal changes in PAI-1 levels in 38 NIDDM patients with arteriographically documented CHD (group A), in 20 NIDDM patients without CHD (group B), and in 15 age-matched healthy controls (group C). The PAI-1 levels were determined at 08.00, 10.00, 13.00, 15.00 and 18.00h by using a plasminogen/chromogenic plasmin substrate assay (Table 1). In group A, PAI-1 levels did not show a significant decrease between 08.00 and 18.00h (6.2+/-2.9%). In contrast, in group B, PAI-1 levels decreased during the same interval (26.7+/-12.7%). In group C, we detected a more profound decrease in PAI-1 levels (88.6+/-5.6%), which was significantly higher than in group B ($p<0.01$). Also, both at 08.00 and 18.00h, the PAI-1 levels were higher in group A than in group B ($p<0.01$;respectively) and in both groups the levels were higher than in group C ($p<0.005$; respectively).

Table 1. Diurnal fluctuations of PAI-1 levels (U/ml)

	08.00h	10.00h	13.00h	15.00h	18.00h
Group A	6.3+/-0.4	6.4+/-0.4	6.2+/-0.5	6.1+/-0.3	6.0+/-0.4
Group B	4.8+/-0.5	4.6+/-0.4	4.3+/-0.2	4.2+/-0.2	3.9+/-0.4
Group C	2.8+/-0.2	2.6+/-0.2	2.2+/-0.3	1.7+/-0.1*	0.6+/-0.1**

Means+/-SEM * $p<0.05$ and ** $p<0.01$ (vs 08.00h in group C)

Conclusions: our results have shown the absence of diurnal fluctuations of PAI-1 in NIDDM patients with CHD, in contrast to the NIDDM patients without CHD and healthy controls. The results imply that prolonged exposure to increased PAI-1 levels might be an important determinant influencing the pathogenesis of CHD in NIDDM.

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PROGNOSTIC MARKERS OF VASCULAR DISEASE IN JUVENILE PATIENTS WITH NEWLY ACQUIRED DIABETES - RELEVANCE TO GENETIC MARKERS OF TYPE 1 DIABETES

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The alterations in fibrinolytic system, are believed to play an essential role in the pathogenesis of diabetic vascular complications. We monitored fibrinolytic disorders in 76 juvenile type 1 diabetics (aged 5-17 yr) with a newly acquired and diagnosed type 1 diabetes mellitus. When analysing the heterogeneous group of all diabetic juveniles, the mean antigen levels of plasma plasminogen activator inhibitor (PAI-1), tissue-type plasminogen activator (t-PA) and lipoprotein (a) (Lp(a)) remained within physiological ranges and were not different from those noted for control group. However, in selected patients (13%), who were excluded in so called 'high-risk vascular disease' group, PAI-1 and Lp(a) were drastically increased and greatly exceeded the relevant mean values observed in 'low-risk vascular disease' group (respectively 5-fold, 66.5±31.0 *vs.* 8.1±5.3 ng/ml, and 8-fold, 560±295 *vs.* 72±44.5 μg/ml). The correlation between these most profound alterations in PAI-1 and Lp(a) was insignificant, however these drastic enhancements significantly contributed to the highly significant discrimination of two groups of diabetics. The occurrence of two susceptibility markers predisposing to acquire type 1 diabetes, HLA-DQα52Arg(+) (67% of which 46.5% were homozygotes) coincided in 25.6% of the examined patients, although there was no apparent relation between HLA-DQ haplotypes and the alterations in fibrinolytic parameters. We conclude that high PAI-1 level in the 'high-risk' group might be interpreted as the result of either single or combined effect of endothelium damage and/or platelet release reaction. Further, the impairments in fibrinolytic system observed in selected type 1 diabetic juveniles might result from their proneness to develop haemostatic disorders and not necessarily merely from the direct consequences of metabolic disorders encountered in type 1 diabetes.

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Abnormality of fibrinolytic system in patients with NIDDM

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The influence of the fibrinolytic system on diabetic complications was investigated. Tissue-plasminogen activator-plasminogen activator inhibitor-1 complex (T-PAI-C) is a new marker of fibrinolysis. T-PAI-C was determined in patients with NIDDM(n=50) and normal subjects(n=9). T-PAI-C level were higher in NIDDM(15.3±4.0 ng/ml) than normal subjects (7.8±3.5 ng/ml). With increasing LDL,VLDL and ApoB levels, higher prevalences of nephropathy were observed. LDL,VLDL and ApoB were associated with raised t-PAI-C level. But t-PAI-C level were no significantly different between NIDDM patients with normoalbuminuria(n=16, 14.8±5.9 ng/ml), micro-albuminuria (n=16, 15.3±4.1 ng/ml) and overt proteinuria(n=18, 16.3±4.4 ng/ml). With increasing t-PAI-C levels, higher prevalences of retinopathy were not observed. Glucose, HbA_{1c} and proteinuria were not associated with raised t-PAI-C level. This results indicate that fibrinolysis is accelerated in NIDDM patients with and without diabetic complications. Increase of t-PAI-C could be a primary factor of common genetic background in NIDDM.

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STUDY ON SUSCEPTIBILITY OF SERUM TO COPPER INDUCED OXISTRESS IN DIABETIC PATIENTS

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Oxidative changes of serum added 500μM $CuSO_4$ and incubated at 37°C for 24 hrs (Stage 3) were compared with of serum kept at 4°C (Stage 1) in following components, that is, uric acid, total bilirubin, total protein, albumin, lipids, apo-lipoproteins, total fatty acid fractionation, vitamin E, TBARS and PAG electrophoresis. Serum were obtained from 14 normal controls and various state of 24 NIDDM patients(DM).

The rate of changes (difference between the value of Stage 3 was divided by the value of Stage 1) were shown to be significantly high in uric acid, vitamin E, apolipoproteins and PUFA of DM.

Furthermore, in 15 DM of whom immunologically determined anti AGE antibody levels were signi-ficantly increased in Stage 3 compared with Stage 1.

These data are likely to tell us that diabetic serum are highly susceptible to oxistress.

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THE DETECTION OF APO CIII PROMOTER T→C -455 POLYMORPHISM IN INSULIN RESPONSE ELEMENT

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One of the most important problems in diabetes is investigation of molecular bases of high level of triglycerides. W.Li et al.(J.Clin.Invest.1995,96:2601-2605) showed the strong influence of the T→C -455 polymorphism of insulin response element of apo CIII promoter to the triglyceride level. The main aim of our work was to create a simple method for detection of that polymorphism. For that purpose two oligonucleotid primers were sintezed. The downstream primer was 25-mer 5' - ATC TCA GCC TTT CAC ACT GGA ATT T - 3', as upstream primer we used 25-mer 5' - GTC TTC TGT GCC TTT ACT CCA AAG A - 3', ends up one nucleotide short of the polymorphism site, and contains, as the 3'-penultimate nucleotide, a G rather than the native C residue. Thus, elongation of this primer must lead through a site-directed mutagenesis (C→G in the coding strand) to the MboI site GATC formation in case of the T at position 455 (CATC→GATC), but not in case of the C at the same position (CACC→GACC). Genomic DNA was PCR amplified with two described primers, cycling conditions were: initial cycle: 93°C 1.5 min., 52°C 1 min., 72°C 1 min., 29 cycles 93°C 1 min., 56°C 1 min., 72°C 1 min., final extension 72°C 5 min. Restriction products were electrophoresed in 10% polyacrilamide gel. Possible patterns were 154 bp (no restriction, C in 455 position), 131 bp, and 23 bp long(in the case there was T in 455 position). Using this approach, we analyzed 61 children from three schools of Saint-Petersburg. The frequencies of T and C alleles were 55.7% and 44.3% respectively.

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LIPID PEROXIDATION IN DIABETIC PATIENTS: RELATIONS TO HYPERTRIGLYCERIDAEMIA, OBESITY AND HYPERGLYCAEMIA

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The relations of hypertriglyceridaemia, obesity (body mass index, BMI) and hyperglycaemia (HbA1C levels) to LDL oxidation were studied in 62 diabetic patients. The patients having an additional disease to diabetes, such as a systemic infection, or using immunosuppressive drugs were excluded from the study. Diene conjugates (DC) were measured in LDL and serum samples to estimate the level of lipid peroxidation. In NIDDM patients (n=43) serum triglyceride levels correlated positively with the total amount of oxidized LDL (LDL-DC; r=0.58, p<0.001), with the proportion of oxidized form in LDL (LDL-DC/LDL; r=0.72, p<0.001), and with the serum level of diene conjugates (S-DC; r=0.74, p<0.001). BMI of NIDDM patients correlated positively with LDL-DC (r=0.36, p<0.05), and in the analysis of the patients with serum LDL-cholesterol less than 6.0 mmol/l (n=25), also with LDL-DC/LDL (r=0.44, p<0.05) and S-DC (r=0.68, p<0.001). Serum triglyceride levels of IDDM patients (n=19) correlated with S-DC (r=0.88, p<0.001). The HbA1C values of the IDDM patients with serum LDL-cholesterol less than 6.0 mmol/l (n=15) correlated positively with LDL-DC (r=0.60, p<0.05) and LDL-DC/LDL (r=0.56, p<0.05). The present study demonstrates connections between hypertriglyceridaemia and LDL oxidation, as well as obesity and LDL oxidation in NIDDM patients. A weak association was found between HBA1C levels and LDL oxidation in IDDM patients with LDL-cholesterol less than 6.0 mmol/l. The present results suggest that in hypertriglyceridaemic and obese NIDDM patients, LDL oxidation may be an important risk factor of atherosclerosis.

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EFFECT OF CHRONIC INTRAPERITONEAL VS SUBCUTANEOUS INSULIN DELIVERY ON TRIGLYCERIDE SECRETION RATE IN STZ-DIABETIC RATS.

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The effect of chronic insulin treatment by continuous delivery from an implant via the intraperitoneal (IP) vs the subcutaneous (SC) route ($3U \cdot day^{-1}$ x 21 days) on hepatic triglyceride secretion rate (TGSR) was examined in streptozotocin (STZ) diabetic rats. Two control groups were used: untreated STZ-diabetic (UD) rats, and nondiabetic (N) rats. All groups were allowed ad lib access to standard Purina chow and to drinking water. TGSR was calculated from the linear increase in plasma triglyceride levels following lipase inhibition by Triton WR-1339. Untreated STZ-diabetic rats had lower insulin, higher glucose and TG levels, and lower body weight (BW) than nondiabetic rats. However, their FFA levels were similar to controls perhaps because their fat stores were depleted. Insulin treatment (either IP or SC) restored fasting plasma glucose, TG, and BW to normal independent of the route of insulin administration and did not significantly alter FFA levels. Both insulin-treated groups were hyperinsulinemic. Peripheral insulin levels were greater in the SC treated group (SC 273 ± 46 $\mu U \cdot ml^{-1}$; IP 98 ± 25; N 70 ± 19; SC vs IP or N, $p < 0.001$). Portal insulin levels were greatest in the IP treated group (IP 474 ± 91 $\mu U \cdot ml^{-1}$; SC 217 ± 51; N 260 ± 86; IP vs SC or N, $p < 0.05$). Despite elevated TG levels, TGSR was reduced in the untreated STZ-diabetic rats (UD 0.44 ± 0.06 mg/min; N 0.68 ± 0.05; $p < 0.05$). TGSR was restored to normal following insulin administration, regardless of route (SC 0.67 ± 0.08 mg/min; IP 0.67 ± 0.09). This occurred despite the 2-fold increase in portal insulin levels of the IP group compared to the SC group. CONCLUSIONS: **1)** In STZ-diabetes, TGSR is decreased, probably due to insulin deficiency. **2)** As the increase in TG levels in STZ-diabetes is not due to increased TGSR, it may result from defective TG clearance. **3)** Chronic hyperinsulinemia in STZ-induced diabetes normalizes TGSR. **4)** The route of insulin delivery does not affect TGSR in these rats.

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MEMBRANE FLUIDITY IS IMPROVED BY GLUCOSE CONTROL IN DIABETIC PATIENTS

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Diabetes is associated with a reduction in membrane fluidity which could be implicated in the pathogenesis of diabetic vascular disease. Yet, the role of glucose control on membrane fluidity remains debated. Thus we analyzed in 7 type 1 and type 2 diabetics the incidence of near normoglycaemia induced by continuous intravenous (Day 1 to D 10) or subcutaneous (D 10 to D 20) insulin infusion on the behaviour of the membrane phospholipids by physicochemical changes on the lipidic dynamics of erythrocytes and lymphocytes membranes. Fluorescence polarization (FP) and electron spin resonance (ESR) were used to respectively measure the FP anisotropy (r) of diphenylhexatriene (DPH) in lymphocytes membranes, the correlation relaxation time (τ c) of a 16 nitroxyde stearic acid (16 NS) probe and the degree of order (S) with 5 NS probe embedded in the membranes of intact erythrocytes. These parameters are directly related to the membrane fluidity (η) at the molecular level of resolution. Throughout the study, in conjunction with the reduction of HbA1c and fructosamine, the seven patients exhibit a progressive increase of the membrane fluidity, associated to a decrease of the degree of order. Every patient exhibits a constant trend to a progressive change of both fluidity and order, from D10 to D 20 when compared to basal value, reaching a mean range from 2 to 20% at D 20.

These preliminary results indicate that glucose control in diabetic patients can improve membranes properties and may presumably restore an optimum fluidity related to the membrane physiological roles.

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Lipid metabolism in the hepatocellular nuclei of the diabetic rats.

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The nuclei contain different lipids. However, regulation of metabolism of that lipid pool remains almost unexplored. The aim of the present study was to examine effect of insulin deficiency on incorporation of the blood-borne 14C-Palmitic Acid into different lipid classes and on the content of different fractions of phospholipid in the nuclei isolated from the liver of control, 2 and 7 day streptozotocin-diabetic rats. The rats were not treated with insulin. Incorporation of the label into the fraction of phospholipids and 1,2-diacylglycerols increased considerably already in 2 days after administration of streptozotocin and remained further unchanged in 7 days. Radioactivity of the fraction of triacylglycerols decreased in 2 days and also remained further unchanged. Incorporation of the label into the fraction of free fatty acids, monoacylglycerols and cholesterol esters was unchanged in 2 days and decreased in 7 days after administration of streptozotocin. The content of total phospholipids was markedly reduced in 7 day diabetic rats and it was accounted for by a reduction in each fraction (sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine and cardiolipin). Specific activity of each phospholipid fraction increased significantly though not uniformly. The largest increase was noted in the fraction of phosphadidylcholine (8 fold) and the smallest in the fraction of phosphatidylethanolamine (50% increase). It is concluded that diabetes profoundly deteriorates lipid metabolism in the hepatocellular nuclei and that, in this way, it can effect certain nuclear functions.

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A RELIABLE FLOW CYTOMETRIC ASSAY FOR FUNCTIONAL LOW DENSITY LIPOPROTEIN RECEPTORS IN HUMAN LYMPHOCYTES.

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A simple, specific flow cytometric method has been developed for the diagnosis of familial hypercholesterolemia (FH) caused by mutation of the low density lipoprotein receptor (LDLR) gene. Using this method, the LDLR activity in 45 patients from 18 Japanese FH families was analyzed. In 43 of 45 FH patients, LDLR activity was 55±18% (mean±SD) compared to that of control subjects (100.9±10.6%). In 2 of 45, showed normal activity of 84% and 123%, respectively. Subsequently, PCR products of the LDLR gene were screened by DGGE. Abnormal band patterns were observed in PCR products of exon 4, 7, 9, 12 and 17. PCR products in patients with ab-normal band pattern were subcloned and sequenced by an automated DNA sequencer. Four known mutations of a 21 bp insertion in codon 200-206 of exon 4, C317S in exon 7, intron 12+2 T to C and K790X in exon 17 were identi-fied. Furthermore, three new mutations were identified: a insertion of C at 390nt in codon 109 of exon 4 and a 7 bp deletion of GGGAAGT in codon 98-100 of exon 4, which caused a frameshift, resulting in the introduction of a stop codon 50 and 85 amino acids (aa) further downstream, respectively. Also, 5 bp inser-tion of GGACC at 1247nt in codon 416 of exon 9 causes a frameshift, creating a stop codon 13 aa further downstream. It is suggested that these mutations introducing a stop codon resulted in a premature termination of the LDLR protein expressed as the class 1 of receptor-negative mutations. Detection of each mutation in their family members showed that mutations were causa-tive for FH. The patients with these known and new mutations showed about half the activity of normal controls but 2 of 6 patients with K790X showed normal activity. These studies suggested that a flow cytometric pro-cedure of the LDLR activity might be specific and reliable for the diagnosis of FH and a useful tool to distinguish from other hypercholesterolemia.

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INFLUENCE OF INSULIN AND DIABETES ON THE SECRETION OF CHOLESTERYL ESTER TRANSFER PROTEIN (CETP) FROM HAMSTER ADIPOSE TISSUE

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Cholesteryl ester transfer protein (CETP) promotes the exchange of cholesteryl ester and triglycerides between HDL and triglyceride-rich lipoproteins. Increased CETP activity may explain the low HDL in diabetes. The present study demonstrates that plasma CETP activity was increased in streptozotocin-induced diabetic Syrian hamsters. Incubation of adipose fragments from these animals resulted in increased secretion of CETP activity into the medium and a reciprocal decrease in CETP activity in tissue homogenates. In normal animals, intraperitoneal administration of regular insulin (2 U/100 g body weight) reduced the levels of plasma CETP activity within 1-2 h and that was associated with a reduction of CETP activity released from adipose tissue. Chronic administration of insulin increased plasma CETP activity due to increased plasma triglycerides without a significant alteration in the release of CETP activity from adipose tissue in vitro. The results of the present study suggest that the levels of CETP activity generated from adipose tissue and that in plasma are augmented in insulin-deficient hamsters. Acute administration of insulin reduces the release of CETP activity from adipose tissue which may be partially responsible for the reduction in circulating CETP. Thus, insulin appears to be a negative regulator of CETP secretion in adipose tissue, which may explain insulin's positive effect on plasma HDL (This study is supported by Canadian Diabetes Association and Heart and Stroke Foundation of Canada).

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LPL MASS IS DECREASED BY ENDOTHELIAL CELL DAMAGE IN NIDDM PATIENTS WITH NEPHROPATHY

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We examined relationship between heparin-releasable lipoprotein lipase (LPL) mass and von Willebrand factor (vWF), an indicator of endothelial cell damage , to elucidate the mechanism for hypertriglyceridemia (HTG) in NIDDM with nephropathy. NIDDMs (n=65) with normo-, micro-, and overt- albuminuria (AU) and non-diabetic controls with normo AU (n=29) were studied. LPL mass was measured in postheparin plasma by Sandwich EIA, and vWF by ELISA. Dietary TG response was determined in plasmas before and 3,6, 9 h after ingestion of milk fat. Age, BMI and HbA1c were comparable between the three NIDDM groups. Both fasting TG and dietary TG response were significantly increased in micro-AU and further increased in overt-AU compared to those in NIDDM with normo-AU or controls. LPL was decreased and vWF was increased with the pregression of nephropathy. Reduction in LPL was markedly correlated with vWF ($r=-0.694$, $p<0.0001$) in total NIDDMs. These results suggest that functional LPL attached to heparan sulfate proteoglycan is decreased by widespread endothelial cell damage in NIDDM with nephropathy. We propose this as a new mechanism for HTG in NIDDM with nephropathy.

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THE EFFECT OF JTT-501 ON SERUM TRIGLYCERIDES IN OBESE ZUCKER FATTY RATS

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Purpose: JTT-501 is a new oral hypoglycemic agent which improves blood glucose control through improving insulin resistance. It was also found that this agent decreases triglycerides (TG) in the blood. The aim of the present study was to clarify the mechanism of decreased TG in obese Zucker fatty rats after the treatment of JTT-501. Methods: After an oral administration of JTT-501 (100 mg/kg, once per day) to obese Zucker fatty female rats, which shows insulin resistance with impaired glucose tolerance and hypertriglyceridemia, and SD female rats for 7 days. Serum TG, glucose, insulin, post-heparin plasma lipoprotein lipase (LPL) and TG secretion rate (TGSR) using Triton 1229 were determind after overnight fast. Results: In obese Zucker fatty rats, serum TG (392.5±145.4 vs 1331.6±438.1 mg/dl, $p<0.0001$) and glucose (86.0±25.6 vs 145.1±66.2 mg/dl, $p<0.01$) were significantly decreased after the treatment. On the other hand LPL (195.9±47.9 vs 59.2±60.7 ng/ml, $p<0.002$) were significantly increased. Serum insulin level and TGSR did not change. In normal SD rats, plasma LPL (250.3±26.9 vs 189.0±16.5ng/ml, $p=0.0005$) was significantly increased and serum insulin (6.8±1.9 vs 13.2±8.4 μU/ml, $p<0.02$) was significantly decreased after the treatment. Conclusion: The decreased serum TG levels were closely associated with increased LPL but not with TGSR in obese Zucker fatty rats. The improvement of insulin resistance may contributed to the increased plasma LPL, resulting in decreased TG. The results of normal SD rats also suggest that this agent has the potential to increase plasma LPL.

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AUTOANTIBODIES AGAINST OXIDIZED LDL ARE INCREASED IN IDDM

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Plasma lipid and lipoprotein concentrations do not generally differ between patients with IDDM and normal subjects, although the risk of macrovascular disease is markedly increased in IDDM. We determined the concentration of autoantibodies against oxidized LDL (ratio between antibodies to native vs oxidized antibodies, oxLDLab) in 38 patients with IDDM (HbA_{1c} 8.4±0.2 %, S-LDL cholesterol 2.9±0.1, S-HDL cholesterol 1.3±0.1, S-triglycerides 1.1±0.1 mmol/l) and 33 normal subjects (HbA_{1c} 5.1±0.1 %, $p<0.001$, 2.8±0.1, 1.3±0.1, and 0.9±0.1 mmol/l, NS for all lipids, respectively). OxLDLab were 1.5-fold higher in the IDDM (1.81± 0.15) than in the normal subjects (1.23±0.07, $p<0.001$). None of the IDDM patients had evidence of macrovascular disease. When the IDDM patients were divided according to their mean oxLDLab level (1.8), patients with high oxLDLab (2.87±0.22, n=13) did not differ from those with low oxLDLab (1.26±0.06, n=25) with respect to age (37±2 vs 38±2 yrs), duration of IDDM (21±2 vs 25±2 yrs), LDL cholesterol (2.8±0.2 vs 3.0±0.1 mmol/l), HbA_{1c} (8.6±0.3 vs 8.3±0.2 %), body mass index (24.2±0.9 vs 24.8±0.6 kg/m^2), S-creatinine (108±8 vs 99±5 μmol/l), urinary albumin excretion rate (377±102 vs 349±212 μg/min) or degree of retinopathy. The patients with IDDM and high oxLDLab:s had, however, signs of more severe autonomic neuropathy than those without (E/I ratio 1.1±0.02 vs 1.3±0.04, $p<0.001$; Valsalva ratio 1.3±0.04 vs 1.6±0.07, $p<0.001$; orthostatic test -18.1±6.2 vs -3.8±2.9 mmHg, $p<0.05$; reflex vaso-constriction to cold -24±6 vs -42±4 %, $p<0.02$). These data provide the first evidence of increased oxLDLab concentrations in patients with IDDM. Unexpextedly, high levels were significantly associated with the presence of autonomic neuropathy but not with other microvascular complications.

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THIS ABSTRACT HAS BEEN WITHDRAWN BY THE AUTHOR.

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INCREASED CHOLESTERYL ESTER TRANSFER PROTEIN (CETP) SPECIFIC ACTIVITY IN A POPULATION OF 126 NIDDM PATIENTS.

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Cholesteryl Ester Transfer Protein (CETP), responsible for the transfer of cholesterol esters (CE) and triglycerides (TG) between lipoproteins, plays a determinant role in lipid metabolism. So far, the few studies on CETP activity in NIDDM have been performed in a very small number of patients and have given controversial results. The aim of our study was to evaluate plasma CETP activity and its main determinants in a large population of NIDDM patients, using normal HDL particles as CE donor. One hundred and twenty six NIDDM patients (66 men, 60 women) were studied. These patients were treated by diet ± oral hypoglycemic drugs. CETP activity was measured as the capacity of the plasma to promote the transfer of radiolabeled CE from HDL_3 to the endogenous apoB-containing fraction of the native plasma. CETP mass concentration was measured by ELISA. Specific activity of CETP, calculated as the ratio of plasma CETP activity to CETP mass was significantly higher in NIDDM patients than in controls (125 ± 67 vs 62 ± 15 %/h/μg). In the whole diabetic population, plasma CETP activity was positively correlated with (VLDL+LDL) cholesterol (r=0.36;p<0.0001), BMI (r=0.24; p=0.006) and negatively with HDL cholesterol (r= -0.25;p=0.004). However, the correlations were different in the normotriglyceridemic NIDDM (TG<150 mg/dl; n=68) and in the hypertriglyceridemic NIDDM (TG≥ 150 mg/dl; n=58) patients. In both groups, a correlation with (VLDL+LDL)chol was found. Triglycerides (r=0.35;p=0.003), BMI (r=0.32; p=0.006) and HDLchol. (r= -0.36; p=0.002) were correlated with CETP activity only in the normoTG group, and HbA1c (r= 0.26; p=0.04) only in the hyperTG group. In the multivariate analysis, (VLDL+LDL)chol. was the only significant determinant of CETP activity in the whole diabetic population (t=3.02;p=0.003) and in the hyperTG group (t=5.4; p<0.0001) when, in the normoTG group, both (VLDL+LDL)chol. (t=2.71;p=0.008) and HDLchol. (t=-2.36; p=0.02) were significant determinants. **In conclusion**: 1) CETP specific activity is significantly increased in NIDDM. This is likely to promote the generation of atherogenic modified lipoproteins in NIDDM. 2) (VLDL+LDL) chol. level is the main determinant of CETP activity in NIDDM; HDL chol. is a significant determinant only in normotriglyceridemic NIDDM patients.

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TRIGLYCERIDE INDUCED DIABETES MELLITUS DUE TO LIPOPROTEIN LIPASE GENE POLYMORPHISM. F.L.Henriksen, [‡]G.Mingrone, L.N.Krogh, M.Hørder. [‡]G.Gasbarrini and H.Beck-Nielsen. Odense University Hospital, Odense, Denmark and [‡]University Cattolica S. Cuore, Rome, Italy.

Two sisters (sib1: 18 and sib2: 15 years old) developed hyperchylomicronemia 14 years old. Diffuse eruptive xanthomas and recurrent attacks of acute pancreatitis ensued. Both developed an insulin-resistant diabetes mellitus (NIDDM). which were intractable on peroral antidiabetics and insulin. A modified biliopancreatic diversion was employed in both sisters. After the operation triglycerides decreased to nearly normal and the eruptive xanthomas, pancreatitis and NIDDM disappeared. The lipolytic enzyme lipoprotein lipase (LPL) has a cental role in the metabolism of triglyceride rich chylomicrons and very low density lipoproteins (VLDL). Decreased LPL activity caused by LPL gene polymorphism or mutations are possible reasons for this triglyceride induced diabetes mellitus. After a 14-h overnight fast Heparin 100 IU/kg is injected intravenously, 15 minutes later post-heparin blood were collected and catalytic LPL activity measured. GC-clamped PCR-amplified DNA from the promotor region and exon 1 through 10 of the LPL gene were screened for nucleotide substitution by denaturing gradient gel electrophoresis (DGGE). Exons with abnormal DGGE pattern (4 band pattern) were sequenced by an automated laser fluorescence sequencer. HDL, HDL_2, HDL_3 cholesterol were quantified by a polyethylene glycol precipitation kit Quantolip, Immuno. Postheparin plasma LPL activity were low in the whole family 76.0 mU/ml in sib1, 117.1 in sib2, 205.7 in a healthy younger sib3, 101.7 in the mother and 145.0 mU/ml in the father. The most of the family presented extremely low HDL cholesterol concentrations (HDL, HDL_2, HDL_3), (0.51 mM, 0.17 mM, 0.34 mM) in sib1, (0.63, 0.19, 0.44) in sib2, (1.12, 0.32, 0.80) in sib3, (1.24 , 0.38, 0.86) in the mother, (0.58, 0.14, 0.44) in the father. Two polymorphies were found in the LPL gene, in exon 4 (GAG to GAA) coding for the same aminoacid Glu^{118} segregating in both the father, sib1, sib2, sib3; in exon 8 (ACC to ACA) coding for the same aminoacid Thr^{361} segregating in both the mother, sib1, sib2; and a stop mutation was detected in exon 9 (TCA to TGA) coding for the change of Ser^{447} to termination segregating in both the mother and sib3. Extremely low LPL activity. HDL cholesterol and the same two LPL gene polymorphisms in sib1 and sib2 support the hypothesis about genetically decreased LPL activity, hypertriglyceridemia and NIDDM.

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DYSLIPIDEMIA IN NON-DIABETIC SUBJECTS FROM FAMILIES WITH TYPE 2 DIABETES

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Non-diabetic subjects from families with type 2 diabetes have a prevalence of dyslipidemia similar to that observed in type 2 diabetic patients. In order to establish the type of dyslipidemia we have examined lipoprotein abnormalities in 20 non-diabetic subjects with family history of type 2 diabetes. The obtained results were compared to the results of the control group (20 type 2 diabetic patients of the same age and in a good glycaemic control). All tested subjects were non-obese but hypertensive. In all subjects measurements of total cholesterol (TC), LDL-cholesterol (LDL-C), HDL cholesterol (HDL-C), triglycerides (TG), apo A, apo B and lipoprotein (a) in the serum were performed. According to obtained results TG levels were elevated in both groups but significantly higher in the type 2 diabetic group (p=0,01), while levels of HDL-C were significantly lower in the both groups. We also found that lipoprotein (a) levels were normal to low in the both groups. Hypertriglyceridemia, which is a frequent trait in type 2 diabetic patients may explain the variability of lipoprotein (a) levels. Other observed parameters were normal.

In conclusion we consider that according to similarities in dyslipidemia to type 2 diabetic patients non-diabetic subjects with family history of type 2 diabetes have to be under careful medical control for prevention of cardiovascular complications.

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LPL MASSES ARE MAJOR DETERMINANTS OF CHOLESTERYL ESTER (CE) TRANSFER AND LIPOPROTEIN CORE WEIGHT RATIO (CWR) IN TYPE II DIABETIC PATIENTS

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The aim of our study was to evaluate the role of LPL on CE transfer rate (CETR) and plasma lipoprotein CWR (TG/TG+CE) in diabetic patients. Subjects were 30 type II diabetics treated by diet alone and compared to the 30 age-, sex-, body mass index-matched controls. Post heparin LPL masses were measured by monoclonal antibody. CETR was evaluated by the method of substrate dependent. Plasma CETP concentration was measured by anti-peptide antibody. Plasma total cholesterol (TC) and TG levels were comparable between the two groups. CETR was significantly lower in the type II diabetics compared to controls (42±21 vs 70±30 nmol/ml/h, $p<0.01$). CETP masses were not different between the two groups (1.52±0.45 vs 1.50±0.39 μg/ml). VLDL and HDL CWR were significantly higher in the type II diabetics (0.921± 0.040 vs 0.780±0.051, $p<0.01$, 0.325±0.142 vs 0.214±0.091, $p<0.01$, respectively). In type II diabetics, CETR correlated positively with plasma VLDL-TC ($r=0.476$, $p<0.01$) and apo B ($r=0.446$, $p<0.02$), inversely with HDL-TC/TG ratio ($r=-0.461$, $p<0.02$), apo AI/AII ratio ($r=-0.651$, $p<0.001$) and LPL masses ($r=-0.448$, $p<0.02$). Furthermore LPL masses were inversely correlated with HDL-CWR ($r=-0.476$, $p<0.01$) and the 24/h urinary C-peptide excretion ($r=-0.425$, $p<0.02$). In conclusion, plasma LPL concentration was closely associated with the net mass transfer of CE, lipid compositions of acceptor or donor lipoprotein and endogenous insulin secretion in the plasma of type II diabetic patients.

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HYPERTRIGLYCERIDEMIA AND LOW HDL CHOLESTEROL IN JAPANESE PATIENTS WITH NIDDM

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The abnormalities in serum lipids commonly observed in diabetic patients are possible risk factors for cardiovascular disease. So we investigated the frequency of hypertriglyceridemia (h-TG) and low HDL-cholesterol (HDL-C) level, and the relation between these dislipidemia, BMI and blood glucose control in Japanese NIDDM patients, and then the influence of strict diet therapy for 2 weeks on these dislipidemia. 511 patients with NIDDM (259 male, 252 female ; 29-89 years of age) were studied. The prevalence of h-TG (> 1.69mmol/l) was 32.5% (83 of 257) in male and 24.2% (61 of 252) in female. The frequency of h-TG increased paralleling an increase of BMI in both male and female. On the other hand the prevalence of low HDL-C (< 0.91mmol/l) was 39.7% in male and 27.2% in female, there was no correlation between frequency of low HDL-C and BMI in male and female. The frequency of h-TG in poorly controled (FBS 180mg/dl and HbA1c > 8.0) obese (BMI > 24.2) patients was 7.1 fold higher than that in good controled (FBS < 140mg/dl and HbA1c < 7.0) non-obese (BMI < 24.2) patients in male, 5.4 fold in female, but frequency of low HDL-C was 1.9 fold higher in male and 2.5 fold in female (see table).

The percentage of diabetic patients with h-TG and low HDL-C

			TG		HDL-C	
			male	female	male	female
non-obese	good	control	8.5 (%)	10.3	26.0	20.7
	poor	control	42.8	23.8	47.8	38.1
obese	good	control	46.3	20.7	41.0	24.1
	poor	control	60.0	56.0	50.0	52.0

Strict diet therapy significantly reduced serum TG (from 2.21 ± 0.45 to 1.53 ± 0.29), but HDL-C did not change significantly (from 0.95 ± 0.17 to 1.02 ± 0.16). Conclusion. The frequency of h-TG in NIDDM was strongly correlate to BMI and blood glucose control, but that of low HDL-C was not correlate to BMI and weekly association to blood glucose control.

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Lp(a) IS A RISK FACTOR OF MACROANGIOPATHY, BUT INDEPENDENT FROM DIABETES

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Introduction: Lp(a) is an accepted risk factor of coronary heart diesease (CHD) and seems also to play an important role in the pathogenesis of peripheral arterial occlusive disease (PAOD). The influence of diabetes on Lp(a) levels is controversly discussed.

Subjects and methods: Therefore we measured Lp(a) in 11 IDDM patients without late complications (group A: 8m, 3f;27+/-6 years), in 9 IDDM patients with diabetic microangiopathy (B:6m,3f;39+/-16),47 IDDM adulescents (C:29m, 18f;13+/-3) and 113 age adapted controls (D:44m,69f;44+/-18) as well as in 9 non-diabetics with PAOD stage IIb-IV (E:7m,2f;62+/-7),in 21 NIDDM with PAOD (F:14m,7f;72+/-6), in 10 NIDDM with microangiopathy (G:6m,4f; 67+/-8),in 9 NIDDM without microangiopathy (H:5m,4f;57+/-14) and also 62 age adapted controls (I:20m,42f;62+/-8). The determination was performed by enzyme immuno assay (Immuno,Germany)

Results: The levels of Lp(a) were: A:24+/-25 (HbAlc:8.7) B:17+/-20 (10.6), C:20+/-4 (8.6), D:18+/-22 (5.2), E:46+/-26 (5.3), F:35+/-34 (7.9), G:19+/-39 (8.8),H:18+/-19 (8.2) I: 23+/-29 (5.4). A significant ($p<0.05$) elevation was only seen in patients with PAOD independent from diabetes. There was no correlation with HbAlc.

Conclusion: In accordance with other studies we show that Lp(a) is beside CHD also a risk factor of PAOD. Diabetes, its duration and the metabolic control have no influence.

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LIPIDS AND OTHERS CARDIOVASCULAR RISK FACTORS IN ABORIGIN POPULATION FROM CHILE

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Cardiovascular disease (CVD) remains as the primary cost in terms of mortality and morbidity in industrialized nations. The relationship between lipoprotein disorders and atherosclerosis, specially of the coronary arteries, has received special attention in the last decade. In the different ethnic groups in the world, there are a great variability in the prevalence of CVD and in the lipoprotein profile. OBJECTIVE: Determine the lipid profile in two aborigin populations from Chile. METHODOLOGY: Two populations were analyzed: Mapuches (n = 64) from the south of Chile and Aymaras (n = 56) from the north of the country. We determine: anthropometric measures (BMI), plasma total cholesterol (TC) and HDL, triglicerides (TG) and blood pressure (BP). Data are expressed as mean ± standard deviation (DE) or percentages. Statistical analysis was performed by t-test and. RESULTS: Means and DE from The Mapuche group comparing to Aymara group yielded the following figures: TC: 191 ± 43 mg/dl vs 172 ± 53 ($p<0.05$); HDL: 62 ± 21 vs 45 ± 18 ($p<0.001$); BMI: 28 ± 5 vs 25 ± 5 (Kg/m^2) ($p<0.01$); BP: 139/86 vs 123/76 mmHg ($p<0.01$); TG:156 vs 143 mg/dl (NS). In the Mapuche group, 34% of the sample showed TC levels over 200 mg/dl while this characteristic was seen in 16% of Aymaras ($p<0.05$), TG concentration over 150 mg/dl was 30% in Mapuche group and 32% inAymara group(NS). BMI>30: 25% in Mapuche group and 13% in Aymara group($p<0.01$).
CONCLUSION: Mapuche population shows higher levels of TC and BP than Aymara population, although the latter presents higher concentration of HDL compared to the Mapuche group.

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FAMILY CLUSTERING OF ELEVATED PLASMA LP(a) IN IDDM.

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The blood concentration of apolipoprotein(a) [LP(a)] is a quantitative genetic trait in the general population, and elevated levels associate with increased cardiovascular risk. Poor glycaemic control has been suggested to increase LP(a) levels in IDDM, but whether this can confound the parental component in LP(a) levels is unclear. We measured LP(a) in 95 patients (42 M, 53 F) with duration of IDDM ranging between 1 and 31 years (median = 7) and all their parents. Albumin excretion was higher than 20 µg/min in 8 patients. The distribution of LP(a) concentration was independent of sex in the parents (5.9±0.8 and 6.6±0.9 mg/dl, p=ns) and was similar in the patients (6.8±0.8 mg/dl, mean±SEM). Glycated haemoglobin levels ranged between 5.1 and 15.3% (median = 8.0) but were unrelated with LP(a) levels. Likewise, no correlations were found with blood glucose, age, duration of diabetes, urinary albumin excretion and BMI, but also total cholesterol and triglycerides. There was no correlation in LP(a) concentrations between spouses ($r^2=0.02$, p=ns), but there was a strong relationship between midparental and offspring levels ($r^2=0.25$, $p<0.0001$) that was independent of glycaemic control. A similar pattern of relationships was observed for major blood lipids. We suggest that the presence of IDDM and poor glycaemic control may not confound the parental, perhaps inherited component of LP(a) levels. Whether raised LP(a) may predict cardiovascular events also in IDDM remains to be determined by prospective studies.

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DYSLIPIDEMIA AMONG DIABETICS IN AN URBAN POPULATION IN INDONESIA. Sarwono Waspadji, Pradana Soewondo, Imam Soebekti, Maryantoro Oemardi and Sidartawan Soegondo, Div. of Endocrinology and Metabolism, Dept. of Med., Fac. of Med., Univ. of Indonesia, Salemba 6, Jakarta 10430, Indonesia.

Among diabetics, dyslipidemia is one of the important factors for the development of premature coronary artery disease. Data on lipid profile among diabetics are often conflicting. Population studies on lipid profile among diabetics are rarely conducted in Indonesia. The aims of this study were to determine lipid profile among diabetics, to compare the results with lipid profile among diabetics found in our previous epidemiological study conducted 10 years earlier, and also to compare with lipid profile among diabetic clinic attendees. A survey was conducted in an urban subdistrict in Jakarta with a total population of 34,645. Adult members of the family (age >15 years) who were randomly recruited (5 %, stratified cluster random) to join this survey. Diabetes mellitus was determined using WHOcriteria. Lipid profiles were measured using commercial kit (enzymatic methods). Altogether there were 58 diabetics (5.7%)among 1019 eligible respondents. The peak prevalence of diabetes mellitus was found in the 4th-6th decades. High TC (> 240 mg/dl) was found in 24.1 % and high TG (> 200 mg/dl) in 29.3 % of the diabetics. The mean TC and TG levels among diabetics were 211±52 mg/dl and 202±173 mg/dl respectively. In our previous survey done 10 years earlier in an urban population adjacent to the present study site using similar methods and criteria, we found 44 diabetics among 2704 respondents. The peak prevalence of diabetes mellitus was also found in the 4th-6th decade's group. The mean TC and TG in this diabetic population were 241±52 mg/dl and 183±95 mg/dl respectively. Data on lipid profile compiled from diabetic clinic Cipto Mangunkusumo hospitals' attendees in 1995 showed the prevalence of high TC and high TG to be 25% and 17.8 % respectively, while the mean TC and TG were 234±64.6 mg/dl and 186±104.9 mg/dl. We concluded that although the prevalence of diabetes mellitus increases, the pattern of dyslipidemia among diabetics remains similar.This means that the prevailing abnormal lipid profile among diabetics is caused by diabetic condition, not due to the changing dietary pattern of the population.

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OVERESTIMATION OF LOW-DENSITY CHOLESTEROL BY THE FRIEDEWALD FORMULA DUE TO Lp (a) IN DIABETIC PATIENTS.

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Serum low-density liporotein cholesterol (LDL-C) and lipoprotein (a) [Lp(a)] have been considered independent cardiovascular risk factors; however the calculation of serum LDL-C by the Friedewald formula includes the cholesterol associated with Lp(a). Therefore, serum Lp(a) levels might act as a confounding factor in epidemiological as well as interventional studies. The main aim of the present study was to evaluate whether the overestimation of LDL-C by the Friedewald formula could be relevant in diabetic patients. For this purpose we included 118 consecutive diabetic patients (46 type 1 and 72 type 2) with serum triglycerides lower than 400 mg/dl. Total cholesterol and total triglycerides were tested on unfrozen samples by an automated enzymatic method. HDL-C was determined by assaying the cholesterol concentration in the supernatant obtained after precipitating lipoproteins with density lower than HDL by a mixture of phosphotungsting acid and magnesium chloride. Lp(a) was measured by ELISA. Diabetic patiens were classified according Lp(a) serum concentrations (mg/dl) as follows : 0-15, group A (n=73); 15-30 group B (n=33) ; 30-45 group C (n=8) and >45 group D (n=4). LDL-C was calculated following the Friedewald formula. Corrected LDL-C was obtained by subtracting the cholesterol portion of Lp(a) [estimated as 0.3 x Lp(a) mass] from the levels of cholesterol calculated by the Friedewald formula. Results : a linear correlation was observed between Lp(a) and both, uncorrected and corrected LDL-C ($r=0.30, p<0.001$ and $r=0.23, p<0.01$; repectively). The overestimation of serum LDL-C levels (mg/dl) using the Friedewald formula for the diferent groups of patients were : 1.5±1 for group A, 6.3±1.3 for group B, 10.6±1.3 for group C and 21.6±3.8 for group D. Our results suggest that overestimation of LDL-C by the Friedewald formula is not negligible in diabetic patients with high serum levels of Lp(a) .

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DYSLIPIDEMIA AND CORONARY HEART DISEASE IN NATIVE ASIAN INDIANS WITH NIDDM.

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Genetic and environmental factors contribute to the higher prevalence of NIDDM, insulin resistance syndrome (X), and coronary heart disease (CHD) in both native and migrant Indians,. Total cholesterol (TC), HDL-cholesterol (HDLC), triglycerides (TG) and LDL-cholesterol (LDLC) were quantitated (mg/dl) in 231 consecutive NIDDM subjects, and results correlated with other clinical and biochemical parameters, including urine albumin excretion (UAE: mg/24h; immunoturbidometry). The most striking abnormality was the very high prevalence of low ("high risk") HDL-C (19% and 43% in males and females respectively); central obesity, inactive life style, premature menopause and infrequent estrogen replacement therapy, besides possible genetic factors may be contributory. The overall prevalence of incipient (IDN) and overt (ODN) diabetic nephropathy were 68% and 11% respectively. In patients with all CHD (22%) and major CHD (5%; myocardial infarction, heart failure, coronary revascularisation), there was a clustering of risk factors including higher LDLC, diabetes duration, systolic BP and UAE. UAE demonstrated a striking positive correlation with LDLC, reflecting association of atherogenic lipid profiles with DN. Conclusion: Dyslipidemia, including low HDLC, is highly prevalent in Asian Indians (especially females) with NIDDM; DN and other coronary risk factors (hyperinsulinemia, hypertension, hyperglycemia etc.), likely account for the very high burden of CHD and mortality.

LIPID RISK % PREVALENCE (URINE ALBUMIN EXCRETION : mg/24h)

TYPE	N	DESIRABLE	BORDERLINE RISK	HIGH RISK
L DLC	201	68 (174)	24 (189)	8 (411)
TG	231	57 (213)	30 (164)	13 (129)
HDLC(M)	143	28 (275)	53 (246)	19 (135)
HDLC(F)	88	21 (63)	36 (76)	43 (94)

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LIPOPROTEIN(a)[Lp(a)] AND PROGRESSION OF ALBUMINURIA IN TYPE 2 DIABETES - RELATIONSHIP TO FIBRINOGEN LEVEL

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To determine the potential role of Lp(a) for the development of albuminuria and to investigate the link between Lp(a) and fibrinogen(Fbg) levels in a 4.5-year follow-up study in patients with non-insulin-dependent diabetes(NIDDM). Sixty-nine patients with NIDDM were selected by the following criteria: age less than 70 years, urinary albumin excretion index(UAEI) less than 300 mg/g·creatinine(Cr) on 3 separate occasions over a 3-month period and regular attendance in our outpatient clinic. The mean values obtained in the initial and last 3 months in the follow-up were calculated as the values of baseline and end point, respectively, and the mean values(m) of the variables and the change(△) from baseline throughout the follow-up were also used for data analysis. The patients were divided into the following two groups: patients with baseline Lp(a)≧25 mg/dl(group H, n=24) and those with Lp(a)<25 mg/dl(group N, n=45). No differences in age, sex, BMI, duration of diabetes, and the proportion of smokers, hypertension and diabetic retinopathy were found between the groups. With regard to intragroup differences, only log UAEI significantly($p<0.05$) increased in the H group at follow-up. Comparing the two groups, Fbg at baseline and follow-up or mFbg in the H group were significantly($p<0.05$) higher than those in the N group. The values of both m and △log UAEI in the H group were signuficantly($p<0.01$) greater than those in the N group. Multiple regression analysis revealed that systolic blood pressure, Lp(a) and total cholesterol at baseline, and △ of HbA1c, HDL-c, Fbg and 1/Cr were significant independent variables for △log UAEI ($p<0.05$). Logistic analysis demonstrated only baseline Fbg concentrations($p=0.038$) as a predictor of high Lp(a) values. These results indicate that raised Lp(a) value is a significant risk marker for the progression of albuminuria in NIDDM, independent of the other risk factors examined, and that baseline Fbg level is a strong predictor of high Lp(a) level.

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RISE IN PLASMA TRIGLYCERIDES : A SIMPLE EARLY INDICATOR OF OXIDATIVE STRESS IN URBAN ASIAN INDIANS.

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Presently urban Asian Indians are a high risk group for coronary heart disease (CHD), non-insulin dependent diabetes mellitus (NIDDM) and dyslipidemias such as raised plasma triglycerides (TG) low high density lipoprotein cholesterol (HDL-c) and raised total cholesterol (Tc) / HDL-c ratio. Above metabolic disorders are associated with insulin resistance and possibly have a common pathogenic mechanism. Oxidative stress is implicated in the pathogenesis of both CHD and NIDDM. The same may apply to dyslipidemias. However, rise in TG may appear much earlier than other metabolic defects. We studied 42 unselected subjects (M=23, F=19), age (47.84 ± 6.9 yr.) to see if dyslipidemias or rise in TG correlate with parameters of oxidative stress. Care was taken to see that none of the subjects had any antioxidant supplements. Lipids were measured by enzymatic methods and expressed as mg/dl while oxidative stress was assessed by measuring plasma reduced glutathione (GSH) by method of Beutler et al and expressed as μ moles / gmHb. The values with Mean (± SD) and correlation with lipids are as under :

	MALES	r	FEMALES	r
GSH	5.51 (2.07)		5.32 (2.40)	
Tc	176.70 (31.24)	- 0.38	218.89 (61.23)	- 0.34
TG	187.17 (187.81)	- 0.56	187.16 (169.64)	- 0.34
HDL-c	47.04 (10.53)	NS	51.31 (9.66)	NS
Tc/HDL-c	3.95 (1.14)	- 0.37	4.40 (1.54)	- 0.2

Results show significant correlation of GSH, a parameter of oxidative stress with values of TG, Tc and Tc / HDL-c. Since rise in TG appears much earlier this may be a simple early indicator of oxidative stress.

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SERUM LIPOPROTEINa AND DIABETIC MACROANGIOPATHY.

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To investigate the possible role(s) of Lp(a) in diabetic macroangiopathy, the relationship between serum Lp(a) levels and echographycally assessed intima-media thickness (IMT) and plaque formation in the common carotid and femoral artery (CA & FA) was evaluated in 193 cases of DM and non-DM,aged 23 to 88 yr. Arterial morphology (IMT and plaques) was evaluated with a Hitachi EUB-555. Serum Lp(a) was determined by turbidimetry. The severity of arterial plaque formations was expressed according to the score of point 0: no plaque, 1: unilateral or 2: bilateral lesions.

Results and Conclusion ; 1) Serum Lp(a) levels showed a positive correlation to the IMT, in either CA or FA, but the statistic analysis did not reach a significant value. 2) In stepwise multi-variate regression analysis, IMT and plaque formations were significantly associated with age and smoking. 3) When the subjects were confined to non-smoker-NIDDM, aged 40 to 65 yr, serum Lp(a) levels showed a significant correlation to CA-IMT ($r=0.555$, $p< 0.02$). No significant correlation was found between serum Lp(a) and IMT in non-smoker-non-NIDDM.

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INDEPENDENT ASSOCIATIONS OF GLUCOSE-INTOLERANCE AND DYSLIPIDAEMIA WITH MORTALITY: THE HOORN STUDY

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Glucose-intolerance and dyslipidaemia are related abnormalities, and both associated with increased mortality. The question is whether the combination of glucose-intolerance and dyslipidaemia is associated with higher mortality. We studied the association of glucose-intolerance, triglyceride-levels (TG) and HDL-cholesterol (HDL) with mortality in a population-based cohort study of 2484 men and women, aged 50 to 75 years, with a follow-up duration of six years. Causes of death of the deceased (n= 177) were retrieved by reviewing the medical records, and classified according to ICD-9. Relative Risks (RR) were estimated by Cox proportional hazards regression models. All models were adjusted for age and sex. Lipid categories were defined as 'favourable' (top HDL-tertile and lowest TG-tertile), 'unfavourable' (lowest HDL-tertile and top TG-tertile) or 'intermediate' (other combinations). In 168 out of 177 the cause of death could be retrieved. When glucose-intolerance and lipid categories were included in one model, dyslipidaemia and abnormal glucose-tolerance were found to be independent risk factors for mortality. Increased mortality could be attributed to ischaemic heart disease (IHD) and sudden death (SD). Of interest is that no significant interaction between lipids and glucose-tolerance was found.

	Relative Risk (95% CI) compared to NGT and 'intermediate' lipid group				
	IGT	NDM¶	Known DM	Favourable	Unfavourable
Mortalty	(254)	(118)	(87)	(464)	(435)
Total (n= 175)	1.1 (0.7-1.8)	1.7 (1.0-2.9)	3.1 (1.9-5.1)	0.7 (0.4-1.1)	1.3 (0.9-3.8)
IHD+SD* (n= 49)	1.5 (0.6-3.5)	3.0 (1.3-7.1)	4.7 (2.0-11.0)	0.8 (0.3-2.0)	1.4 (0.7-2.7)

¶ Newly diagnosed DM at baseline. * ICD-9 codes 410-414, 427.4, 427.5, 798.

CONCLUSION: In a general caucasian population, abnormal glucose-tolerance and dyslipidaemia (high TG and low HDL) are independent predictors of mortality due to ischaemic heart disease and sudden death.

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Lipoprotein (a) levels in diabetic subjects with and without ictus cerebri.

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Non Insulin Dependent Diabetes Mellitus (NIDDM) is associated with a much higher prevalence (about 2-4 folds) of coronary heart disease and ictus cerebri, compared with health subjects. In elderly people affected by NIDDM, duration of the disease and glucose serum levels are the main predictive factors for cerebro-cardiovascular disease. We studied the influence of lipoprotein (a) [Lp(a)], an indipendent risk factor for vascular disease, in NIDDM patients either affected or not affected by ictus cerebri. Seventy NIDDM patients (34 males and 36 females; mean age 72 4 ± 8.78 years) have been assessed and divided into two groups: group A included 36 NIDDM subjects (20 males, 16 females; mean age 72.44 ± 3.63 years; mean duration of the disease 8.66 ± 7.34 years), while group B was composed of 34 NIDDM (14 males and 20 females; mean age 72.5 ± 12.14 years; mean duration of the disease 20.29 ± 9.95 years) affected by ictus cerebri. The following parameters have been evaluated in all subjects: glycaemia, total and HDL cholesterol levels, trygliceride values, glycosilate hemoglobin levels. Statistical analysis was performed using Student's t test. Trygliceride and Lp(a) serum values were analyzed by Wilcoxon's non parametric test. Bivariate regression analysis was applied to the extrapolated data. Patients included in the group B presented higher glycaemia and glycosylated hemoglobin serum levels and more altered lipid pattern than group A subjects. No significant differences in Lp(a) serum levels were observed between these two groups. However, there was a significant positive correlation of Lp(a) levels with glycosilate hemoglobin in the group B. On the basis of this finding we postulated that glycosilation of Lp(a) may enhance its atehrogenic potential.

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Relationship between postprandial triglyceride (TG) response and metabolic vascular risk factors in healthy subjects.

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The postprandial (PP) TG response has been shown to be increased in coronary artery disease patients and in type II diabetics. A higher PP TG response could be related to a complex metabolic disorder including insulin resistance. We studied 50 normolipemic subjects (TC levels < 240 and TG < 200 mg/dl) aged 20-60 (31 w and 19 m) without any history of diabetes, hypertension or vascular disease, before, and 2, 5, and 8 hours after a standardized mixed meal (1145 kcal, 53 g/lipids). Subjects in the highest quartile of the PPTG response (calculated as incremental PP area), showed a trend to higher age, BMI, glycemia and TC/HDL-C ratio as well as a lower glycemia/insulin ratio, when compared with subjects in the other 3 quartiles, but only differences in age were statistically significant ($p = 0.002$). When analyzing the quartiles of the fasting TG distribution the same not significant trends were observed. In contrast, subjects in the highest quartile of the 8th hour postprandial TG value had significantly lower HDL-C ($p = 0.01$) and higher TC/HDL-C ratio ($p = 0.001$). Also, these subjects showed abnormal mean fasting insulin levels and glucose/insulin ratio (20.6 mUI/L and 4.66 respectively). Median Q1-Q3 8th h PP TG values were respectively 98, 72 and 147 mg/dl. A low late postprandial TG value may indicate both normal TG rich lipoprotein production and clearance. In contrast, we suggest that a high value could, better than the fasting TG, discriminate subjects with metabolic abnormalities that increase the vascular risk. Glucose tolerance and fibrinolytic capacity in these groups is currently under analysis.

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LIPOPRROTEIN (a) LEVELS AND GLYCEMIC CONTROL IN NIDDM SUBJECTS.

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We examined the effects of 6 and 12-months improved glycemic control on Lp(a) concentrations in 32 poorly controlled NIDDM subjects. 6 subjects were treated by α-glucosidase inhibitor, 8 by insulin and 18 by sulfonyl urea. LP(a) was measured by monoclonal anti-LP(a) antibody technique, HbA1c by HPLC and Fructosamine by calorimetric method Results are shown by mean±SE.

※<0.01, ※※<0.05 compared with pre-treatment values.

		Before	6 months	12 months
FPG	(mg/dl)	241±17	139±5※	139±6※
HbA1c	(%)	10.3±0.4	6.8±0.3※	6.9±0.2※
FRA	(mM/L)	381±14	287±10※	304±12※
Lp(a)	(mg/dl)	28.9±3.2	20.5±2.7※	18.4±1.9※
T-chol	(mg/dl)	211±8	193±7※※	199±8
HDL-chol	(mg/dl)	50±4	50±4	47±4
TG	(mg/dl)	128±13	107±10	130±12
Apo A1	(mg/dl)	116±4	127±5	138±5
B	(mg/dl)	100±5	93±5	106±7
E	(mg/dl)	5.1±0.3	4.7±0.3	5.9±0.4※※

LP(a) concentrations did not differ among subjects treated by α-glucosidase inhibitor, insulin and sulfonyl urea. We conclude that in NIDDM subjects, LP(a) concentrations are related to the degree of glycemic control.

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PREVALENCE OF LARGE VESSEL DISEASES AMONG ADULT DIABETICS IN SOUTHERN TAIWAN-POPULATION BASED STUDY

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In a population-based study in Southern Taiwan during the years 1995-1996, 1200 subjects aged 20 years or older were screened for diabetes mellitus. Response rate was 80%. The prevalence of diabetes melllitus was 9.9%. Diabetics had higher prevalence of large vessel diseases than nondiabetics. ($P<0.05$) Prevalence of ischemic heart disease (IHD), leg vessel disease (Leg VD) and stroke were 22.8, 2.0 and 5.1% for diabetics, and 11.8, 1.6 and 1.5% for non-diabetics, respectively. Selected cardiovascular variables in diabetics (non-diabetics) were systolic blood pressure (SBP) 138.2±26.3 (114.8±23.4) mmHg, diastolic blood pressure (DBP) 78.5±10.8 (60.0±13.1) mmHg, body mass index (BMI) 26.1±3.5 (23.3±3.5) kg/m^2 and high density lipoprotein cholesterol (HDL-C) 44.9±13.8(50.6±13.4) mg/dl. Diabetics had higher SBP, DBP, BMI but lower HDL-C concentrations than non-diabetics. ($P<0.05$) Conclusions: 1. Compared with western NIDDM, the prevalence of large vessel disease were lower among our patients. 2. Diabetics had higher prevalence of large vessel disease than non-diabetics. 3. Diabetics had high blood pressure, body mass index but lower HDL-C levels than non-diabetics.

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PROSPECTIVE STUDY OF LIPOPROTEIN(a) AND THE RISK OF MYOCARDIAL INFARCTION IN NIDDM WITHOUT VASCULAR COMPLICATIONS

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To assess whether lipoprotein(a) [Lp(a)] is a risk factor for macroangiopathy in non-insulin dependent diabetic patients (NIDDM) without any diabetic complications and they had neither history nor sings or symptoms of cardiovascular and cerebrovascular disease. We studied 56 NIDDM without diabetic complications who were followed for 5~6 years. The serum Lp(a) levels were measured by ELISA. The distributions of Lp(a) in the subjects were identical to that of controls, while the median Lp(a) levels in the subjects were significantly higher than that of control(23.6mg/dl vs 13.0mg/dl; $p<0.05$). The subjects were divided two groups, one(N=36) had low Lp(a) levels(below 20mg/dl) and other(N=30) had high Lp(a) levels (above 20mg/dl). There were no significant differences between the two groups with respect to age, HbA1c, BMI, cholesterol and uric acid. Of the 30 patients with high Lp(a), 4 experienced a myocardial infarction(MI), while 2 of 36 patients with low Lp(a) had same episode. There were no significant differences in HbA1c, cholesterol, triglyceride values between NIDDM who developed incident MI events compared with who did not. Of the 30 patients with high Lp(a), 2 experienced a cerebral infarction(CI), while 4 of 36 patients with low Lp(a) had same episode. There were no significant differences in HbA1c, cholesterol, triglyceride values except blood pressure between NIDDM who developed incident CI compared with who did not. We founded Lp(a) is a significant risk factor for cardiovascular disease but is not a risk factor for cerebrovascular disease.

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HYPERLIPIDEMIA AND OBESITY AMONG DIABETICS AT JUBAIL MILITARY HOSPITAL

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OBJECTIVE Evaluate the pattern of dyslipidemia among diabetics, attending the Primary care clinic.
DESIGN: Retrospective study.
SETTING: Primary health care department of the Armed forces hospital at King Abdul Aziz Naval Base, Jubail, Eastern Saudi Arabia.
SUBJECTS: All diabetics registered and followed up at the Primary Care clinic.
METHODS: 282 diabetics were registered in this clinic and interviewed by Primary care doctors. Weight and height were recorded, fasting blood glucose level and cholesterol level were measured after 12 h fasting.
RESULTS: The study revealed that among 90% NIDDM and 10% IDDM. 22.7% were having a normal Body Mass Index (BMI) 40% overweight, and 36.5% obese. Females had a significantly higher BMI than males (P< 0.005).Total cholesterol was more than normal in 26%, LDL in 27% and Triglyceride in 11% but HDL lower than normal in 38 %.. However mixed hyperlipidemia was seen in (5%) of the study population.
CONCLUSION: Obesity was high among diabetic patients and require special attention as well as dyslipidemia.

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ASSOCIATION OF APOPROTEIN E POLYMORPHISM ON LIPOPROTEIN (a) LEVELS IN TYPE 2 DIABETIC PATIENTS.

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Apolipoprotein E (apoE) polymorphism is known to modulate cholesterol and triglycerides serum levels in diabetic as well as in non diabetic subjects. However little is known concerning its possible influence on Lp(a). In a groupe of 406 NIDDM patients aged 59.48 ± 10.78 years, with a BMI of 28.89 ± 5.34 kg/m2 and HbA1c=8.23 ± 1.88%, Lp(a)=21.19 ± 32.0 mg/dl with a median value of 81 mg/l, we determined 6 apoE genotypes. Patients were separated in 3 groups (A:2/2 and 2/3), (B:3/3), and (C:3/4 and 4/4) with 231 males (respectively A=37, B=136, C=57) and 175 females (A=30, B=113, C=29) and compared according to their Lp(a) serum levels assayed by immunonephelometry. Increasing significant differences were found between A and B (8.8 ± 15.3 vs 19.6 ± 26.0mg/dl, p<0.01), A and C (8.8 ± 15.3 vs 20.6 ± 27.0, p<0.02) in males, in females differences were found between A and C (14.5 ± 16.2 vs 50.6 ± 71.6mg/dl, p<0.05). These data were confirmed after logarithmic transformation of Lp(a). Thus apoE polymorphism seems to be responsible for a significant modulation of Lp(a) plasma levels although differently in male and female diabetic patients. This phenomon could be of interest in the understanding of Lp(a) catabolism and may be of importance in the comprehension of macroangiopathic complications.

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INSULIN RESISTANCE AND DYSLIPIDEMIA IN HEMODIALYSIS PATIENTS

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Dyslipidemia and insulin resistance are commom among patients with end-stane renal failure. The aim of this study was to evaluate lipid and lipoprotein profile, decreased tissue insulin sensitivity /expressed as fasting plasma insulin>18 mU/L/, and to investigate the role of insulin resistance on lipid levels in hemodialysis patients. We studied 39 patients /24F,15M/, 20 with decreased tissue insulin sensitivity /fasting insulin 29+8,2 mU/L/ and 19 with better tissue insulin sensitivity /fasting insulin 9,7± 4,6/, with similar age and duration ofhemodialysis. First group of patients had higher fasting glucose levels /5,4± 0,7 vs 5,1±0,6 mmol/L,p=0.05/, fasting triglyceride levels /2,7±1,6 vs 2,1±1,0,p<0,05/. Fasting apolipoprotein AI was lower in the first group /1,3+0,2 vs 1,7+0,3,p< 0,05/, but there were no differences in fasting apolipoprotein B100,HDL-ch,LDL-ch,Lp/a/, between two groups. There were significant positiv correlation between fasting insulin and triglyceride levels /r=0.580,p<0,05/,significant negativ correlation between insulin and HDL-ch /r=-0.489, p<0,05/ and fasting insulin and Apo A1 /r=-0.410,p<0.05/, in the first group only. In the second group there was significant negativ correlation between triglyceride and HDL-ch/r=-0,391,p<0,05/ only. We concluded thatdecreased tissue insulin sensitivity is associated with hipertriglyceridemia and lower Apo A1 and HDL-ch, in our hemodialysis patients, as in other insulin resistant statments.

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INDEPENDENT CORRELATION BETWEEN LIPOPROTEIN (a) AND ANGIOGRAPHIC CORONARY ARTERY DISEASE IN NIDDM.

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Lipoprotein (a) (Lpa) is an established risk factor for Coronary Artery disease (CAD) in non-diabetic subjects. The correlation between serum levels of Lpa in NIDDM patients with CAD is not yet clearly established. One of the best methods for the assessment of the severity and extension of CAD is coronary angiography (CA). We examined the correlation between serum levels of Lpa and CAD in NIDDM. 43 patients with NIDDM with CAD participated in this study. Mean age 59.7±5.7, Mean BMI 27.789±3.41, 60% suffered from hypertension (A.H.) and 75% were smokers.
Patients under lipid lowering therapy and patients with albuminuria were excluded. The estimation of the degree of stenosis and obstruction in the 11 segments of coronary artery tree (in CA) was made according to the Gensini Score (G.S.). We also measured the ejection fraction (E.F.), total serum levels of cholesterol, triglycerides, fractions of cholesterol, apolipoproteins A and B, Lp(a) [Lp(a) was measured with the immunoturbidimetric method], HBA_{1C} and fibrinogen.
The mean value of Lpa was 37.69±29.74 mg/dl (range 9-132), mean GS 55.72±41.88 (range 4-168). The bottom tertile of GS (range 4-18) had Lp(a) 18.18±7.77 and the upper tertile of GS (range 80-168) had Lpa 51±49.05
The multifactorial analyses between G.S. and all the other parameters indicated statistically significant correlation between GS and Lp(a) (r=0.374, p<0.01), serum level of fibrinogen and GS (r=0.425, p<0.005), serum level of LDL cholesterol and GS (r=0.291, p<0.05).
There was also statistically significant correlation between E.F. and Lp(a) (r=-0.339, p<0.025) and E.F. and fibrinogen (r=-0.345, p<0.02).We concluded that there is significant and independent correlation between serum levels of Lp(a) and the severity and extention of C.A.D. in patients with NIDDM.

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INSULIN INSENSITIVITY, MINIMAL MODELLING AND HEPATIC LIPASE ACTIVITY IN THE DYSLIPIDAEMIA OF TYPE 2 DIABETES

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NIDDM is commonly associated with insulin resistance and dyslipidaemia characterised by hypertriglyceridaemia and low HDL. Previous studies of insulin insensitivity have employed insulin measurements by conventional radioimmunoassay. We investigated insulin action and secretion in relation to hepatic lipase activity in 23 NIDDM subjects (median age 58 (IQR 46-65) years, BMI 25.0 (24.1-29.0) kg $\cdot m^{-2}$) using an insulin-modified frequently sampled intravenous glucose tolerance test. Specific insulin was measured by ELISA , minimal modelling was used to measure insulin sensitivity (Si) and glucose effectiveness (Sg). Hepatic lipase (HL) activity was measured in postheparin plasma. Si correlated negatively with BMI (r=–0.46, p<0.05), Sg (r=–0 52, p<0.05) and with fasting triglyceride (r=–0.71, p<0.001) but not with total cholesterol nor HDL-cholesterol HL correlated positively with triglyceride (r=0.43, p<0.05) and negatively with HDL-cholesterol (r=–0.46, p<0.05) and glucose tolerance (r=–0.42, p<0.05) but not with fasting glucose, Si or Sg. There was a negative corelation between HL and glucose tolerance (r=–0.42, p<0.05). Fasting triglyceride was positively correlated with fasting insulin (r=0.50, p<0.05) and first phase insulin secretion (r=0.46, p<0.05). Using a specific insulin assay, we have shown that hypertriglyceridaemia is closely associated with insulin insensitivity and increased hepatic lipase activity which may in turn lower HDL-cholesterol.

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LIPOPROTEIN(a) LEVEL AND VASCULAR COMPLICATIONS IN NON-INSULIN DEPENDENT DIABETES MELLITUS

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Lp(a) has been shown to be an independent risk factor for atherosclerotic vascular disease in subjects without diabetes. But, in patients with NIDDM, Lp(a) levels have been reported to be normal or increased. In this study, we examine the following issues : the relationship between Lp(a) levels and development of micro- and macrovascular complications and the relationship between Lp(a) and other risk factors of vascular complications in patients with NIDDM. For determination of Lp(a), $ApoA_1$, $ApoB_{100}$, triglyceride, total- and HDL-cholesterol, blood samples were collected after an overnight fast from ninety-five patients with NIDDM (41 women and 54 men). Lp(a) levels in patients with diabetic retinopathy (n=41) were significantly higher (53.6±7.9 vs 27.5±5.3mg/dL, p=0.008) than those in patients without retinopathy (n=33). And also Lp(a) levels in patients with macrovascular complication(s) (n=21) were significantly higher (52.3±11.0 vs 32.2±4.3mg/dL, p=0.047) than those in patients without macrovascular complication(s) (n=71). $ApoB_{100}/ApoA_1$ ratio in patients with Lp(a) level≥30mg/dL (n=34) were significantly higher (1.20±0.09 vs 0.86±0.04, p=0.002) than that in patients with Lp(a) level<30mg/dL (n=59). LDL-cholesterol levels in patients with Lp(a) level≥30mg/dL (n=34) were significantly higher (131.5±9.37 vs 108.1 ±5.24mg/dL, p=0.020) than those in patients with Lp(a) level<30mg/dL (n=59). And Lp(a) levels were not related with sex, age, body mass index, HbA_1c, smoking history and 24-hour urine protein amount. These results suggest that Lp(a) levels seem to be associated with microvascular and macrovascular complications in patients with NIDDM.

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RELATIONSHIP BETWEEN INSULIN RESISTANCE AND ABNORMAL LIPID PROFILE IN GREEK OBESE ADOLESCENTS

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The present investigation was undertaken to identily metabolic variables in childhood which may reflect independent atherogenic risk factor in obese pediatric population. Anthropometric data reflecting overweight, fat distribution were evaluated in 41 obese adolescents and concentrations of apolipoproteins and lipids, IGF-1, C-peptide were selected. Insulin resistance was assessed by fasting insulin level and sum of the insulin values during 2 hours, after an O.G.T.T. in all subjects and compared with 27 lean age-matched normal children. Insuline level was determined by RIA. Results from the two groups were ajusted for puberty, age and sex and were correlated with multivariate analysis, Lipids and lipoprotein evaluated used z=x-x´/SD. In obese vs non-obese mean value/SD for age (11.78 vs 10.95), Bone age (11,98 vs 9,18) BMI (31,40 vs 18,76 kg/m^2) W/h (0,80 vs 0,77) Results: In obese vs nonobese mean value HDL (42,6±15,84 vs 44,02±15,3) LDL (98,57±27,34 vs 95.89±40,04), CHOL (166,69±24,39 vs 152,84±40,04), TRYG (106,35±45,14 vs 76,56±41,4), Lpa (31,6±36.77 vs 29,92±37,0), IGF1 (389,85±227,26 vs 269,52±175,97). The obese had significant higher values IGF1 P<0,011, Tryg, P<0.04, APO-B, P<0,027 and T-CHOL, P<0,05. Mean fasting-insuline-value (29,54 IU/L±SD 13,89 vs noobese (10,11 IU/L±SD 7,73, P<0.01) and sum insulin 489,3 IU/L±SD 296,6 vs 208,55 IU/L±SD 178,1, P<0,01). Fasting-insulin-levels indepentendly of age and BMI were significantly correlated with CHOL (r=0,53, P<0,0005) Lpa (r=0,498, P<0,008) and 2h insulin with LDL (r=0,35, P<0,028) and TRYG (r=0,457, P<0,009). There was a significant difference between obese and nonobese boys and girls regarding HDL and insulinaemia. HDL was positively correlated to fasting and 2-h ins: in nonobese boys. (r_z=0,41, P<0,053, r_z=0,35, P<0,053) and girls (r_z=0.5, P<0,028, 2h r_z=0,39, P<0,07) and negatively related to obese boys (r_z=-0,53, P<0,35, 2h r_z=-0,49, P<0,043) and girls (r_z=-0,46, P<0,06 and 2h r_z=-0,37, P<0,092). **CONCLUSION:** Insulin resistance in obese Greek young subjects is the key of atherogenic metabolic abnormality.

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LP(a) PHENOTYPES IN JAPANESE NIDDM

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In this study, we determined Lp(a) concentrations and phenotypes in group of 121 randomized NIDDM patients under 65 y.o. and 200 controls to investigate differences of apo(a) isoform between patients and controls, and relationship between apo(a) isoform and diabetic nephropathy. Lp(a) phenotyping was performed by using SDS PAGE and classified according to Utermann's classification. Serum Lp(a) concentrations were 18.4±1.4 mg/dl (mean± SE) in the patients and 14.7±1.2 mg/dl in controls (p<0.05,t-test). Lp(a) phenotype frequencies in the patients and controls were significantly different (p<0.0001,x^2-test). F:0 (0)%, B:0 (0.5)%, S1:0.8 (2.5)%, S2:4.1 (4.5)%, S3:43.0 (28.5)%, S4:12.4 (40.5)%, O:3.3 (5.5)%, F/S4:0 (0.5)%, B/S3:0.8 (0.5)%, S1/S3:4.1 (1.0)%, S1/S4:1.7 (1.5)% S2/S3:9.9 (4.5)%, S2/S4:3.3 (5.0)%, S3/S4:16.5 (5.0)% ;[patients (controls)]. We classified NIDDM patients to 3 groups by qualitative urinary protein (group A:(-) n=91, group B:(±)and(+) n=22, and group C:(2+)and(3+) n=8). Each serum Lp(a) concentrations were 17.0±1.6, 19.4±2.7, and 31.6±8.8 mg/dl (p<0.05:group A vs. Group C). Lp(a) phenotype frequency of group C was S3:n=5, S2:n=1, S1/S3:n=1,and B/S3:n=1. That frequency was significantly different to group A (p<0.05,x^2-test). These data suggest that NIDDM patiens have another apo(a) isoform frequency than non-diabetics, and apo(a) isoform relate with aggravation of diabetic nephropathy.

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ROLE OF BODY WEIGHT AND GLYCAEMIC CONTROL IN THE DYSLIPIDAEMIA OF NON-INSULIN DEPENDENT DIABETES MELLITUS.

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Dyslipidaemia, in particular raised serum triglyceride and low HDL cholesterol, is characteristic of patients with non-insulin-dependent diabetes mellitus (NIDDM). Controlled clinical trials under strict laboratory conditions have demonstrated that improved glycaemic control can also improve lipid values. In fact, the National Cholesterol Education Panel has stated that control of hyperglycaemia is the key to improving the lipoprotein pattern in diabetes. In this study we aimed to determine whether improved glycaemic control under routine clinical conditions significantly impacted on lipid values in a diabetic out-patient population.

260 patients with NIDDM (151M, 109F, age 63 ± 11 yr), who were not taking lipid-lowering therapy were followed for 12 months with measures of fasting serum lipids, glycaemic control and body weight. During this time, 21 patients were commenced on lipid-lowering therapy leaving 239 patients who formed the study group. Overall there was no change of total cholesterol (5.64 ± 1.25 vs 5.59 ± 1.12mM), triglyceride (2.37 ± 2.70 vs 2.22 ± 1.74mM), serum glucose (9.6 ± 3.3 vs 9.4 ± 3.1mM) or HbA1c (7.3 ± 1.75 vs 7.16 ± 1.52mM). Patients whose HBA1c improved (8.39 ± 1.99 vs 6.60 ± 1.37%:P<0.001) had a significant albeit small decrease of total cholesterol (5.83 ± 1.48 vs 5.54 ± 1.23mM, p<0.01), with no change in HDL cholesterol or serum triglyceride. Conversely subjects whose BMI was significantly lower at follow up (28.9 ± 5.1 vs 27.5 ± 4.8kg/m2) had a significant decrease of serum triglyceride (2.53 ± 2.28 vs 2.09 ± 1.64mM; p <0.01), and increase of HDL cholesterol (1.10 ± 0.28 vs 1.17 ± 0.33mM, p<0.01). The major factor determining change of triglyceride and HDL cholesterol was the change of body weight (p< 0.001 and p<0.01 respectively), while the change of fasting glucose levels predicted the change of serum cholesterol (p< 0.01). We conclude that improved glycaemic control in the absence of weight reduction is insufficient to produce a major change of the abnormal lipoprotein pattern of NIDDM. Consideration should therefore be given to the early introduction of lipid-lowering therapy in patients with NIDDM, even those whose glycaemic control is suboptimal.

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LP(a) AND OTHER CARDIOVASCULAR RISK FACTORS IN CHINESE NIDDM PATIENTS WITH AND WITHOUT ALBUMINURIA

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Lp(a) is a recognized risk factor for cardiovascular diseases. Its concentrations are increased in IDDM patients with proteinuria. However, it remains unresolved whether levels are elevated in NIDDM. To address this issue and other cardiovascular risk factors in these patients, we have evaluated 164 Chinese NIDDM newly referred to the Diabetes Specialist Clinic, which serves as a tertiary referral centre to the region. Data are expressed as mean ± SEM, with the exception of Lp(a) and triglyceride which have a skewed distribution and are thus expressed as geometric mean x/÷ antilog SEM. Diabetic patients had Lp(a) concentrations comparable to 132 normal controls (164.7 x/÷ 1.1 mg/L Vs 148.4 x/÷ 1.1 mg /L). Among diabetics, 43 % had abnormal albuminuria as defined by albumin:creatinine ratio of ≥3.5 mg/mmol.. There was no significant difference in the Lp(a) values between the albuminuric and normoalbuminuric group (165.9 x/÷ 1.1 mg/L Vs 175.9. x/÷ 1.1 mg/L). Albuminuric patients had significantly higher fasting glucose (10.9 ± 0.6 mmol/L Vs 9.1 ± 0.4 mmol/L, p<0.01) and HbA1c (9.2 ± 0.3 % Vs 7.8 ± 0.3 %, p=0.001) than normoalbuminuric patients. They also had higher systolic BP (145 ± 4 mmHg Vs 133 ± 4 mmHg, p<0.05) and mean BP (123 ± 3 mmHg Vs 113 ± 3 mmHg, p<0.05). The prevalence of hypertension (systolic BP≥140 mmHg or diastolic BP ≥90 mmHg) was also significantly greater in the albuminuric group (68% Vs 27 %, p=0.001). Albuminuric and normoalbuminuric patients, however, had comparable BMI (24.5 ± 1.4 Kg/m^2 Vs 24.9 ± 0.6 Kg/m^2), waist:hip ratio (0.93 ± 0.01 Vs 0.92 ± 0.02), total cholesterol (5.83 ± 0.17 mmol/L Vs 5.67 ± 0.15 mmo/L), LDL-C (3.70 ± 0.15 mmol/L Vs 3.63 ± 0.12 mmol/L), HDL-C (1.27 ± 0.05 mmol/L Vs 1.33 ± 0.05 mmol/L) and triglyceride (1.62 x/÷ 1.1 mmol/L Vs 1.43 x/÷ 1.1 mmol/L). In conclusion, neither NIDDM nor development of diabetic nephropathy significantly effects Lp(a) concentrations in these Chinese subjects. In line with previous studies, albuminuric patients are at greater risk of cardiovascular disease as evidenced by their poorer glycaemic control and elevated blood pressure.

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INFLUENCE OF BODY FAT AND ITS REGIONAL DISTRIBUTION ON PLASMA LIPIDS AND GLYCAEMIC CONTROL IN NIDDM.

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Aim of the present study was to evaluate whether the presence of obesity and a central pattern of fat distribution are significantly associated with plasma lipid profile and glycemic control in patients with NIDDM. We have examined 1,639 noninsulin-treated patients with NIDDM (aged 31-91 yrs), randomly selected from those attending the Diabetes Centers of Verona and sorroundings. Patients were grouped in quintiles of either body mass index (BMI) or waist/hip ratio (WHR). Plasma levels of triglycerides, total and LDL-cholesterol significantly increased across the quintiles of BMI (p<0.001), while HDL-cholesterol significantly decreased (p<0.001). However, when allowance was made for sex, age, WHR, fasting insulin concentration, diabetes duration, treatment (diet vs oral agents), and inverse of creatinine, only total cholesterol (p=0.02) and LDL-cholesterol (p<0.01) levels retained a significant trend to increase with the increase of BMI. Plasma levels of triglycerides (p<0.001) increased, and HDL cholesterol (p<0.05) decreased across the quintiles of WHR, but these trends were no longer statistically significant after adjusting for BMI and all other potential confounders. Glycated hemoglobin (HbA_{1c}) did not change significantly with the increase of either BMI or WHR. These results suggest that in noninsulin-treated patients with NIDDM, an excess of body fat, irrespective of its regional distribution, is independently associated with a more atherogenic plasma lipid profile. On the contrary, neither an excess of body fat nor a central pattern of fat distribution are independently associated with a worse glycaemic control.

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LIPOPROTEIN (a) IN URBAN SOUTH INDIAN NIDDM PATIENTS WITH ISCHAEMIC HEART DISEASE

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High rates of ischaemic heart disease (IHD) occuring at a younger age have been well documented in several migrant Asian Indian populations. Recently elevated Lipoprotein (a) (Lp-a) levels have been shown to be associated with IHD in Asian Indians. However there is no data on Lp (a) levels within the Indian subcontinent. We compared the levels of Lp(a) in NIDDM patients with and without IHD and non-diabetic control subjects without IHD (n=16 in each group). The mean Lp(a) levels were significantly higher (P<0.001) in NIDDM with IHD (55.4 ± 34.1 mg/dl) compared to NIDDM without IHD (24.2 ± 23.9 mg/dl) and control subjects (24.4 ± 19.5 mg/dl). Thirteen of the 16 NIDDM with IHD (81.3%) compared to 4/16 (25%) of NIDDM without IHD and 5/16 (31.3%) of the control group had Lp(a) values above 30 mg/dl. Lp(a) levels showed a much stronger correlation with IHD than conventional lipid markers like total cholesterol, LDL, VLDL, and HDL cholesterol and serum triglyceride. Lp(a) appears to be a very good marker of IHD in South Indian NIDDM patients.

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OVERWEIGHT AND OBESITY. 1985- 1995. PATHOLOGIC ENTITIES ASSOCIATED

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We wanted to establish the frequency of association between lipid disorders, arterial hypertension, diabetes mellitus and impaired oral glucosa test in a group of patients with overweight and obesity. We realized a descriptive study with the information of 604 patients above 19 years old with different degrees of obesity. Variables of study were: arterial hypertension (values ≥ 140/90), lipid disorder (cholesterol and tryglicerids ≥ 200 mg/dL), diabetes mellitus (basal glucemia ≥ 140 mg/dL or ≥ 200 mg/dL at any time of the day and two hours later of realizing glucose oral tolerance test) and impaired oral glucose test (values between 140 and 199 mg/dL two hours later of the test). We used univariate analysis and Epi-Info software. We found lipid disorders in 96.96% of the total group, 96.32% in women and 100% in men (p NS). Arterial hypertension was observed in 39.36% of total group, 37.02% in women and 53.65% in men (p < 0.01). Diabetes mellitus was present in 42.69% of total group, with 39.52% in women and 63% in men (p < 0.01). Finally, we observed an impaired oral glucose test in 22.28%, 22.97% in women and 16.66% in men (p NS). Data collected corroborate the enormous impact of this group of pathologies in morbimortality of patients with overweight and obesity, inside the concept of plurimetabolic syndrome. It is necessary to realize analytical studies to determine the factors that explain the differences observed.

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RELATION OF LIPOPROTEIN(A) AND DEVELOPMENT OF DIABETIC MICROANGIOPATHY

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Lipoprotein(a)(Lp(a)) has been reported to be related to atherosclerotic vasucular diseases. Many studies have indicated that the serum levels of Lp(a) in diabetic patients are elevated to be associated with higher incidence of macroangiopathy such as ishemic heart diseases. In this study we investigated the relation between Lp(a) and development of microangiopathy in 120 NIDDM patients(69.4 ± 0.7 y.o.:M ± SE)followed for more than 10 years. In 79 patients without retinopaty at the beginning of the observation , 45 patients remained unchanged in no-retinopathy(NDR) but 26 patients finally worsened to simple retinopathy(SDR) and 8 patients to pre-or proliferative retinopathy(PDR) .Though 74 out of 109 patients without proteinuria at the start of follow－up showed normoalbminuria(NOR) in the end, 37 and 8 patients were developed to microalbuminuria (30 ～ 300mg/gcr.)(MIC)and macroalbuminuria(MAC) , respectively. Lp(a) levels were 25.2±3.0mg/dl in NDR, 22.3±3.4 mg/dl in SDR , 29.8±7.4 mg/dl in PDR. There were no significant differences of Lp(a) levels among three different diabetic retinopathy stages. On the other hand , the serum levels of Lp(a) were 23.0±2.4 mg/dl in NOR , 27.9± 3.8mg/dl in MIC, 46.3± 14.2mg/dl in MAC. There existed significant differnces in serum Lp(a) concentrations among three stages of diabetic nephropathy. In conclusion the risk of macroangiopathy must be much more taken into consideration because of elevated serum Lp(a) levels in diabetic nephropathy.

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CLINICAL SIGNIFICANCE OF ACCUMULATED VISCERAL FAT IN PATIENTS WITH NON-INSULIN DEPENDENT DIABETES MELLITUS.

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It is generally recognized that the accumualtion of visceral fat is closely associated with hypertension, lipid metabolism disorder and impaired glucose tolerance, and also it is one of important factors to develop the atherosclerosis. We challenged to elucidate whether the accumulation of visceral fat would be involved in diabetic complications in patients with non-insulin dependent diabetes mellitus (NIDDM) or not. Studies were performed in 60 NIDDM patients (31 men, 29 women, 54.1 ± 2.1 (mean ± SE) y-o) and 490 healthy subjects. The thicknesses of the preperitoneal fat layer (P-fat) as visceral fat and subcutaneous fat layer (S-fat) were measured using ultrasonography method by Sizuki et al. and then the relationships between those fats and the metabolic markers or the grade of diabetic complications were analysed. S-fat in female remarkably increased that in male in all subjects. P-fat in NIDDM patients significantly increased compared to that in healthy subjects. P-fat in patients treated with insulin were significantly reduced compared to that with diet alone. No significant relathionship was found between both fats and metabolic markers such as FPG and HbA_{1c}. A significantly positive correlation was shown P-fat with MNCV in male, and also P-fat in female showed similar results with SNCV and R-R time interval variation. The tendency of negative correlations were obtained between both fats and urinary albumin excretion. P-fat in NIDDM patients with proliferative retinopathy was markedly reduced compared to that in those in no retinopathy. Daily urinary CPR values revealed a significantly positive correlation with P-fat.

Conclusions: The present data suggest that visceral fat would be a negative modulator against diabetic complications derived from microangiopathy and it might be regulated by the amount of insulin secretion from the pancreas.

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HAND AND SHOULDER ABNORMALITIES IN DIABETIC PATIENTS: ASSOCIATION WITH SERUM LIPIDS

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Hand and shoulder abnormalities have been shown to be common in conditions like diabetes and coronary heart disease, which are related to the lipid abnormalities. The aim of our study was to evaluate whether limited joint mobility (LJM), Dupuytren's disease (DD) and shoulder capsulitis (SC) are associated with lipid abnormalities in diabetic patients. We examined 291 type 1 (age±SD 33.2±10.0 years) and 139 type 2 (61.3±12.3 years) diabetic patients in terms of LJM, DD, SC, serum total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C) and triglycerides (TG). Differences were regarded significant at p-level <0.05. Prevalence of LJM, DD and SC were 58, 14 and 10% in type 1 and 60, 14 and 22% in type 2 diabetic patients, respectively. The mean TC concentrations were higher in type 1 diabetic subjects with moderate (5.5±1.4 mmol/L) and severe LJM (5.2±0.6 mmol/L) than in those without LJM (4.7±1.1 mmol/L). In type 2 patients HDL-C values were higher in subjects without LJM (1.3±0.4 mmol/L) than in subjects with moderate (0.9±0.3 mmol/L) or severe LJM (0.9±0.3 mmol/L). No significant association between LJM and serum TG concentration was found in type 1 or 2 patients. TC (5.4±1.4 vs. 4.8±1.1 mmol/L) and TG (1.5±1.2 vs. 1.0 ±0.6 mmol/L) concentrations were higher in type 1 diabetic subjects with than in those without DD, whereas no associations were found in type 2 diabetic subjects. No significant association between DD and HDL-C was found in type 1 or 2 diabetic subjects. The mean TC concentration was higher (5.5±0.7 vs. 4.8±1.2 mmol/L) in type 1 patients with SC than in those without, whereas no association was found in type 2 patients. No significant associations between SC and mean HDL-C or TG concentrations were found in type 1 or 2 diabetic subjects.

Conclusions: High TC and TG are associated with hand and shoulder abnormalities in type 1 diabetic subjects, whereas only LJM was associated with HDL-C in type 2 diabetic subjects.

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ATHEROGENIC SERUM FATTY ACID PROFILE IN TYPE 2 DIABETICS WITH HYPERTRIGLYCERIDEMIA

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Hypertriglyceridemia significantly accelerates the atherogenic risk in diabetics, especially in non-insulin dependent diabetes mellitus (NIDDM). There is growing evidence that beside serum lipids the process of atherogenesis is seriously influenced also by the abnormal serum and tissue fatty acids (FA) composition. The aim of the study was therefore to assess the changes in serum FA composition in NIDDM patients with diverse types of hyperlipidemia (HLP). The groups of NIDDM patients, treated with oral antidiabetic drugs, consisted of 21 subjects (10 men) with normal serum lipids (DM-NLP), 11 cases (4 men) with hypercholesterolemia (DM-CH), 43 (14 men) with hypertriglyceridemia (DM-TG) and 39 patients (16 men) with combined hypercholesterolemia and hypertriglyceridemia (DM-HLP). 21 (10 men) age-matched healthy control (C) subjects were also investigated. Serum lipid levels and glycemia were determined with the aid of an automatic biochemical analyzer. Fatty acid analysis of total serum lipids was carried out by gas chromatography using a capillary column with a highly polar stationary phase. The statistical significance of differences among the groups were evaluated with the ANOVA test. There were no differences between the groups in the age of subjects (mean±SEM 63.4±0.7 yrs) and in the duration of diabetes (10.1±0.43 yrs). The significantly different serum FA composition was found in DM-TG and DM-HLP groups compared to others. In particular, the proportion (wt%) of total saturated FA increased compared to that of the C (C: 30.48±0.49 vs DM-TG: 32.60±0.34, DM-HLP: 32.74±0.34; P<0.01). They had also a higher content of total monounsaturated FA in comparison to other groups (C: 27.67±0.44, DM-NLP: 27.67±0.76, DM-CH: 27.53±1.09 vs DM-TG: 30.80±0.44, DM-HLP: 31.84±0.59; P<0.01), and lower content of total polyunsaturated FA of the omega-6 series (C: 37.90±0.58, DM-NLP: 37.23±1.07, DM-CH: 37.72±1.04 vs DM-TG: 32.49±0.52, DM-HLP: 31.56± 0.72; P<0.01). The latter was mainly due to a decreased content of C18:2n-6 in these groups (C: 27.71±0.34, DM-NLP: 27.19±0.94, DM-CH: 27.12±1.16 vs DM-TG: 23.05±0.45, DM-HLP: 23.07± 0.52; P<0.01). From the long chained omega-6 FA, a lower content of C20:3n-6 and C20:4n-6 in the DM-HLP group in comparison to normolipidemics was noted. The data on n-3 polyunsaturated FA revealed only a lower content of C18:3n-3 in diabetics when compared to controls. In conclusion, the increase of serum saturated FA level at the cost of polyunsaturated FA, especially those of n-6 serie, may together with higher level of serum triglycerides significantly contribute to the increased atherogenic risk commonly found in hypertriglyceridemic NIDDM patients.

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NON-DIETARY DETERMINANTS OF POSTPRANDIAL TRIGLYCERIDE VALUES IN NORMOTRIGLYCERIDEMIC TYPE II DIABETICS.

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In Type II diabetes, fasting hypertriglyceridemia has been shown to be a risk factor for cardiovascular disease. The aims of this study were to assess the determinants of postprandial triglyceride (TG) levels and the association between fasting TG levels and postprandial TG levels in normotriglyceridemic (fasting TG ≤ 2.2 mmol/l) subjects with Type II diabetes. We included 23 (11m/12f) euthyroid, diet or tablet-treated subjects, aged 61±7.9 years and with a BMI of 30.4±5.3 kg/m^2. The HbA1c was 6.0±1.3% and mean fasting TG levels ranged from 0.6 to 2.2 mmol/l with a median value of 1.5 mmol/l. All subjects performed self-monitoring of their TG values with an Accutrend GCT (Boehringer Mannheim, Mannheim, Germany). The following values were computed from their self-monitored TG (STG) data: A mean fasting (F-STG), mean bedtime (BT-STG) and a mean incremental area under the curve (A-STG). The laboratory measured fasting TG level correlated significantly with the F-STG (r =0.65, p=0.001) and with the BT-STG (r =0.54, p=0.011), but not with the A-STG. Waist-to-hip ratio (WHR) correlated significantly with BT-STG (r =0.55, p=0.01), but not with fasting TG, F-STG or A-STG. Thyroid stimulating hormone (TSH) correlated significantly with BT-STG (r =0.55, p=0.01) and A-STG (r =0.55, p=0.009), but not with fasting TG or F-STG. In conclusion, in normotriglyceridemic subjects with Type II diabetes the fasting TG levels correlate strongly with self-monitored fasting and bedtime TG values, but not with the incremental area under the curve. Body fat distribution (WHR) and TSH levels, even in these euthyroid subjects, were important determinants of postprandial TG levels. The clinical significance of these findings remains to be demonstrated.

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GENDER INFLUENCE ON LDL SUBFRACTION DISTRIBUTION IN TYPE II (NIDDM) DIABETIC PATIENTS.

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Predominance of small and dense LDL (phenotype B) is considered a risk factor for cardiovascular disease. We evaluated the influence of gender on LDL subfraction distribution in NIDDM patients before (HbA1c 9.8±2.0%) and after (HbA1c 5.9±1.0%) the optimization of glycemic control with insulin therapy. Thirty-three NIDDM (19 males, age 56.4±7.0 years, Body mass index (BMI) 26.4±3.5 kg/m^2; 14 postmenopausal females, age 63.9±11.4 years, BMI 25.2±3.5 Kg/m^2) and 25 age-sex-BMI matched control subjects were studied. Six LDL subfractions were isolated by density gradient ultracentrifugation and classified as the quotient LDL_1-LDL_3/LDL_4-LDL_6 (phenotype A >1.8, AB 1.1-1.8, B < 1.1). Differences were assessed by Mann-Whitney test. In NIDDM patients, during both stages of glycemic control, no differences between men and women were observed in the percentage of LDL subfractions and the phenotype. However, compared with non-diabetic women, NIDDM women showed a lower percentage of LDL_2 (31.0±3.6 vs 24.4±8.7%, p<0.05) and a higher percentage of LDL_5 (6.3±1.5 vs 10.0±4.8%, p<0.05) and LDL_6 (4.2±0.8 vs 6.1±2.3%, p<0.05) that resulted in a lower phenotype ratio (3.4±1.1 vs 2.4±1.7, p<0.05). No differences were observed between non-diabetic and NIDDM men during both stages of glycemic control. In conclusion, these findings may contribute to explain the loss of protection for cardiovascular disease in diabetic women.

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DIABETIC MICRO- AND MACROANGIOPATHY. HOMOCYSTEINE AND LDL-TRIGLYCERIDES COMPARED TO OTHER LIPOPROTEIN-RELATED RISK FACTORS

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We analysed lipids, lipoproteins and homocysteine in 200 female and male patients with type I and type II diabetes mellitus and in age-matched healthy controls. Blood samples were drawn after an overnight fasting period. In contrast to healthy controls, diabetic patients had higher HbA1c values and type II diabetics were overweighted. In female type II diabetics, serum triglycerides were significantly higher than in female controls (2.47 vs 1.58 mmol/L, $p < 0.05$); in contrast, the increase in triglycrides in male type II diabetics was not significant (2.79 vs 1.96 mmol/L). In both, female (f) and male (m) diabetic patients, HDL-cholesterol was significantly reduced compared to controls. In type II diabetic patients serum triglycerides as well as HDL-cholesterol correlated with the severity of the diabetic macroangiopathy. None of the standard serum lipid measurements correlated with microangiopathic lesions. However, LDL-triglycerides were significantly elevated in female and male diabetic patients with severe macroangiopathic (f: 0.49 vs 0.39; m: 0.42 vs 0.35 mmol/L) and microangiopathic lesions (f: 0.48 vs 0.41; m: 0,41 vs 0.33 mmol/L). Moderately elevated homocysteine levels were reported as a risk factor for peripheral vascular and premature coronary artery disease. In this study, however, diabetic patients showed significantly decreased homocysteine concentrations compared to healthy controls (f: 12 vs 15; m: 13 vs 17 μmol/L). In the group of diabetes patients, severity of macroangiopathic lesions correlated with increasing homocysteine levels (f: 15 vs 11; m: 15 vs 12 μmol/L); there was no correlation between homocysteine and microangiopathic lesions. We conclude that, in contrast to conventional lipid-related risk indicators, LDL-triglycerides could become a powerful predictor for microangiopathy in diabetic patients. The finding that homocysteine is generally lower in patients with impaired glucose tolerance should be accounted for if homocysteine is used to assess an individual's risk for atherosclerosis.

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CHANGES IN LDL_A AND LDL_B IN POORLY CONTROLLED PATIENTS WITH NON-INSULIN DEPENDENT DIABETES MELLITUS

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This study attempted to reveal atherogenesis specificity in NIDDM patients considering lipoprotein heterogenity and the strong evidence for direct involvement of LDL subpopulations in particular LDL_B subfraction's potential for involvement in atherosclerosis.
36 poorly controlled NIDDM patients (HbA_{1c} > 10%) were compared with 36 sex and age matched healthy control subjects. LDL subclasses were separated by sequential ultracentrifugation. ApoAI and ApoB were assayed using immunoturbidimetric tests, cholesterol (Chol) and triglyceride (TG) concentrations in plasma, lipoprotein-classes and LDL-subclasses using enzymatic methods. The comparative analysis applied between poorly controlled NIDDM patients and controls reveals: decrease in Chol-contents in LDL_A ($\Delta\%LDL_A$-Chol=-16.9, $p<0.05$) whereas in LDL_B was observed increase ($\Delta\%LDL_B$-Chol= 20.0, $p<0.05$); the increase in TG-contents was different for LDL_A ($\Delta\%LDL_A$-TG = 105.0, $p<0.001$) and LDL_B ($\Delta\%LDL_B$-TG = 56.2, $p<0.001$); the decrease in ApoB-contents is also different for LDL_A ($\Delta\%LDL_A$-ApoB = -47.8, $p<0.001$) and LDL_B ($\Delta\%LDL_B$-ApoB= -22.8, $p>0.05$). Significant changes in plasma TG, total Chol, HDL-Chol ApoAI and ApoB were also observed.
Conclusion: in poorly controlled NIDDM patients were found differences both in the size and Chol- and TG-contents of LDL_A- and LDL_B-subclasses.

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ATHEROGENIC LIPIDS AND LIPOPROTEINS ABNORMALITIES IN IDDM COMPLICATED BY EARLY AND LATE NEPHROPATHY.

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Assumption: Accordingly to epidemiological study in Warsaw (1995) nephropathy increases 9 - folds the risk of cardiovascular death in IDDM. This is much more than it could be explained by traditionally studied risk factors. The specific for diabetic nephropathy, quantitative and qualitative lipids and lipoproteins abnormalities could be considered as the possible explanation for this additional atherogenic risk. **The research aims were:** 1. to evaluate the plasma lipids composition - total cholesterol, VLDL - cholesterol, HDL - cholesterol, LDL - cholesterol, total triglycerides, triglycerides in VLDL, triglycerides in LDL, protein content of LDL; 2. to determine the oxidizability of isolated LDL (oxidation test in vitro). **3 groups of the IDDM were studied**: a. 20 patients with overt nephropathy; b. 15 patients with microalbuminuria; c. 20 patients without nephropathy (as control group). All patients were hospitalised for the study. **Results:** I. Statistically significant differences in plasma lipids were found: 1. in microalbuminurics vs controls - LDL cholesterol; 2. in proteinurics vs controls a) total cholesterol, b) LDL cholesterol, c) total triglycerides, d) triglycerides in VLDL; 3. in proteinurics vs microalbuminurics a) total cholesterol , b) total triglycerides, c) triglycerides in VLDL. II. Statistically significant differences in LDL oxidizability in vitro were found: 1. in proteinurics vs controls a) lag phase, 2. in all three groups - maximal rate of LDL oxidation. **Conclusion**: In microalbuminurics (pre-clinical nephropathy) and proteinurics (overt nephropathy) IDDM subjects similar atherogenic abnormalities of lipids and lipoproteins composition and oxidizability were found. This suggests, that atherogenic risk in diabetic nephropathy occurs very early and is not simply related to the degree of proteinuria.

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LDL oxidation in type 2 diabetic patients with microalbuminuria.

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The aim of this study was to evaluate if there is a relationship between LDL oxidation and microalbuminuria, that is considered an early marker of vascular involvement in type 2 diabetes. Twenty-four type 2 diabetics, 12 out of them microalbuminuric (MA+) and 12 normoalbuminuric (MA-), and 12 control subjects were selected. Microalbuminuria was defined as an urinary albumin excretion rate of 30-300 mg/24h. There was no difference in the three groups for age, gender, and blood pressure values. As index of in vivo LDL oxidation, we measured the percentage of LDL^- by ion-exchange HPLC. In vitro susceptibility to oxidation of LDL was evaluated following the kinetic of coniugated diene formation in presence of Cu++ ions (lag time). Metabolic control was significantly worse in diabetics MA+ in comparison with diabetics MA- (fructosamine 341±71 vs 279±53 mM/L; $p<0.02$). Diabetics MA+ had triglyceride level significantly higher in comparison with diabetics MA- (2.21± 1.01 vs 1.15± 0.39 mmoli/L, $p<0.01$) and controls (2.21± 1.01 vs 1.18± 0.61 mmoli/L, $p<0.01$). Diabetics MA+ had significantly higher percentage of LDL^- in comparison with diabetics MA- (5.2±1.7 % vs 3.1±1.2 %; $p<0.01$) and controls (5.2±1.7 % vs 2.34±1.03 %; $p<0.001$). LDL isolated from diabetics MA+ had a significantly shorter lag time in comparison with diabetics MA- (79±11 vs 97±9 minutes; $p<0.05$), and controls (79±11 vs 120±24.5 minutes; $p<0.001$). A significant linear correlation was observed between LDL^- and some parameters of metabolic control such as fructosamine ($r=0.45$, $p<0.05$) and triglycerides ($r=0.65$, $p<0.001$). An inverse correlation was found between lag phase and fructosamine ($r=-0.50$, $p<0.01$) and triglyceride ($r=-0.69$, $p<0.001$). These data show that type 2 diabetics with microalbuminuria have evidence of an increase oxidation in vivo and oxidability in vitro of LDL in association with a poor metabolic control. We propose that an increased lipoprotein oxidation could promote endothelial lesions in type 2 diabetics with microalbuminuria.

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RELATIONSHIP BETWEEN SEQUENTIAL CHANGES OF MICROPROTEINURIA AND SERUM LIPIDS IN NIDDM

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There are many risk factors to deteriorate diabetic nephropathy. To determine these factors, we compared sequential changes of microproteinuria with clinical backgrounds in NIDDM. We studied the levels of microproteinuria (microalbuminuria by immunoturbidity method and transferrinuria by latex agglutination method) in 204 diabetic patients. We divided them into three groups by the levels of microproteinuria; Normal group (N, n=77): normoalbuminuria (<20mg/g Creatinine) and normotransferrinuria (<1.0mg/g Creatinine), Mild group (M, n=43): normoalbuminuria and high transferrinuria, High group (H, n=84): high albuminuria and high transferrinuria. After 3-year observation, they were divided into three groups according to sequential changes of microproteinuria; (1) Deteriorated group: 23 cases (N→M, N→H, M→H), (2) Unchanged group: 78 cases (N→N, M →M), (c) Improved group: 48 cases (H→M, H→N, M→N). Among these three groups, we compared clinical backgrounds (Age, Duration, Blood pressure, Body mass index, FPG, HbA1c, Total-cholesterol, Triglyceride, HDL-cholesterol, BUN and Creatinine). In the deteriorated group, triglyceride ($P<0.01$) and body mass index ($P<0.05$) were significantly increased and HDL-cholesterol ($P<0.05$) was significantly decreased compared with other two groups. We conclude that the deterioration of microproteinuria in NIDDM will be associated with lipids metabolism.

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RELATIONSHIP BETWEEN LDL PARTICLE SIZE AND ATHEROSCLEROSIS IN THE PATIENTS WITH NIDDM.

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This study was conducted to determine the relationship between atherosclerotic diseases and LDL particle size in NIDDM. Method: Included in the present study were 125 NIDDM patients treated in this diabetes center, 102 male and 23 females aged 60.1±7.7 years with BMI of 23.7± 3.6. They were divided into 4 groups: 62 with ischemic heart disease (CHD), 17 with cerebral infarction (CVD), 20 with arteriosclerosis obliterans in the lower limbs (ASO), and 26 with no sclerotic disease(C). The healthy volunteers were used as control(N). Plasma lipid and lipoprotein levels and LDL particle size (Kraus & Bruke method) were determined using over nigt fasting blood samples. Results:The LDL particle size was 25.4±0.9, 25.4 ± 0.6, 25.4 ± 0.8, 25.2 ± 0.7, and 26.1 ± 0.7nm respectively, in Groups CHD, CVD, ASO, C and N. It was smaller in all 4 NIDDM groups than in Group N. The percentage of Pattern B was statistically significantly higher in all 4 NIDDM groups than in Group N. The plasma lipid and lipoprotein profile showed a TG-rich tendency in the NIDDM groups compared to Group N. In conclusion, LDL particles were small in NIDDM patients regardless of the presence or absence and the type of atherosclerotic diseases. This seemed to be related to the fact that lipoprotein profile was TG-rich.

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LIPIDE STATUS OF POORLY REGULATED DIABETES : OBESE TYPE 2 WITH AND WITHOUT MACROANGIOPATHY , TYPE 1 AND TYPE 1 WITH NEPHROPATHY

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Determinants of hyperlipidaemia in diabetes mellitus are numerous: age, obesity, glycaemic control, nephropathy etc. According to PROCAM study results, different dislypidemias have disproportional grades of risks for macroangiopathy. Group of 20 obese(BMI=30,4=/-4,9) type 2 Diabetics without Macroangiopathy (OD2-M), 20 obese (BMI=32+/-5,1) type 2 Diabetics with Macroangiopathy(OD2+M), 20 nonobese type 1 Diabetics (D1) and 10 nonobese type1Diabetics with Nephropathy (D1+N) were compared according to first level lipidogram. Patients were not smokers, and except D1+N group were normotensive. Duration of diabetes was 6,4+/-2,5 y. for OD2-M; 6,1+/-3,2 y. for OD2+M ; 8,2+/-3,1 y. for D1 and l3+/-4,4 y for DI+N group. HbAlc was high and not significantly different: OD2-M =10,5+/-2,56%,OD2+M =9,83+/-4,3%, D1 =11,23+/-2,7% and D1+N=10,2+/-2,6%(p>0,05).Values for triglicerides / cholesterol/ LDL/HDL were: OD2-M 1,7/5,62/3,62/1,31 mmol/l; OD2+M 3,94/6,36/3,91/1,19 mmol/l; D1 1,88/6,68/3,95/1,33 mmol/l; D1+N 3,28/9,26/6,49/1,12 mmol/l (without SD here). Basal C-peptide levels were significantly higher in DM2+M group than in DM2-M (1,73+/-0,29 vs 1,21+/-0,23 nmol/l , p<0,01) . Conclusive differences were found between significantly higher level of triglicerides in DM2+M than in DM2-M,positively correlated with higher C-peptide levels(p<0,01); significantly (but less) higher cholesterol as well as LDL level in D1 than in DM2-M(p<0,05) but not DM2+M group(p>0,05); significantly higher triglicerides,holesterol and LDL in D1N group comparing to all other groups (p<0,01). HDL cholesterol was not found significantly different between groups (p>0,05). It was concluded that in chronically hyperglycemic patients hypertrigliceridaemia with higher insulin (resistance) is risk factor of macroangiopathy in OD2 patients, moderate hypercholesterolaemia in D1 patients ,and frank hypercholesterolemia and hypertrigliceridemia in D1+N.

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SMALL, DENSE LDL AND EARLY STAGE OF DIABETIC NEPHROPATHY

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We have previously reported the decrease in LDL particle size in NIDDMs with microalbuminuria compared to that in those with normoalbuminuria. Therefore, the present study was conducted to examine the influence of LDL size on the development of early diabetic nephropathy monitoring urine FDP(fibrin/fibrinogen drgradation products),a new candidate for earlier marker for the nephropathy. We examined 144 NIDDMs without hypertension and 142 non-diabetic healthy volunteers (N). Urine (early morning) FDP was measured by chemiluminescent enzyme-linked immunoassay with dioxetane derivative substrate for the labelled alkaline phosphatase. The detection limit is lng/ml, which is approximately 1/100 of conventional turbidimetric method. Abnormally high level of urine FDP (above mean+2SD of group N) was found in 68% (45/66) of NIDDMs with normoalbuminuria. Furthermore, 90% (30/33) of those with micro- or macroalbuminuria had elevated urine FDP. When NIDDMs with normoalbuminuria were divided into two groups according to thier urine FDP levels, there were no significant differences in either mean age, BMI, duration of diabetes, smoking rate, blood pressure, HbAlc, FBG or plasma lipids. The only one discriminater was LDL size ; the group with abnormally high urine FDP levels (n=23) had smaller LDL compared to that with normal urine FDP levels (n=22) (25.4±1.3 vs 26.3±1.2 nm, p<0.05). Together with our previous observation it is concluded here that small sized LDL is deeply involved in the development of early diabetic nephropathy.

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APOLIPOPROTEIN E (APO E) GENE POLYMORPHISM IN SARDINIAN NON INSULIN DEPENDENT DIABETIC PATIENTS (NIDDM)

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The preliminary aim of our study was to compare the frequency of apo E alleles between the homogeneous Sardinian population and what is reported for the rest of Italians in the mainland (James RW, Arterioscl Thromb 1993). Additionally we compared the Centre (the most archaic area)with the rest of Sardinia, as we found a difference in the frequency of the Gly40Ser amino-acid variant of the glucagon receptor gene between these Sardinian regions(Tonolo et al, Diabetologia 1997 in press). All subjects were of Sardinian ancestry for more than two generations. In multiplex NIDDM families at least two affected siblings and, if available, unaffected siblings were included. Additionally unrelated NIDDM and non diabetics subjects were also enrolled. All are currently characterised for BMI, diabetic complications (retinopathy, nephropathy, ischemic heart disease) and hypertension. Blood for DNA, apoA1, apoB, serum total and HDL cholesterol, triglycerides, HbA1c, and urinary albumin excretions rate were also obtained. So far a total of 950 subjects were enrolled. We report here the preliminary data on the first 517 subjects in whom apoE alleles were determined. No apparent allele distribution differences were evident so far between NIDDM and non NIDDM, and data (presented as % and analysed with χ^2)are cumulated.

Apo E	All	North	Centre	South	*=p<0.01vs Mainland
ε2	3.6 *	5.0	1.9	0.9	§=p<0.05 North vs
ε3	89.2 *	87.3 §	94.8	89.8	Centre; #=p<0.05
ε4	7.3	7.7	3.3 #	9.3	South vs Centre

Total cholesterol as well LDL cholesterol and apolipoprotein B were lower, but not significantly, in subjects with the ε2 allele. Our preliminary data indicate: 1)ApoE alleles frequency in Sardinia are different from the mainland, 2)the Centre of Sardinia is different from the North and the South, holding one of the lowest prevalence of ε4 in the world, 3) dietetic therapy in NIDDM may mask the effects of apoE polymorphism on lipid metabolism, 4)we are in a good position to analyse the relations between apo E alleles and cardiovascular disease in Sardinian NIDDM.

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APOLIPOPROTEIN E GENOTYPE AND CHOLESTEROL SYNTHESIS IN NORMOLIPIDEMIC TYPE II DIABETIC PATIENTS

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The polymorphism of the apolipoprotein (apo) E gene locus influences up to the 16% of the variance of total serum cholesterol in the normal population. Subjects carrying the E4 allele show decreased cholesterol synthesis and LDL receptor activity than those carrying the E3 allele. So far, the effect of apo E polymorphism on cholesterol synthesis in diabetic patients is unknown. We studied 14 type II non-obese diabetic patients in good metabolic control, with normal renal function and treated with diet and oral hypoglycemic agents. The patients were divided in two groups according to their Apo E genotype. Apo E 3/3 group: 4 males and 4 postmenopausal females aged 60±8 years, BMI 27±1.7 Kg/m^2, HbA1c 6.3±1%, Tot. Chol. 199±19 mg/dl, HDL Chol. 51±11 mg/dl, Tg. 140±54 mg/dl. Apo E 4/3 group: 3 males and 3 postmenopausal females aged 63±3.5 years, BMI 27±2 Kg/m^2, HbA1c 6.9±0.3%, Tot. Chol. 203±17 mg/dl, HDL Chol. 51±16 mg/dl, Tg. 157±49 mg/dl. Apo E genotype was determined on DNA extracts by restriction isotyping of PCR products. The 24/h urinary mevalonate excretion, an index of whole body cholesterol synthesis, was evaluated by GC(CI^-)MS after exctraction by CH_2Cl_2:PrOH (9:1). The mean mevalonate excretion was 1.43±0.18 µmol/24h in the Apo E 3/3 group and 1.20±0.13 µmol/24h in the Apo E 4/3 group (n.s.). In conclusion our preliminary data demonstrate no differences of cholesterogenesis between the two most common Apo E genotypes in a selected group of non-obese, normolipidemic type II diabetic patients under optimized metabolic control.

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EFFECT OF INSULIN ON LOW-DENSITY LIPOPROTEIN OXIDABILITY IN NIDDM PATIENTS .

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Hyperinsulinemia and increased oxidative modification of low-density lipoproteins (LDL) have been implicated as independent risk factors for atherosclerosis, the most common cause of mortality and morbidity of patients with non-insulin-dependent diabetes mellitus (NIDDM). The aim of our study was to evaluate the effect of acutely induced hyperinsulinemia on LDL susceptibility to in vitro Cu^{2+} induced oxidative modification in 15 NIDDM patients and 11 healthy control subjects. A 10-hour isoglycemic hyperinsulinemic clamp (75 µU/ml) and a time-controlled study with saline infusion were performed in all subjects. Compared to healthy subjects NIDDM patients showed a shorter lag time of diene production (67±19 vs 74±10 min; $p<0.05$), increased oxidative modification of protein moiety measured by emission fluorescence spectra at 430 nm with excitation at 355 nm (EFS) (402±130 vs 281±115; $p<0.02$), although the differences in thiobarbituric acid reactive substances (TBARS) (50 0±26 vs 45.5±26 nmol/mg of LDL protein) did not reach statistical significance. While acute hyperinsulinemia resulted in a decrease in lag time (-18%; $p<0.01$), an increase in TBARS (+ 46%; $p<0.001$), and a rise in EFS (+51%; $p<0.01$) in controls, the LDL oxidability-enhancing effect of insulin was not significant in diabetics. We conclude that NIDDM is associated with increased LDL susceptibility to oxidative modification. While acutely induced hyperinsulinemia enhances LDL oxidability in healthy subjects it does not aggravate LDL oxidability in NIDDM patients, suggesting factors other than insulin play a role in diabetes.

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INSULIN THERAPY IN TYPE 2 DIABETES.

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The aim of our open randomized controlled study was to assess the influence of intensive and combined insulin therapy on insulin and lipid profile in NIDDM patients with secondary failure (SF) to sulphonylureas (SU). The study involved 32 patients: (10m, 22f., mean age 58,9± 0,9 y., BMI - 21,8±1,04 kg/m^2). Patients were divided into 2 groups: 1.basal-bolus insulin regimen (Actrapid® HM + Monotard® HM), (n=18); 2.combined basal insulin/glibenclamide therapy (Monotard® HM as basal insulin) (n=14). Lipid metabolism and pancreatic function assessment was conducted at baseline state, and at the end of 4 and 12 week period. Marked fall in basal and postprandial glycaemia was achieved after 4 week period in both groups. Basal free insulin level didn't change in patients of the 1st group, but significantly increased in the 2-nd group. Both regimes caused marked postload hyperinsulinaemia. Plasma levels of Cholesterol (Chol.), Tryglycerides (Tg.) and LDL-chol. were elevated in all patients. At the end of the 12 week period Tg and VLDL chol. levels decreased significantly till normal levels in both groups. Chol. level significantly decreased at the expense of LDL and VLDL fractions and reached normal level only in the 1-st group. Tg and VLDL chol. levels decreased in these patients at the end of 4 week period, while at the end of the study these atherogenic parameters were lower, than in control group. In the 2-nd group Tg and VLDL chol. levels decreased till normal ranges only at the end of 12 week period, while total and LDL chol. levels at the same study point were higher than those in control group. Marked fall in apoB/apoA1 ratio was observed at the end of the study in the 1-st group, while in combined regime, decreasing after 4 week period this parameter reached initial values at the end of the study. Phosfolipid content of HDL was improved after 12 week period in both groups. Lp(a) level was constant in both groups, irrespective of treatment mode. Finally, intensive insulin therapy during 12 weeks caused optimal antiatherogenic changes in lipid profile of NIDDM patients with SF, in spite of marked persisting hyperinsulinaemia.

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THE EFFECT OF INTENSIVE INSULIN THERAPY ON THE SERUM LIPID PROFILE IN DIABETIC PREGNANT WOMEN.

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Intensive insulin therapy has improved the course of pregnancy and delivery in women with diabetes. Additional evidence shows that hyperinsulinemia exert effect on lipid metabolism, which undergoes specific modifications in these patients. The present study assessed the serum lipid profile in 54 pregnant women and 35 non-diabetic pregnant women with respect to the following parameters: triglycerides (TG), total cholesterol (TCh), HDL-cholesterol (HDL-Ch), LDL-cholesterol (LDL-Ch), apolipoprotein A1 (apo A1), apolipoprotein B (apo B). The metabolic control was determined by serum fructosamine concentration. Serum lipid profiles in women at the end of I and III trimester (TR) of pregnancy is shown in table:

	Non-	diabetic	Insulin	treated
	I TR	III TR	I TR	III TR
Fructosamine (mM)	2.18	1.93	2.89	2.73
TG (mM)	1.48	3.85	1.09	2.55**
TCh (mM)	6.30	8.87	4.65*	7.96
HDL - Ch (mM)	2.03	3.33	1.26*	1.57***
apo A1 (mg/dl)	209.1	234.8	158.8**	168.0***
apo B (mg/dl)	113.2	167.9	95.6	164.5

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

In diabetic pregnant women serum levels of TG, TCh, HDL-Ch, apo A1 and apo B were lower than those in non-diabetic pregnant patients. The reduced TG level may result from inhibition of lipoprotein lipase due to large doses of insulin. The decreased in HDL-Ch and apo A1 does not seem so clear. However, it may be caused by resistance of hepatic lipase to insulin, or to down regulation mechanism occurring in states of hyperinsulinemia.

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BEZAFIBRATE AND SIMVASTATIN COMBINATION THERAPY FOR DIABETIC DYSLIPIDEMIA. EFFICACY AND SAFETY

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Diabetic dyslipidemia is a principal cause of cardiovascualr morbidity and mortality. Hypolipidemic drugs.- fibrates and statins, have been shown to correct diabetic dyslipidemia and reduce cardiovascular morbidity. In this study 142 patients (age 35-73) diagnosed as suffering from NIDDM (Hg A_1C above normal levels) on diet or oral hypoglycemic therapy with diabetic dyslipidemia which persisted in spite of glycemic control were assigned to simvastatin 20 mg/day for 3 months, followed by a combination therapy of 400 mg Bezafibrate + 20 mg Simvastatin per day for a period of 6 months. On statin alone, LDL-C decreased by 29% triglycerides by 16% HDL-C increased by 4.2% and no significant change was seen in Lp(a) and Fibrinogen levels. Combination therapy decreased LDL-C by 28% ($p<0.001$), triglycerides decreased by 40% ($P<0.0001$), HDL-C increased by 17.6% ($p<0.01$), Lp(a) decreased by 10% ($p<0.05$), Fibrinogen decreased by 10% ($p<0.05$), 11 patents had increased C.P.K. serum levels 3 patients stopped treatment due to C.P.K elevation and minor muscle pain. Adherence to therapy was 85% at 6 months on the combination regimen and 98% on statins monotherapy. Our results suggest that in diabetic dyslipidemia a combination therapy may be beneficial due to improved atherogenic and thrombogenic profile.

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LDL MODIFICATIONS IN WELL CONTROLLED DIABETIC PATIENTS: EFFECTS OF PHYSICAL TRAINING

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Our group previously reported that the atherogenic electronegative LDL subform (LDL-) is increased in diabetic patients and closely related to glycemic control in IDDM patients.We evaluated the effect of a 3 month physical training program (3-5 days/week, 1852±387 kcal/week)on LDL composition, the percentage of LDL - and LDL susceptibility to oxidation in fourteen type I (7 male, 7 female, age 25 ± 6 years, BMI 23.8±3 kg/m^2, HbA1c 6.6±0.8% (reference range 4.6-5.8%) and thirteen type II diabetic patients (9 male, 4 female, age 55±5 years, BMI 26±6 kg/m^2, HbA1c 7.3±1%). LDL was isolated by sequential ultracentrifugation and LDL - by anion exchange in a Fast Protein Liquid Chromatography device. LDL susceptibility to oxidation was measured by dienes formation induced by $CuSO_2$ (Lag phase). Differences were assessed by Wilcoxon test. After three months of physical training, cardiorespiratory capacity (VO_2 max and O_2 pulse) improved significatively ($p<0.05$), and no changes were observed in BMI and HbA1c. The percentage of LDL - decreased from 16.2± 7.4% to 14.04±5% ($p=0.06$). No changes in LDL components (total and free cholesterol, protein and phospholipid) and in Lag phase were observed (43.6±6.7 min to 43.3±7 min).In conclusion, physical training may contribute to reduce the atherogenic risk in diabetes by means of decreasing the proportion of electronegative LDL subform.

(FIS 95/1252).

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EFFECTS OF CILOSTAZOL ON SERUM LIPOPROTEIN PROFILES IN PATIENTS WITH NIDDM

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Recently, the lipid lowering effects of cilostazol in hyperlipidemic subjects has attracted attention. A study to evaluate the effects of cilostazol on lipoprotein metabolism of 12 NIDDM subjects with dyslipidemia was conducted. One hundred and fifty mg per day of cilostazol was administered orally for three 4-week periods. Serum lipid and apolipoprotein concentrations were measured during at the end of 4-week period. Cholesterol content in the remnant like particles (RLP-c) was measured by using monoclonal anti apo B-100 and anti apo A-1 immunoaffinity mixed gels. LDL particle size was analyzed based on a new parameter (LDL-migration index, LDL-MI) which was calculated by dividing the distance from the VLDL peak to the LDL peak by the distance from the VLDL peak to the HDL peak on a PAGE densitogram. Results: The triglyceride content in serum showed a significant decrease and the HDL-c content increased significantly. RLP-c decreased from 11.8 to 4.0 mg/dl. LDL-MI showed a significant decrease from 3.9 to 3.4. These data suggest that cilostazol may have some beneficial effects on lipoprotein metabolism by normalizing LDL particle size in NIDDM patients with dyslipoproteinemia.

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EFFECTS OF VOGLIBOSE AND ACARBOSE ON FASTING AND POSTPRANDIAL PLASMA LIPIDS IN NIDDM PATIENTS

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Both, impairment of glucose and lipid metabolism are components of the insulin resistance syndrome. The aim of this study was to investigate whether the α-glucosidase inhibitors voglibose and acarbose influence plasma lipids in diabetics. 170 hospitalized NIDDM patients were randomly allocated to six parallel groups and received 7 days treatment with either three times daily voglibose (0.5, 1, 2, 5 mg), acarbose (100 mg) or placebo. Blood samples were taken before and 2, 3 and 4 hours after a carbohydrate rich standard meal on day -1 and day 7 to analyse phospholipids (PL), triglycerides (TG), cholesterol (C) in the serum and in VLDL, LDL and HDL as well as apolipoproteins (apo) AI, AII, B (total, VLDL, and LDL), CIII, E, and Lp (a). The effects of the treatments were analysed by one-factorial analysis of variance of the difference (day 7 *minus* day -1). 157 patients (mean age 54 years; 135 male) could be analysed. *Fasting lipids*. Voglibose decreased fasting TG (-8 % and -16 % at 1 mg and 5 mg per day), VLDL-TG (-8 % and -12 %), VLDL-C and VLDL-PL; none of these changes, however, was significantly different from placebo. Acarbose had no effect on fasting TG. Voglibose (5 mg) decreased apo E (-13 %, $p < 0.05$) and apo CIII (-7 %, $p = 0.170$). There were no consistent changes in LDL-C, apo B and Lp(a). Voglibose did not change fasting HDL-C, but acarbose lowered HDL-C from 0.95 to 0.90 mmol/l (-6 %, $p < 0.05$). *Postprandial lipids*. On day -1, total TG increased in all groups by 12 % on average 2 hours after the test meal. The 2 hours postprandial TG were dose-dependently reduced by voglibose (from 2.1 to 1.6 mmol/l at 5 mg, $p = 0.02$). Consistent changes were obtained with 5 mg Voglibose for VLDL-TG (-16 %, $p < 0.05$), VLDL-C (21 %, $p = 0.07$), VLDL-PL (-22 %, $p < 0.05$), apo E (-13 %, $p < 0.01$) and apo CIII (-6 %, $p < 0.05$); there were only slight reductions in the acarbose group. We conclude that voglibose is more potent than acarbose in reducing fasting TG-rich lipoproteins and the postprandial TG response.

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THE EFFECTS OF CIPROFIBRATE ON PLASMA LIPIDS AND FIBRINOGEN

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Increased levels of fibrinogen seems to be an independent risk factor for cardiovascular disease. The aim of the present study was to evaluate the effect of ciprofibrate on plasma lipids and fibrinogen in NIDDM dyslipidaemic patients.24 patients (mean age 65±6, gender 10 ♂/14♀).Their current antidiabetic therapy: 1 diet only, 14 diet+sulfonyluria(SU), 1SU+Metformin, 4SU+Insulin and 5Insulin. They, if after at least one month dietary treatment period, didn't normalize their lipidaemic profile(Total cholesterol (TC)>5.2mmol/L and or Trigycerides(Trg)>1.7mmol/L)start ciprofibrate treatment, 100mg daily for 6 months.Before and at the end of the 2nd,4th and 6th month, their body weight (BW),plasma,glucose,HBA_{1C},TC,HDL,Trg and also transaminases alkaline phospatase,γGT, creatinine, uric acid and CPK were measured.Statistical analysis was done by paired samples T-test.Results:(Mean±SD before(0) and at the end of 6th month(6) respectively and% change). Significantly reduced levels of a.Fibrinogen(10.79±2.6→8.99±2.17μmol/L, -16.6%, p<0.0001),b.T.C (7.23±2.4→5.57±0.65mmol/L,-23%, p<0.0001), c.LDL (5.03±2.38 3.70+0.69mmol/L, -26.3%, p<0.0001). d.Trg (2.02±0.97→1.13±0.38, - 44%, p<0.0001). HDL increased (1.14±0.26→1.39±0.36mmol/L, +17%, p=0.0015), plasma glucose reduced in a smaller degree(7.6±1.7→7.0±1.6mmol/L, -8%, p=0.02.BW and HbA_{1C} didn't change significantly.There was not elevetion of transaminases, creatinine and CPK.In conclusion: Ciprofibrate-100mg daily for 6 months-significantly improves lipidaemic profile of dyslipidaemic NIDDM patients. Also reduces fibrinogen levels without toxic effects on liver and muscles.

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LIPOPROTEIN CHANGES DURING SUBCUTANEOUS OR INTRAPERITONEAL INSULIN IN IDDM PATIENTS ON PERITONEAL DIALYSIS

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Intraperitoneally administered insulin is regarded as the most physiological replacement therapy of insulin. Compared to subcutaneous insulin this leads to lower peripheral insulin concentration and equal or better glycemic control. The effects on serum lipids, a major risk factor of atherosclerosis, are conflicting. This study was undertaken to evaluate the effects of subcutaneous vs. intraperitoneal insulin on serum lipoproteins in type I diabetic patients on continuous ambulatory peritoneal dialysis (CAPD). Eleven patients participated in the study. Serum lipids, apoproteins A-I, A-II and B, and HDL subfractions were measured. While on CAPD all patients were first treated with subcutaneous and then with intraperitoneal insulin. The metabolic studies were repeated after three months on either treatment. Intraperitoneal insulin decreased HDL-cholesterol ($p<0.01$) and increased LDL/HDL ratio ($p<0.01$) significantly. HDL-cholesterol reduction was almost entirely seen in the HDL3 fraction ($p<0.05$) that consists of the smallest and densest particles. Apoprotein A-I, the major apoprotein of HDL, decreased during intraperitoneal insulin treatment ($p<0.05$), and the ratio of apo A-I/HDL increased ($p<0.05$). Apo B/apo A-I ($p=0.06$) and apo A-I/apo A-II ($p<0.01$) ratio diminished with the use of intraperitoneal insulin. Glucose control was better during intraperitoneal than during subcutaneous insulin (HbA_{1c} 8.13 ± 0.39 and 9.49 ± 0.43 %, respectively, $p<0.01$). Intraperitoneal insulin, while inducing lower peripheral insulin concentration and better glycaemic control than subcutaneous insulin had deleterious effects on the lipoprotein profile among CAPD patients. The significance of these changes in modifying the risk of atherosclerosis is unknown.

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EFFICACY AND SAFETY OF FLUVASTATIN TREATMENT IN KIDNEY TRANSPLANT, DIABETIC RECIPIENTS; 1-YEAR FOLLOW UP

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Organ transplant recipients treated with steroid+cyclosporine often develop dyslipidemia which may significantly contribute to the increased cardiovascular mordity and mortality of these patients. Therefore, we studied the effect of fluvastatin treatment (Lescol, Sandoz, 20 mg/day) on serum lipid levels in 21 diabetic patients (13 men, 8 women, age range 31-63 years, BMI 25.9±4.5 kg/m2) who had underwent a successful kidney transplantation and had dominating hypercholesterolemia. Following an 8-week period of diet (daily cholesterol intake <250 mg) fluvastatin was applied subsequently for 1 year. Diet alone did not cause significant change in serum lipid levels. Fluvastatin reduced the total cholesterol, LDL-cholesterol and triglyceride levels from 7.70±0.87, 4.87±1.05 and 2.84±0.85 to 6.40±0.70, 3.52±0.69 and 2.64±0.86 mmol/L, $p<0.001$, <0.001 and <0.05, resp., while the level of HDL-cholesterol increased from 1.12±0.28 to 1.52±0.39 mmol/L, $p<0.001$. Lipoprotein-A1 increased from 1.52± 0.29 to 1.67±0.36, lipoprotein-B decreased from 1.27±0.20 to 1.14±0.15 mmol/L, $p<0.05$ and <0.01. These maximum improvements were achieved by the 12th week of fluvastatin therapy and no further significant changes were observed until the end of the study year. Serum concentration of Lp(a) remained unchanged throughout the study. Liver enzymes (ASAT, ALAT) did not increase. No side effects (myositis, myoglobinuria) was observed. Serum level of cyclosporine (using stable doses throughout the year) was 199±89 ng/ml at start and 206±28 ng/ml at the end of the year, i.e. no increase due to interference with fluvastatin was found. Thus, fluvastatin seems to be a very effective and safe drug for the treatmet of kidney transplant diabetic patints receiving steroid+cyclosporine immunosuppressive therapy.

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IMPROVEMENT OF HYPERVISCOSITY SYNDROME IN DIABETIC PATIENTS BY HEPARIN-INDUCED EXTRACORPOREAL LDL PRECIPITATION (HELP)

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In contrast to other selective procedures for extracorporeal LDL precipitation, HELP can also effectively lower plasma fibrinogen and improve blood rheology. We compared the effectiveness of short-time HELP treatment, applied on a weekly basis in patients with: hyperlipoproteinaemia (A) and NIDDM with hyperlipoproteinaemia (B). Changes of metabolic and hemorheologic parameters during 5 consecutive HELP treatment were calculated as interval values (value after HELP and before next HELP divided by two) and expressed as percentage of the value before starting HELP($\bar{X}\pm SD$):

	A (Hyperlipoproteinaemia)	B (NIDDM+ hyperlipoproteinaemia)
LDL-C (mM/l)	-13% ± 7	-37% ± 6
TC (mM/l)	-28% ± 7	-34% ± 4
HDL-C (mM/l)	+2% ±11	-15% ± 5
TG (mM/l)	-43% ± 7	-28% ± 8
Fib (g/l)	-7% ± 6	-15% ± 6
ErcF (µl/sec)	+9% ± 7	+26% ± 5
PV (mPa.s)	-16% ± 1	-25% ± 3

Correlation between plasma viscosity and fibrinogen were r=0,758, p<0,01 and r=0,827, p<0,003 for A and B respectively. Our results demonstrate that HELP treatment immediately corrects hyperviscosity syndrom and hyperlipoproteinaemia as well, suggesting the usefulness of this method when a quick and drastic fluidification of blood is required.

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EFFICACY OF FENOFIBRATE TREATMENT IN POLYMETABOLIC SYNDROME (PMS) IS ASSOCIATED WITH SERUM INSULIN LEVELS

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Dyslipidemia, arterial hypertension (HT), central obesity (CO), impaired glucose tolerance (IGT), hyperinsulinemia and insulin resistance are the major features of the PMS. The aim of the study was to compare the effect of fenofibrate treatment in dyslipidemic patients with PMS divided 1) according to presence or absence of IGT and 2) according to insulin levels: above or below the third quartile of serum insulin distribution in the examined group. Material included 37 dyslipidemic males with PMS (at least two of the following: HT, CO or IGT), aged 20 - 70 years, with serum total cholesterol (TC) > 6.5 mmol/l, or triglycerides (TG)>3.0 mmol/l or HDL -cholesterol < 0.9 mmol/l, treated with micronised fenofibrate 200 mg daily. Serum lipids and glucose were evaluated enzymatically, insulin by RIA. After 12 weeks of therapy serum lipids changes were similar in both groups of patients:with normal glucose tolerance (NGT) and IGT: serum TC decreased by 12.5%, TG by 37.3%, and HDL-cholesterol increased by 10.0% of the baseline level in the IGT group and by 9 3%, 22 5% and 17.8% respectively in the NGT group. The fasting insulin and insulin area under the curve during the oral glucose tolerance test (OGTT) decreased in both IGT (by 30.2% and 16.1%) and NGT (by 18.5% and 6.4% respectively), while changes in fasting and area under the curve of glucose during OGTT were different (p<0.01): decrease was observed in the IGT group only. In the group with the fasting insulin values > 3rd quartile of insulin distribution serum TC decreased by 11.7% TG by 32.1% and HDL increased by 3.8%, while in the rest of the group the changes were 10.6%, 27.6% and 20.4% respectively. The difference in increase of HDL-cholesterol was significant (p<0.02). The results suggest that in dyslipidemic patients with PMS fenofibrate therapy 1) is associated with favourable changes in glucose profile in IGT patients and 2) the serum lipids response is associated with fasting insulin levels: the increase of cholesterol in antiatherogenic lipoprotein fraction HDL is significantly higher in patients with lower insulin levels.

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FEASIBILITY OF INTENSIVE LIPID LOWERING IN PATIENTS WITH DIABETES MELLITUS.

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Macrovascular disease causes premature morbidity and mortality in diabetic patients. Agressive lipid lowering may prevent macro-angiopathy. The aim of our study was to investigate the feasibility of intensive lipid lowering in diabetic patients and to determine the effect of this therapy on the composition of plasma lipoproteins.Thirty-six IDDM and 59 NIDDM patients were included in an open study at the diabetes clinic. Target values for plasma lipids were: LDL-cholesterol < 2.6 mmol/l, triglycerides < 1.7 mmol/l and HDL-cholesterol > 0.9 mmol/l for men and > 1.1 mmol/l for women. After 6-12 weeks of diet, lipid lowering medication (statin / fibrate / acipimox) was prescribed and extended until all target levels were reached. Mean baseline lipid levels for IDDM and NIDDM were: total cholesterol 5.6 and 5.8 mmol/l, LDL-cholesterol 3.6 and 3.7 mmol, HDL-cholesterol for men 1.1 and 1.0 mmol/l, for women 1.4 and 1.2 mmol/l, and triglycerides 1.7 and 2.2 mmol/l, respectively. Target values were reached in 66% of the patients. Total cholesterol decreased by 1.3 mmol/l (95% CI 1.0 - 1.5) in IDDM and 1.7 mmol/l (95% CI 1.4 - 2.0) in NIDDM patients, LDL-cholesterol by 1.2 mmol/l (95% CI 0.8 - 1.5) and 1.3 mmol/l (95% CI 1.0 - 1.5) and triglycerides by 0.7 mmol/l (95% CI 0.3 - 1.0) and 1.1 mmol/l (95% CI 0.9 - 1.4), respectively. HDL-cholesterol increased only in men and women with IDDM: by 0.15 mmol/l (95% CI 0.01 - 0.28) and 0.34 mmol/l (95% CI 0.02 - 0.71), respectively. The cholesterol/triglycerides ratio decreased significantly in VLDL in IDDM and in IDL in NIDDM. The ratio increased significantly in HDL in NIDDM patients. In conclusion, these results show that intensive lipid lowering in diabetes mellitus is feasible and affects the composition of plasma lipoproteins, in agreement with improved lipoprotein metabolism and a less atherogenic lipid profile.

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EFFICACY AND SAFETY OF FLUVASTATIN IN PATIENTS WITH NON INSULIN DEPENDENT DIABETES ON INSULIN THERAPY AND DYSLIPIDEMIA.

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The incidence of dyslipidemia in patients with Non Insulin Dependent Diabetes (NIDDM) is high, as is cardiovascular morbidity and mortality. This double blind randomised placebo controlled study was initiated to evaluate the safety and efficacy of fluvastatin in NIDDM patients on insulin therapy and dyslipidemia (LDL>4.0mmol). Eighty-one NIDDM patients on insulin therapy entered the study (age 61±9 years, male/female=0,57). All patients started with 4 weeks of diet (ADA/EDA recommendation), then they were randomised between placebo and fluvastatin 40mg OD. The study groups were identic with respect to age, BMI, sex, cardiovascular history and laboratory evaluations. The following results were obtained:

	week 0	week 12		
	Baseline	placebo	fluvastatin	
HbA_{1c}	6.2 mmol/l	6.5	6.4	
TC cholesterol	6.6 mmol/l	+ 2.2%	-16,2%	(p<0.001)
triglyceriden	1.8 mmol/l	+ 8.6%	-4.9%	(p<0.05)
HDL cholesterol	1.2 mmol/l	- 1.9%	-1%	(ns)
LDL-cholesterol	5.0 mmol/l	+ 2.8%	-25.1%	(p<0.001)
Lp (A)	262 mmol/l	+ 10.6%	+2.6%	(ns)
$ApoA_1$	1.7	no changes		(ns)
ZUNG-scale	34	35	33	(ns)

In this double blind, randomised, placebo controlled study patients with NIDDM on insulin therapy and dyslipidemia were treated with fluvastatin 40mg OD. LDL-cholesterol and triglyceriden decreased significantly in the treatment group. We observed no change in patients mood (ZUNG scale). Adverse events were equally distributed between the two study groups. Conclusion: Fluvastatin treatment for dyslipidemia in NIDDM patients is effective and safe.

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EFFECT OF THE WEIGHT REDUCTION ON GENE EXPRESSION OF LIPOPROTEIN MODIFYING PROTEINS.

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Low HDL-cholesterol concentration and hypertriglyceridemia are more prevalent in obese persons than in subjects with normal body weight. To study the regulation of serum HDL-cholesterol and triglyceride levels, we conducted a weight reduction trial among 33 obese, postmenopausal, middle-aged women. The intervention group (13 subjects) and control group (20 subjects) were comparable according to their age, body weights, serum lipid, plasma glucose and serum insulin concentrations. Conventional laboratory methods were used for analysis of lipids and lipoproteins. Cholesterol ester transfer protein (CETP) activity was measured. A quantitative RT-PCR method was established to measure the gene expression of CETP (biopsies from liver and adipose tissue obtained at the end of the weight reduction period) and lipoprotein lipase (LPL, adipose tissue only). The reduction of the body weight among the intervention subjects averaged 4.7±0.5 kg. Serum HDL-cholesterol concentration was reduced (–0.06±0.03 mmol/l, $p<0.01$), and triglyceride (–0.14±0.09 mmol/l) and total cholesterol concentrations (–0.14±0.12 mmol/l) tended to be reduced. Plasma CETP activity was not affected by the weight reduction, even though the CETP gene expression was lower both in liver (–29%) and adipose tissue (–25%) compared with controls. In the intervention group the LPL gene expression in the adipose tissue was 50% lower than in controls ($p<0.05$). After the weight reduction a highly significant negative correlation was noted between the serum HDL-cholesterol concentration and the amount of CETP mRNA in adipose tissue ($r= -0.76$, $p=0.01$), whereas a positive correlation between the serum triglyceride and CETP mRNA ($r=0.69$, $p<0.05$) was found. In conclusion, weight reduction affects lipid metabolism at the gene expression level, and adipose tissue itself seems to have a significant role in the altered lipid transfer.

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EFFECT OF GLYCATED FIBRONECTIN ON PROLIFERATION AND IGFBP4 SECRETION BY CULTURED HUMAN AORTIC SMOOTH MUSCLE CELLS (SMC)

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The late products of nonenzymatic glycosylation, namely AGEs, contribute to several long-termed complications of diabetes, including vascular disease, where SMC proliferation plays an important role. PDGF has been considered a competence factor for this cell type and IGF-I can work as a progression factor for the mitogenic effect of PDGF. The objective of this study was to evaluate the effect of an AGE-modified matrix protein (fibronectin) on SMC proliferation as well as on IGF-I-IGFBP4 system after stimulation with PDGF-BB. Immortalized human aortic SMC (AALTR-16, provided by Dr. JK Dougall, Fred Hutchinson Cancer Res. Ctr. Seattle, WA) in culture were plated in three different substrata (plastic, fibronectin and AGE-fibronectin) and stimulated with 10 ng/mL of PDGF-BB for 24-h. Total RNA was extracted and analyzed by ribonuclease protection assay employing riboprobes complementary to IGF-I, IGF-IR and IGFBP4 mRNA. To study the secretion of IGFBP4, conditioned media of untreated and treated cells were submitted to Western Blot using antibody anti-IGFBP4 (UBI. Lake Placid, NY). To evaluate the effect of glycated fibronectin on SMC proliferation, the cells were seeded in 12-well plates coated with either fibronectin or AGE-fibronectin, grown in media supplemented with 2% FCS, PDGF-BB (10 ng/mL), IGF-I (20 ng/mL) and EGF (20 ng/mL) and counted after 4, 10 and 14 days. There was no significant differences in the amount of IGF-I, IGF-IR and IGFBP4 mRNA contents after stimulation with PDGF-BB in comparison to untreated conditions in any of the three substrata. On plastic and fibronectin substrata, the PDGF-BB elicited an increase of 63% and 40% respectively in IGFBP4, while on AGE-fibronectin substratum, PDGF-BB evoked a decrease of 28% in IGFBP4. SMC proliferation on AGE-fibronectin was significantly increased after 14 days in culture. Since IGFBP4 is known to inhibit the IGF-I action, the decrease of IGFBP4 secretion (probably by a post-transcriptional mechanism) induced by PDGF-BB when SMC are plated on AGE-fibronectin substratum can result in elevation of local free IGF-I. This increased availability of IGF-I may exert a role in SMC proliferation and therefore would be involved in the pathogenesis of atherosclerosis in conditions of chronic hyperglycemia. Supported by FAPESP grant 91/3617-8.

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EFFECT OF PIOGLITAZONE ON THE FATTY STRIATED PORTION IN THE ARTERIAL WALL

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<**Purpose**> We have previously reported that pioglitazone can inhibit fat deposition in the aortic wall. In the present study, we investigate the effect of pioglitazone on the compositions between macrophages (M ϕ) and smooth muscle cells(SMC) in the fatty striated portion. <**Methods**> Five male JW rabbits were assigned to group A, fed with a diet containing 0.5% cholesterol (C), and five to group B, fed with a diet containing C and 300 ppm pioglitazone (P). These diets were given at 200 g/day for 10 weeks. Blood was sampled at weeks 0 and 10 for the measurement of serum lipids and other parameters. The descending aorta was removed at week 10. The obtained aorta was sliced vertically to a long axis of the aorta from the site 0.5 cm above the diaphragm. These sections were also stained immunohistochemically (anti-M ϕ RAM11 antibody and anti-SMC Actin 1A4 antibody) for the measurement in the fatty striated portion by image analyzer (Olympus Co.). Moreover, we also measured the lipid deposition area (DL) on the tunica intima of the aorta from 2 to 6 cm above the diaphragm. <**Results**> Among serum lipids examined, T-CHO markedly increased after 10 weeks in both groups without significant intergroup difference, while the perioxide lipid level was significantly lower in group B than in group A. In the fatty striated portion, the number of macrophages was lower in group B than in group A. Use of M ϕ specific and SMC specific antibodies demonstrated that lesions from group B were significantly smaller and less cellular than were the lesions in group A. And DL was also significantly lower in group B (15%), compared with group A (57%). <**Conclusion**>From these results, it was thought that pioglitazone is a useful drug for the prevention and treatment of atherosclerosis through some mechanism, such as antioxidant action, other than enhancement of LPL activity, as previously reported.

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AGEs-LDL LEAD MACROPHAGES TO FOAM CELLS VIA MACROPHAGE SCAVENGER RECEPTOR IN VITRO

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Recent studies showed that modification of LDL with AGEs (advanced glycation end products) leads to loss of its ligand activity for the LDL receptor, suggesting the possibility that the atherogenicity of AGEs-LDL in vivo could be explained by an increase in plasma LDL cholesterol level due to prolonged plasma clearance of AGEs-LDL. However AGEs-proteins are recognized by AGEs-receptors, in particular, by macrophage scavenger receptor (MSR) in macrophages or macrophage-derived cells. Therefore, it is also possible that AGEs-LDL are taken up by macrophages via MSR, which might contribute to the foam cell formation in the early atherosclerotic lesions. This possibility was tested in the present study. (i) modification of LDL with glycolaldehyde (GA) resulted in a time-dependent increase in its negative charge, fluorescent activity and immunochemical reactivity to anti-AGEs-antibodies. These physicochemical and immunological properties resemble those of AGEs-modified proteins. (ii) GA-LDL was actively taken up by mouse peritoneal macrophages, leading to intracellular accumulation of cholesteryl esters. (iii) CHO cells overexpressing MSR showed a high affinity binding not only to acetyl-LDL but also to GA-LDL. These data obtained from in vitro experiments suggest that AGEs-LDL may play a key role in pathogenesis of diabetic macro-angiopathy.

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EFFECT OF PIOGLITAZONE IN INHIBITING LIPID DEPOSITION STUDIES WITH THE INSULIN TOLERANCE TEST

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<**Purpose**>Since the syndrome X was proposed as a risk factor for arteriosclerosis, insulin resistance has been considered to be a serious clinical problem in the treatment of diabetes. In the present study, we investigate the effect of pioglitazone on blood insulin and insulin sensitivity in the presence of hypercholesterolemia for the purpose of clarifying its effect on arteriosclerosis.<**Methods**> Male JW rabbits, weighing about 2.5 kg, were used at the age of 10 weeks. Five animals were assigned to group A, fed with 0.5% cholesterol-containing diet, and five to group B, fed with diet containing 0.5% cholesterol and 300 ppm pioglitazone. These diets were given at 200 g/day for 10 weeks. Blood glucose, serum insulin, and serum lipid levels were measured at the start of feeding (week 0) and at week 10. At the same time, the insulin tolerance test (ITT) was also performed. Moreover, the descending aorta was removed at week 10, and the lipid deposition area (DL) on the inner surface of the aorta was measured. <**Results**>Blood glucose and serum insulin levels remained unchanged at week 10, compared with week 0, and no significant intergroup differences were observed at week 10. Areas under the curves of changes in blood glucose levels (AUC) in ITT also remained unchanged at week 10, compared with week 0, and no significant intergroup difference was observed at week 10. However, DL at week 10 was 57% in group A, compared with 15% in group B, showing a significantly lower DL in group B than in group A.<**Conclusion**> It was revealed that pioglitazone had inhibited lipid deposition in the Aortic wall with hyper cholesterolemia. On the other hand, it could not be determined whether pioglitazone had the effect of improving insulin resistance in the presence of hypercholesterolemia because of the lack of significant intergroup difference in serum insulin or AUC measured in ITT. From these results, it was inferred that inhibition of lipid deposition in the arterial wall by pioglitazone was not achieved by the direct effect of this drug on insulin; however pioglitazone has some other effect on inhibition for lipid deposition.

1703

INVOLVEMENT OF POLYMORPHONUCLEAR LEUKOCYTES(PMN) IN THE OXIDATION OF LOW DENSITY LIPOPROTEIN.

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Oxidized LDL, which is increased in diabetics, has been thought to play central roles in the development of atherosclerosis. However, precise mechanisms of facilitated LDL peroxidation in diabetic state still remain unclear. To investigate the early events of oxidative modification of LDL, we tested the possible role of glycated-LDL(G-LDL) in stimulating leukocytes adhesion to endothelial cells(EC) and involvement of PMN in lipid peroxidation process.G-LDL was made by incubating native LDL(N-LDL) with 100 mM D-glucose for 70 hrs. Oxidatively modified LDL(GOX-LDL) was made by further incubation of G with 50 mM Fe-ADP. Circulating monocytes(M) and PMN were isolated from healthy donors, and incubated with LDLs at 37℃ for 30 min. The degree of lipid peroxidation was measured as TBARS and the superoxide(O^{2-}) was measured by MCLA-dependent chemi-luminescence and H2O2 by a scopoletin method. N-LDL and G-LDL stimulated adhesion of PMN, but not M, to EC. G-LDL caused 1.5 fold increase of O^{2-} generation by PMN pre-stimulated with phorbol 12-myristate 13-acetates (PMA). GOX-LDL caused greater increase O^{2-} generation from PMN than G-LDL. TBARS assay revealed that GOX-LDL received stronger oxidation of its own lipid by PMA-stimulated PMN than N-LDL did(5.72 ± 0.464 vs 2.65 ± 0.122, $p<0.001$). Results suggest that hyperglycemia increases the oxidation of G-LDL via the PMN activation including augmented cell-adhesion and free radical generation.

1704

EFFECT OF PIOGLITAZONE ON ATHEROSCLEROSIS

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<**Purpose**>Insulin resistance is an etiologic factor for NIDDM and also a factor for the aggravation of NIDDM. In the present study, we investigated the effect of pioglitazone on atherosclerosis in the aspects of serum lipids, histology, lipid deposition, and blood pressure. <**Methods**>Five male JW rabbits were assigned to group A, fed with diet containing 0.5% cholesterol (C), and five to group B, fed with diet containing C and 300 ppm pioglitazone (P). These diets were given at 200g/day for 10 weeks. Blood was sampled before (week 0) and 10 weeks (week 10) after the start of feeding for measurement of serum lipids. At the same time, systolic and diastolic blood pressure was measured. The descending aorta was removed at week 10 to observe histological appearances (HE stain and immunohistochemical stain) and also to measure the lipid deposition area (DL) on the unfolded aorta using an image analyzer. <**Results**> As compared with serum lipid levels at week 0, T-CHO significantly increased in both groups, while HDL-C remained unchanged and T-G tended to decrease. There was no significant intergroup difference in T-CHO, HDL-C, or T-G at week 10. Although lipid peroxide(LPO) levels increased at week 10 in both groups, LPO at week 10 was significantly lower in group B than in group A. In histological observation, hypertrophy of the tunica intima was remarkable in group A, but slight in group B. Moreover, DL at week 10 was 57% in group A, compared with 15% in group B, showing a significantly lower DL in group B than in group A. Blood pressure at week 10 did not markedly alter in group A, as compared with week 0, whereas both systolic and diastolic blood pressure tended to slightly decrease at week 10 in group B, as compared with week 0. <**Conclusion**> Although pioglitazone had no effect on T-CHO, HDL-C, or T-G, this drug was confirmed to have inhibitory effects on lipid deposition and hypertrophy of the tunica intima. In addition to these results, the tendency toward decreases in LPO and blood pressure suggested that pioglitazone had inhibitory effects on atherosclerosis.

1705

INSULIN SIGNALING ACCELERATES ENDOCYTIC UPTAKE OF AGEs MEDIATED BY MACROPHAGE SCAVENGER RECEPTOR

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Hyperglycemia is expected to accelerate the formation and accumulation of advanced glycation end products (AGEs) in plasma and tissues, which might be a causative factor for diabetic complications or vascular dysfunctions. Our recent studies showed that macrophage scavenger receptor (MSR) mediates endocytic uptake of AGEs-proteins by macrophages or macrophage-derived cells, implicating a possibility that the AGEs-receptor-mediated endocytic uptake of AGEs in circulation or AGEs deposited extracellularly may contribute to some extent to protect or reduce AGEs-accumulation in vivo. As an extension of this notion, the present study was undertaken to examine effects of insulin signaling on the MSR-mediated endocytic uptake of AGEs-proteins. Coexpression of human insulin receptor (IR) with MSR in Chinese hamster ovary (CHO) cells showed a 1.6-fold accelerating effect of insulin on the degradation of AGE-proteins compared with wild type CHO cells, whereas insulin had no effect on the cells coexpressing MSR with kinase-deficient insulin receptor or insulin receptor lacking binding site for insulin receptor substrate 1 (IRS-1). Furthermore, the insulin-induced endocytic activity for AGEs-proteins were inhibited by phosphatidylinositol-3-OH kinase (PI3 kinase) inhibitors such as wortmannin and LY294002. Thus, it is suggested that insulin might accelerate the MSR-mediated endocytic uptake of AGEs-proteins through a PI3 kinase pathway.

1706

Inhibitory Effect of TROGLITAZONE (CS-045) on Cytokines-Induced Monocyte Chemoattractant Protein-1 (MCP-1) Expression in Human Mesangial Cells.

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Insulin resistance is one of the risk factors for progression of atherosclerosis. In diabetes, atherosclerosis and microvascular complications such as diabetic nephropathy may account for the disabilities and mortality rate of this disease. Recently, the new oral insulin sensitizing drug troglitazone (CS-045) is thought to be hopeful for the treatment of diabetes. Monocyte chemoattractant protein-1 (MCP-1) is a recently identified cytokine, which plays an important role in the pathogenesis of atherosclerosis, including glomerulosclerosis through the induction of monocyte migration. However, the direct effects of CS-045 on the progression of glomerulo -sclerosis have not been clarified yet. Thus, we investigated the effect of CS-045 on the expression of MCP-1 in human mesangial cells (HMCs). HMCs were treated with or without CS-045 (1-10mM) in the presence or absence of various concentrations of cytokines such as tumor necrosis factor-α (TNF-α) (50-500 ng/ml), interleukin-1β (IL-1β) (1-1000 pg/ml) and phorbol myristate acetate (PMA) (5-100 ng/ml), and then the amount of the MCP-1 released from HMCs was measured. Although the release of MCP-1 was increased by various cytokines (TNF-α to 55 fold, IL-1β to 2.7 fold and PMA to 4.9 fold vs control), CS-045 significantly inhibited the TNF-α-induced (49.3%), IL-1β-induced (35.0%) and PMA-induced (33.7%) MCP-1 production. Moreover, Northern blot analysis revealed that there was a decrease of the MCP-1 mRNA level in HMCs treated with CS-045. Our present studies indicated that CS-045 may prevent the progression of glomerulosclerosis by inhibition of MCP-1 expression in HMCs.

1707

MACROPHAGE SCAVENGER RECEPTOR MEDIATES GREATER PART OF THE ENDOCYTIC UPTAKE OF AGEs

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Cellular interaction of advanced glycation end products (AGEs) is mediated by the AGE-receptor. The AGE-receptors so far reported are RAGE, Galectin-3 and MSR (macrophage scavenger receptor). Macrophages or macrophge-derived cells are known to show the highest endocytic activity for AGEs-proteins. Our recent study using CHO (Chinese Hamster Ovary) cells overexpressed with MSR clearly showed that the endocytic uptake of AGE-proteins by macrophages are mediated by MSR (1). To strengthen this contention, the present study was undertaken to examine the interaction of AGE-proteins with peritoneal macrophages from MSR-deficient mice (MSR -/-). In experiments at 37°C, thioglycolate-induced peritoneal macrophages from MSR -/- showed a marked decrease (less than 10%) in the endocytic degradation capacity for ^{125}I-acetylated low density lipoprotein (acetyl-LDL). Under the parallel conditions, the degradation activity of ^{125}I-AGE-bovine serum albumin (BSA) by these MSR-deficient macrophages was less than 30% (2). The remaining endocytic capacity of ^{125}I-AGE-BSA by these MSR-deficient macrophages was not inhibited by acetyl-LDL, but inhibited significantly by AGE-BSA, AGE-hemoglobin or polyanions such as dextran sulfate and polyinosinic acid. These results indicate that approximately two thirds of endocytic uptake of AGE-proteins by macrophages is mediated by MSR, while the remaining part is mediated by other AGE-receptors.

1708

PROSTACYCLIN-STIMULATING FACTOR IS REGULATED BY PROTEIN KINASE C ACTIVATION INDUCED BY HIGH GLUCOSE LEVELS IN DIABETES MELLITUS.

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Prostacyclin (PGI_2) synthesized by the vascular wall is a potent vasoactive prostanoid which regulates vascular tonus and inhibitoin of platelet aggregation. Reduced PGI_2 could play a key role in the development of macrovascular complications in diabetes mellitus. We have purified and cloned a newly identified PGI_2-stimulating factor (PSF) which regulates PGI_2 production by endothelial cells. The purified PSF is an acid-labile and anionic protein, with a molecular mass of 31 kDa on SDS/ PAGE. A cloned cDNA of PSF is 1124 bp which codes 282 amino acids from the putative first methionine with a potential N-glycosylation site at position 171. The expressions of PSF mRNA were decreased in the aorta from streptozotocin-induced diabetic rats (26±5%, n=6) and smooth muscle cells from diabetic patients (35±4%, n=5). The PSF protein expressions are decreased in the human coronary artery from diabetic patients (44±7%, n=5). As hyperglycemia has been shown to activate the protein kinase C (PKC) in the vascular tissues, we investigated the effects of glucose and PKC activation on the expression of PSF in aortic smooth muscle cells. The expression of PSF mRNA was reduced by increasing glucose levels from 5 to 22 mM. PKC agonist (PMA) and high glucose level (22 mM) reduced PSF mRNA expression to 38±8% (n=3) and 27±6% (n=5), respectively, as compared to low glucose level (5.5 mM). PKC inhibitor (GF109203X) abolished the effect of PMA and glucose-induced decrease of PSF mRNA levels. These findings suggest that PSF gene expression could be down-regulated by PKC activation which may be caused by high glucose levels, possibly resulting in the reduction of vascular PGI_2 synthesis in diabetes mellitus.

1709

THE RECEPTOR FOR ADVANCED GLYCATION END PRODUCTS MEDIATES THE CHEMOTAXIS OF SMOOTH MUSCLE CELLS

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Long-term incubation of proteins with glucose leads to advanced glycation end products (AGE). We recently demonstrated the intracellular AGE-accumulation in smooth muscle cell (SMC)-derived foam cells in the atherosclerotic lesions. To understand the mechanism of AGE-accumulation in SMC, we characterized the interaction of AGE-proteins with rabbit arterial SMC. In experiments at 4°C, ^{125}I-AGE-bovine serum albumin (AGE-BSA) showed a dose-dependent saturable binding to SMC. In experiments at 37°C, AGE-BSA underwent receptor-mediated endocytosis and lysosomal degradation. The endocytic uptake of ^{125}I-AGE-BSA was effectively inhibited by unlabeled AGE-proteins, but not by acetylated LDL and oxidized LDL, ligands for the macrophage scavenger receptor (MSR), and amphoterin, a ligand for one-type of the AGE-receptor (RAGE), and 2-(2-Furoyl)-4(5)-(2-furanyl)-1H-imidazole-hexanoic acid-BSA, a ligand for the other AGE-receptors, p60 and p90, indicating that the endocytic uptake of AGE-proteins is mediated by an AGE-receptor distinct either from MSR, RAGE, p60 or p90. To examine the functional role of this receptor, the migratory effects of AGE-BSA on SMC were tested. Incubation with AGE-BSA resulted in the dose-dependent cell migration. This phenomenon was chemotactic, and was significantly inhibited by an antibody against TGF-β, and the amount of TGF-β secreted into the culture medium from SMC was 7-fold higher than that of control, indicating that TGF-β is involved in the AGE- induced SMC chemotaxis. These data suggest that AGE may play a role in SMC migration in atherosclerotic lesions.

1710

THE TRANSCUTANEOUS IMPEDANCE MEASUREMENT FOR ASSESSMENT OF SOFT TISSUES ISCHAEMIA

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Macrovascular disease is a common complication of diabetes mellitus. Accordingly our data 24.3% of patients with foot problem due to blood vessels obstruction are diabetics. Objectiv assessment of the degree of ischaemia is not a solved problem now. At the same time the division ischaemic foot lesious from neuroischaemic is important because of influence clinical management. The measuring of transcutaneous oxygen pressure which is commonly used for mentioned purpose is enough expensive. We proposed the method of transcutaneous impedance measurement for assessment the degree of foot soft tissues ischaemia. It's based on the biological tissues electric resistance. The level of impedance in nondiabetic healthy people with normal major vessels blood flow is 49.1 +- 12.1 Om.30 patients with acute ischaemia and 30 patients with chronic ischaemia were studied. The results of clinical and morphological investigatious were assessed as well as impedance measuring. The localisation of obstruction was varied. Occlusia was caused by diabetic and nondiabetic atherosclerosis, thrombangitis or trauma of artery. It was established the results of the transcutaneous impedance measurement were not dependent on aetiology and localisation of obstruction. The severity of acute and chronic foot ischaemia was correlated to soft tissues electric resistance: I degree - 65.2 +- 0.6 Om; II degree - 66.7 +- 0.8 Om; III degree - 68.3 +- 0.7 Om; IV degree - 69.4 +- 0.3 Om. The level of impedance more then 69 Om was the sign of morphologically confirmed necessity of amputation. We consider described method of soft tissues ischaemia assessment as useful and costsaving test in clinical management of patients with diabetes.

1711

ELEVATION OF SUPEROXIDE DISMUTASE ACTIVITIES IN PATIENTS WITH NIDDM

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Hyperglycemia causes glycation of proteins and produce superoxide anion radicals(O_2^-), which has been postulated to develop diabetic angiopathy. To estimate the production of O_2^-, superoxide dismutases (SODs), O_2^--scavenging enzymes, were measured in 60 NIDDM patients and 50 healthy controls. The NIDDM patients were divided to two groups, the good control(GC; HbA1c≤6.9 %) and the poor control(PC; HbA1c≥7.0 %). The serum SOD activities were examined by electron spin resonance spectrometry using spin trapping method. The total SOD activities were measured and then, fractionated to Mn-SOD and Cu,Zn-SOD activities using CN^-. The total-, Mn- and Cu,Zn-SOD activites were positively correlated with HbA1c and glycated alubumin ($p<0.01$) measured by the automated HPLC analyzer, HA-8131 and GAA-2000 (Kyoto-Daiichi, Kyoto, Japan). Mn- and Cu,Zn-SOD activities were 1.98 ± 0.41 and 1.18 ± 0.55 U/ml in the control, 3.27 ± 1.30($p<0.001$ vs control) and 1.03 ± 0.79(NS vs control) U/ml in the GC, and 3.35 ± 1.55($p<0.001$ vs control) and 1.76 ± 1.20($p<0.05$ vs control) U/ml in the PC, respectively. Significant differences were found both between the control and the GC and between the control and the PC in Mn-SOD activities whereas in the Cu,Zn-SOD activities, significance was found only between the PC and the control.These data suggest that hyperglycemia causes the production of O_2^- and resultant increase in the SOD activities, but a different SOD is recruited depending on the glycemic control.

1712

The effect of n-3 fatty acids on the suppression of atherogenesis in the type 1 diabetic rats: Morphologic study.

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Thirty two male Wistar albino rats were included into the study with the purpose of investigating the effects of n-3 fatty acids on the development of atherosclerotic lesions in diabetic rats.Twenty rats were randomly selected and injected 60mg/kg/sc streptozotocin to make them diabetic. Of 20 rats with diabetes ,10 were given MaxEPA [Eikosapentonoik acid (EPA) + Dekosaheksanoik acid (DHA)] , 10 mg EPA and 7mg DHA per day)] .12 rats were taken into the control group. 6 rats chosen randomly were also on MaxEPA therapy. After 4 months their aorta examined with electronmicroscopy. In TEM findings of the aorta of the diabetic rats the luminal surface of endothelial cells was irregular , dilation of ER was noted, as well as decreased mitocondria number . The nuclei were pycnocytic. On the apical side of endothelial cytoplasm different sized vacuoles were found . At SEM level fibrin and cell wask substances were seen on the luminal surface. When N-3 fatty acids were administrated to diabetic rats TEM findings showed a much more regular surface when compared with the sole diabetic group. The vacuolar degeneration in endothelial cell was lesser . At SEM level a localised zone of degeneration was seen , evaluated as a crater developed after a treatment of N-3 fatty acid at the site of an aterom plaque. In the control group given N-3 fatty acids , TEM and SEM findings were similar with that of the control group which was not on N-3 fatty acids.

In the light of these findings , we can suggest that the use of n-3 fatty acids in order to prevent atherosclerosis in diabetes mellitus is effective and there is no side effects on the normal vessel structure.

1713

OXYDATIVE STRESS AND DIABETES CHRONIC COMPLICATIONS.
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Having as a purpouse research on correlation between diabetes chronic complications and conditions of lipid peroxidation studies were made on content of MDA in blood plasma, erythrocytes membranes and HDL. Positive correlation was determined between MDA content and diabetic angiopathys as well as neuropathys. MDA content had positive correlation with some degree of diabetic neuropathy (r=+0,63), angiopathy of legs (r=+0,53), periferic somatic polyneuropathy (r=+0,50), also vegetative cardiovascular, gastrointestinal and urogenital neuropathy (r=+0,47). Negative correlation was determined between MDA content and erythrocites deformability (r=-0,51). Sustainable and long time diabetes' compensation as well as entherosorption by carbon and natural fibrous sorpents are furthering factors of the lipid peroxidation. These sorpents re-activate the function of hepato-biliar system, further diabetes compensation and slow the lipid peroxidation, thus preventing development of diabetic angio- and neuropathies. Therefore, lipid peroxides are damaging factors for diabetic patients. Entherosorption by carbon and natural fibrous sorpents is a protective way in the progress of chronic vascular and neurologic diabetes complications.

1714

Effects of insulin and Tranilast on cytosolic free Ca^{2+} concentration and DNA synthesis of aortic smooth muscle cells

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In diabetic macroangiopathy, hyperinsulinemia induced by insulin resistance causes atherosclerosis. An essential event in atherogenesis is the proliferation and migration of vascular smooth muscle cells. The effects of insulin and tranilast(TRA), anti-allergic drug (N-(3,4-dimethoxycinnamoyl)anthranilic acid), on cytosolic free Ca^{2+} concentration ($[Ca^{2+}]i$) and DNA synthesis of cultured rat aortic smooth muscle cells (SMC) were investigated. SMC were isolated from fresh thoracic aortic media of 5-week-old male Wistar rats by enzyme dispersion methods and cultured in DMEM supplemented with 10% fetal calf serum (FCS). Cells at passages 5-10 were used in this study. Measurement of cytosolic free Ca^{2+} concentration ([Ca2+]i) was performed by the Ca^{2+} fura-2 fluorescence method. Insulin ($1X10^{-4}M$) significantly increased $[Ca^{2+}]i$ of SMC, from 20nM to 60nM. TRA ($1X10^{-4}M$) significantly inhibited the increase in $[Ca^{2+}]i$ of SMC induced by Insulin, from 40nM to 2nM. After SMC were incubated 48 h in medium without serum, SMC were incubated in media with or without various additions of insulin ($3X10^{-6}M$, $1X10^{-4}M$), TRA ($1X10^{-5}M$, $3X10^{-5}M$, $1X10^{-4}M$, $3X10^{-4}M$, $1X10^{-3}M$), and 0.5 μCi [methyl-^{3}H] for 18 hrs. The [^{3}H]-thymidine uptake was examined. Insulin ($3X10^{-6}M$,$1X10^{-4}M$) significantly increased [^{3}H]-thymidine uptake of SMC (150%,200%) in a concentration dependent manner. TRA inhibited the DNA synthesis of SMC induced by insulin ($3X10^{-6}M$) dosedependently. TRA ($3X10^{-4}M$, $1X10^{-3}M$) significantly inhibited the DNA synthesis of SMC induced by insulin, (40%,30%) respectively.

These findings suggest that Tranilast might inhibit the proliferation of vascular smooth muscle cells induced by hyperinsulinemia and ameliorate diabetic macroangiopathy and prevent atherosclerosis.

1715

OXIDATIVE STRESS IS PRODUCED DURING MEAL IN NIDDM PATIENTS.

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Free radical production has been reported to be increased in patients with diabetes mellitus. In this study a standard meal (600 Kcal, 49% as carbohydrates, 40% as fat, and 11% as protein, containing 100 mg vitamin C and 13 mg vitamin E) was aministered to 10 NIDDM patients (7 males and 3 females; age 54.1±3.5 years, mean±SD; duration of diabetes 10.2±3.2 years; BMI 25.9±2.1 Kg/m^2). Two h after meal, SH groups (484.5±58.4 vs 440.7±48.3 μmol/l, p<0.003), and uric acid (5.3±1.3 vs 4.9±1.1 mg.dl, p<0.001) were significantly decreased, vitamin C increased (3.34±0.9 vs 4.17±1.1 mg/l, p<0.01), while vitamin E unchanged. MDA (2.3±0.6 vs 3.2±0.6 μmol/l, p<0.001) significantly increased, while total plasma radical-trapping activity (TRAP), which evaluates plasma antioxidant capacity due to known and unknown antioxidants present in the plasma as well as their mutual cooperation, was reduced (542.4±71.7 vs 458.4 vs 47.9 μmol/l, p<0.001). LDL oxidizability significantly increased after meal (Lag time: 81.7±23.8 vs 71.5±14.8 min, p<0.03; peak time: 141.6±31.4 vs 127.8±23.7 min, p<0.01; rate: 9.6±1.3 vs 11.5±1.8 μmol/minxgr protein,p<0.001) This finding shows that in diabetic patients during meal an oxidative stress is produced.

1716

IMMUMOHISTOCHEMICAL STUDY OF MYOCARDIAL REMODELING IN DIABETIC RATS

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<Aim>To clarify the effect of diabetes mellitus on remodeling after myocardial infarction,experimental myocardial infarction was induced in diabetic rats,and histological features of their heart tissues were determined by immunohistochemical staining as well as by computed image analysis.<Method&Materials>At six weeks of age male Wistar rats were divided into two groups;one was the diabetic group (85mg/Kg body weight)and the other the healthy control group. Ligation of right coronary artery was performed on rats of both group. Eight weeks after the ligation, the heart of each rat was removed and fixed. Specimens were stained by the Masson-trichrome stain.For evaluation of changes in the extracellular matrix,tissues were also stained by ABC method.Anti-rat antibodies raised in rabbits against fibronectin and collagens, of Type I,III,IV,V and VI were used for the immunohistochemical staining,respectively.For quantitative evaluation of the degree of fibrosis, 80 points were randomly selected in the anterior free wall of the non-injured left ventricles,and the percentage of fibrotic area was calculated by a computer analyzer.<Results>The degrees of deciduation and of regenerative fibrosis as well as the major molecular species of the extracellular matrix did not show any significant difference in areas of myocardial infarction between diabetic group and control one. In the diabetic group,however, the fibrotic pattern developed to a severer degree in areas without myocardial infarction than in the correesponding areas in the control group. Percentage of fibrosis in the areas without myocardial infarction was 2.39±0.39% in the diabetic group.The value was significantly (p<0.05)higher than the corresponding value of 1.58±0.24% in the control group.Immunohistochemical staining showed that the components of the fibrotic areas in the extracellular spaces were collagens of Types III and VI and fidronectin in the areas without myocardial infarction of the diabetic group.

1717

THE GLYCOXIDATION PRODUCT N^{ε}-(CARBOXYMETHYL) LYSINE IS INCREASED IN DIABETES AND AGING

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N^{ε}-(Carboxymethyl)lysine (CML) a major product of oxidative degradation of glycated proteins has been suggested to represent a general marker of oxidative stress and long-term damage to proteins in aging, atherosclerosis, and diabetes. It was our aim to study the occurrence and distribution of CML in these conditions. Therefore, we produced an antiserum which specifically recognizes protein-bound CML. In-vitro studies showed that the formation of CML was dependent on the presence of oxygen and on glycation of proteins. Furthermore, the oxidative formation of CML from glycated proteins was reduced by antioxidants e.g. lipoid acid and vitamin E. Immunolocalization of CML in human skin, lung, heart, kidney, intestine, intervertebral discs and particularly in arteries provided evidence for an age-dependent increase in CML accumulation in distinct locations, and acceleration of this process in diabetes. Intense staining of the arterial wall and particularly the elastic membrane was found. High levels of CML modification were observed within atherosclerotic plaques and in foam cells. The positively stained dermal connective tissue area was 3.1±0.9% in young adult, 22±1.5 in old adult and 56.5±11.3% in old diabetic patients. Corresponding values for arterial walls were 8.3±0.5%; 22.3±0.8% and 25.2±3.8%. The preferential location of CML immunoreactivity in lesions may indicate the contribution of glycoxidation to the processes occurring in diabetes and aging. Determination of CML-content in normal (25) and diabetic (37) sera revealed an increase in diabetes (7.3±2.9 vs. 11.6±4.1 pmol/mg protein). CML serum values correlated weakly to HbA1c (r=0.70). In summary, our observations, however, provide strong evidence for the enhanced formation of CML-modified proteins in aging and diabetes glycoxidation. Furthermore, CML may serve as an indicator for the efficiency of therapeutic approaches for limiting oxidative reactions in human subjects.

1718

MYOCARDIAL HYPERTROPHY IN THE NEWBORN GOTO KAKIZAKI RATS.

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Left ventricle hypertrophy is frequent in diabetic patients and it is not always due to hypertension. In Goto Kakizaki (GK) rats, a model of non-obese genetically-determined diabetes, we have previously shown that a cardiac hypertrophy occurred without any significant increase in blood pressure, but that hemorheological disorders might be involved in cardiac hypertrophy . The aim of the present study was to examine the onset of cardiac hypertrophy and the myocardial chemical composition, using male Wistar rats as controls. After sacrifice of the animals, at 4 or 15 days, or 3, 6, 12, or 18 months, heart was weighed and several biochemical parameters were measured. In the GK rats cardiac weight and cardiac index were significantly higher than in control rats, by 4 days, and the difference was significant at each age. Heart proteins and triglycerides were significantly lower than in control rats. Heart sodium (at 3 months), potassium and magnesium (at 6 and 12 months) and calcium (at 6 months) were significantly lower in the GK rats. Fibronectin was determined in the heart of 6-months animals and was not significantly different in GK and control rats. In conclusion this study suggests that in the GK rats, 1) cardiac hypertrophy may be found very early and is constituted during foetal life ; 2) it occurs independently of any hypertension ; 3) fibrosis is not the main factor involved in cardiac hypertrophy.

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ASSOCIATION BETWEEN LENS PROTEIN GLYCATION AND CATARACT DEVELOPMENT IN DIABETIC RATS

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To assess the temporal association between glycation of lens proteins and the creation of polymeric products believed to contribute to the onset of cataract we followed the formation of early and late glycated adducts in the lenses of hyperglycaemic rats during a period of 5 months. We examined, whether phlorizin treatment of diabetic rats by inhibiting renal tubular glucose reabsorption influences advanced glycation process. The study groups included controls (C),untreated diabetic rats (D), and diabetic rats receiving insulin alone (DI) or in combination with phlorizin (DIP).Lenses were removed at 4 and 20 wk, and advanced glycated products in alkali-soluble lens proteins were determined by their spectrofluorescence (385/335nm).In 20-wk untreated diabetic as compared to control rats, a significant increase was observed in the fluorescence level (3.25±1.02 vs 1.61±0.17 FU/mg, p<0.001), while in 4-wk animals the increase was from 1.26±0.11 FU/mg in controls to 1.80±0.25 FU/mg in diabetics, p<0.001.Daily treatment of 20-wk diabetic rats with insulin alone (2.46±0.48 FU/mg) or in combination with phlorizin (2.30±0.26 FU/mg) did not significantly influence lens fluorescence level. The amount of glucose bound ketoamine linkage was estimated after acid hydrolysis as released 5-hydroxymethylfurfural (HMF).In 20-wk controls, it was slightly higher than in 4-wk controls (0.57±0.31vs0.41±0.20 nmolHMF/mg, respectively).The diabetic group showed a significant increase, however. In 4-wk diabetics, a level of 1.07±0.36 nmol HMF/mg was found, while in 20-wk animals the glycated protein amount rose to 2.46±0.79 nmol HMF/mg.In addition to the increases in glycated content with continuing diabetic hyperglycaemia, significant changes in protein composition of alkali-soluble lenses developed. The SDS-PAGE pattern showed an appearance of protein polymers of heterogeneous size (C-4wk: 3.0±1.1% vs C-20wk:17.9±2.9%; D-4wk:7.3±2.1% vs D-20wk:19.8±3.6%) and the proteins of high molecular weight (HMW).Only a small amount of these HMW proteins was present in controls (C-20wk: 2.5±1.2%) and short-term diabetes (D-4wk: 0.8±0.2%), whereas in long-term untreated diabetes there was a dramatic increase (D-20wk: 30.5±3.2%) with a corresponding decrease in other peaks. All diabetic animals from this group had macroscopically detectable cataractous lenses. The treatment with insulin or insulin/phlorizin followed the HMW protein level of the untreated animals (28.2±4.0% or 27.08±3.3% vs 30.52±3.32%) without beneficial effect.

1720

A SIMPLE METHOD FOR DETECTION OF ELASTIN PEPTIDES IN PATIENTS WITH DIABETIC MACROANGIOPATHY.

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Elastin is a main protein responsible for the elasticity of vascular wall. The elastin degradation is accelerated in atherosclerosis and soluble elastin-derived peptides (EDP) are then released and can reach the circulating blood. In this study was used a simple enzyme-linked immunosorbent assay (ELISA) for measurement of EDP in sera of 25 patients with diabetic macroangiopathy. The microtitre plates were coated directly with diluted (1:10) samples, standards or controls, blocked with 1% solution of bovine albumin to prevent non-specific protein binding to the plates, and EDP assigned with specific rabbit antibody. The specific antibody was detected with peroxidase linked goat anti-rabbit IgG and the color development of o-phenylenediamine was measured at 492 nm. Determinations were carried out in triplicate. Intra-assay variation was less than 7% and inter-assay variation -less than 10%. The new method was compared with sandwich version of ELISA. The mean level of EDP by sandwich version was 276±88 ng/ml vs. 251±76 ng/ml by new variant of ELISA (p>0.05). In conclusion, this modified variant of ELISA is a simple and faster method for detection of elastin degradation in diabetic macroangiopathy.

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CARDIOVASCULAR EFFECTS OF HYPERINSULINISM AND VAGOSYMPATHETIC CONTROL IN THE RAT WITH VENTROMEDIAN HYPOTHALAMIC LESIONS.

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Insulin has a well-demonstrated accelerating effect on heart rate, but its pressor effect is still debated. The cardiovascular effects of insulin mainly result from sympathetic activation. In order to test the cardiovascular effects of endogenous hyperinsulinism, the model of rats with ventromedian hypothalamic lesions (VMH) was used. Nine male VMH rats and 7 male SHAM rats were followed until 18 weeks after operation. In VMH rats, weight was significantly higher than in SHAM rats by the first week after operation, plasma insulin by 24 hours, reaching 224 ± 40 pmol/l at 1 week and being maximal at 8 weeks (476 ± 62 vs 38 ± 3 pmol/l in SHAM rats, $p < 0.001$), blood glucose was significantly lower since the first week. Heart rate (HR) and blood pressure (BP) were monitored by a telemetric method (Data Sciences System) after implantation of an aortic catheter and were weekly recorded during 24 hours. In VMH, HR was significantly lower by the first week ($p = 0.02$), reaching its lowest level at 4 weeks (306 ± 7 vs 399 ± 6 beats/min before operation). Systolic BP increased slowly in both groups and was significantly different only at 8 weeks (VMH : 129 ± 2 vs SHAM : 121 ± 3 mmHg, $p = 0.03$). In VMH rats a positive correlation was found between HR and weight and plasma insulin, and between systolic BP and weight. In conclusion, 1) VMH rats show an early, persistent and significant bradycardia, which is likely to result from an increase in parasympathetic activity and a decrease in sympathetic activity, and a moderate increase in BP associated with weight gain ; 2) both bradycardia and the delayed and moderate increase in systolic BP compared with the rapid and marked increase in plasma insulin suggests that the integrity of sympathetic control is necessary for insulin to induce its hemodynamic effects totally.

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ACE GENE POLYMORPHISM AND ARTERIOSCLEROSIS IN JAPANESE NIDDM PATIENTS

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AIM: There is an insersion/deletion(I/D) polymorphism in the angiotensin converting enzyme(ACE) gene. ACE gene DD type was reported as a possible genetic risk factor for myocardial infarction(MI) in Caucasian and Japanese. We investigated the effect of ACE gene polymorphism on arteriosclerosis and MI in the Japanese diabetic patients. METHODS: Subjects investigated were 200 unrelated Japanese NIDDM patients. We measured the averaged thickness of the intimal plus medial thickness (IMT) of the carotid arteries of subjects using an ultrasound high resolution B-mode imaging. The genotypes of ACE gene were determined polymorphism using PCR amplification of the genomic DNA. Contribution of ACE gene polymorphism to IMT was evaluated with multiple regression analysis. Also we analyzed the association between past history of MI and ACE polymorphism using Kai square analysis.
RESULTS: The frequency of D allele was 0.34, which was lower than that of Caucasian as previous reported. Multiple regression analysis disclosed that existence of DD type increased IMT by 0.12 mm. In those with advanced arteriosclerosis (IMT≥1.3 mm), the prevalence of MI observed in NIDDM with DD type was more than that of nonDD type ($\chi 2=5.2$, $p=0.02$). But this was not the case with those lacking advanced arteriosclerosis(IMT<1.3 mm). CONCLUSION: Co-monitoring of IMT and ACE genotype may be useful for finding high risk individuals for MI in NIDDM patients.

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PREVALENCE, INCIDENCE AND FACTORS CONNECTED WITH ISCHEMIC HEART DISEASE IN IDDM AND NIDDM PATIENTS

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Ischemic heart disease and myocardial infarction are the main causes of death of diabetic patients. To assess the impact of the selected external and internal connected with diabetes mellitus factors the prospective study was started aimed at the prevalence and incidence of ischemic heart disease and myocardial infarction in a large cohort of diabetics. 1643 patients were examined (309 diabetics type I, 1334 diabetics type II). The frequency of ischemic heart disease was 12.9% in diabetes type I and 35.6% in diabetes type II. The frequency of myocardial infarction was respectively 6.1% and 16.9%. In 39.6% of diabetics type II and in 12.5% of diabetics type I ischemia was found in ECG records in the absence of clinical symptoms (silent ischemia). During 3 - year observation 193 cases of ischemic heart disease were newly diagnosed (19 in diabetes type I and 174 in diabetes type II). 61 patients experienced myocardial infarction (7 in diabetes type I and 54 in diabetes type II). Then the relations between prevalence or incidence and the factors: age, sex, duration of diabetes mellitus, body mass index, arterial hypertension, total cholesterol triglycerides and creatinine levels, fasting and postprandial glycemia and 24-hour proteinuria were analysed. The most significant risk factors connected with development of ischemic heart disease were: age, duration of diabetes, postprandial glycemia, arterial hypertension and creatinine level. Conclusion. The study allowed to assess the connection between studied parameters and ischemic heart disease. This could serve for the practical use of the designing of the care program for the people from a group with the highest risk.

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GLN-ARG 192 POLYMORPHISM OF PARAOXONASE GENE IS NOT RELATED WITH CARDIOVASCULAR DISEASES IN PATIENTS WITH NIDDM. YS Kim, WS Kim, JT Woo, SW Kim, IM Yang, JW Kim, YK Choi. Kyung-Hee University, Seoul, Korea

Paraoxonase is located in a high-density-lipoprotein. It has been known to protect lipoproteins from oxidation. Paraoxonase has a genetic polymorphism with single amino acid substitution which arises from a glutamine(A isoform) to arginine(B isoform) interchange at position 192. In French Caucasian, paraoxogenase polymorphism was reported as an independent cardiovascular risk factor in patients with NIDDM. We investigated the relevance of this polymorphism to cardiovascular disease in Korean patients with NIDDM and normal subjects. Of the 66 patients, 30 had confirmed coronary artery disease or cerebral infarction. The other 36 had no history of such diseases and ECG abnormality. Sixty eight normal subjects had no medical history and family history of diabetes. The genotype frequency did not differ between patients with NIDDM and normal subjects(AA: 13.6 % *vs* 16.2 %, AB: 44 % *vs* 33.8 %, BB: 42.4 % *vs* 50 %). There was also no difference in genotype frequency between diabetic group with cardiovascular disease and diabetic group without cardiovascular disease(AA: 13.9 % *vs* 13.3 %, AB: 47,2 % *vs* 50 %, BB: 38,9 % *vs* 36.7 %). Compared with subjects homozygous for the A allele(AA genotype), the odds ratio of cardiovascular disease for subjects homozygous for the B allele was 0.8(95 % CI 1.3 ~ 1.7, $P = 0.784$) and that for those heterozygous for the B allele was 1.3(95 % CI -1.8 ~ 1.2, $P = 0.703$). These data suggest that the polymorphism of paraoxogenase is not an risk factor of cardiovascular disease in Korean patients with NIDDM.

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RISK FACTORS FOR THE ONSET OF CARDIOVASCULAR DISEASE DURING THE TREATMENT OF NIDDM

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The aim of our study was to evaluate the risk factors for the onset of cerebral infarction(CI) and myocardial infarction(MI) in NIDDM, retrospectively. 【METHOD】 In 92patients (33 with CI (DM-CI), 22 with MI (DM-MI) and 37 with no evidence of CI or MI (Control) during 3 years) ,the following risk factors were analyzed : age, sex, duration of DM, alcohol intake, smoking, BMI, blood pressure, serum lipid and HbA1c levels before the onset of CI or MI. 【RESULTS】 Male was significantly higher in DM-MI ($p<0.05$) than those in DM-CI . There were no significant differences in age, alcohol intake, smoking and duration of DM among 3 groups. In DM-CI, BMI (24.4 ± 4.0Kg/m^2), systolic and diastolic blood pressure (152.2 ± 18.9/86.7 ± 12.9mmHg), and serum triglyceride(TG) (167.8 ± 90.1mg/dl) were significantly higher ($p<0.01$) than those in the other 2 groups. Serum HDL-cholesterol (HDL-C) was significantly lower ($p<0.01$) in DM-CI (40.0 ± 8.7 mg/dl) and in DM-MI (42.1 ± 9.7mg/dl), and LDL-cholesterol (LDL-C) was significantly higher ($p<0.01$) in DM-CI (136.2 ± 32.4mg/dl) and in DM-MI (135.0 ± 32.0 mg/dl) than those in Control (63.6 ± 23.5mg/dl, 117.3 ± 24.0 mg/dl, respectively).However, total cholesterol showed no significant difference among 3groups. HbA1c level was significantly increased during 3 years in DM-CI. 【CONCLUSION】 Male, obesity, hypertension, high TG and LDL-C, low HDL-C, and HbA1c level are important predictors for the onset of cerebral infarction in NIDDM. Moreover, high LDL-C and low HDL-C are also predictors of myocardial infarction.

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MARKERS OF COAGULATION AND FIBRINOLYSIS ARE THE PREDICTIVE FACTORS OF CORONARY HEART DISEASE IN PATIENTS WITH NIDDM

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AIM: Coronary heart disease (CHD) is one of very important complications in patients with diabetes mellitus and is recently more and more notable because of its prevalence. However, its prediction still remained to be difficult. We investigated whether several markers of coagulation and fibrinolysis could be predictive factors of CHD in patients with DM. **METHOD:** 59 patients with NIDDM (mean age: 61.4 ± 11.0 years, 27 male and 32 female) were followed for 4 years. At the beginning of this study , blood samples were collected for measuring HbA1c, tissue factor (TF), thrombomodulin (TM) , fibrinogen (FBG), antithrombin III (ATIII), factor VII activity (FVIIa), tissue plasminogen activator (tPA), plasminogen activator inhibitor-1 (PAI-1), PA-PAI complex (PAIc), α_2-anti-plasmin-plasmin complex (PIC) . Urine samples were also collected for evaluation of urinary albumin index (UAI) calculated by dividing urinary albumin by urinary creatinine. In each patient, ECG was performed at the beginning and once a year during this study. The subjects were divided into 2 groups by onset of CHD(ischemic change of ECG, angina pectoris or acute myocardial infarction) during this study as follows: group H (onset (+)) and N(onset(-)). Differences of HbA1c, UAI and the markers between 2 groups were tested by Mann-Whitney test. Discriminant analysis was performed for investigating more contributable markers of the discrimination. **RESULT:** 11 patients(18.6%) had new onset of CHD. Significant differences between H and N were observed as follows; HbA_1c(8.53 vs.9.85%, $p<0.05$) · UAI(216.83 vs.1785.2mg/g·Cre, $P<0.001$) · TM(15.40 vs.24.39FU/ml, $p<0.025$) · FBG (242.5 vs.291.3mg/dl, $p<0.01$) · FVIIa (106.0 vs.128.1%, $p<0.01$) · PIC(58.14 vs. 62.50ng/ml, $p<0.025$) (mean, N vs. H, p value) . Discriminant analysis showed that PIC($p<0.025$), ATIII ($p<0.025$) and UAI ($p<0.01$) contributed to the discrimination. **CONCLUSION:** PIC and ATIII may be significant predictive factors of CHD in patients with NIDDM.

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EFFECTS OF PHYSICAL EXERCISE ON CARDIOVASCULAR RISK FACTORS IN WELL CONTROLLED DIABETIC PATIENTS

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The aim of the study was to evaluate the effect of physical training on cardiovascular risk factors in well-controlled diabetic patients. Fourteen normotensive type I (7 male, 7 female, age 25 ± 6 years, BMI 23.8±3 kg/m^2, HbA1c 6.6±0.8% (reference range 4.6-5.8%) and thirteen type II diabetic patients (male 9, female 4, age 55±5 years, BMI 26±6 kg/m^2, HbA1c 7.3±1%, 84 % normotensive) were included in a supervised physical training program (3-5 days/week, 1851±387 kcal/week) during 3 months. Parameters studied included fat distribution, glycemic control, lipid profile, blood pressure and plasma homocysteine levels. Satisfactory compliance was achieved in most of the patients. Differences were assessed by paired t-test and Wilcoxon test. VO_2 máx increased significatively ($p<0.005$). After three months, no significant changes were seen in HbA1c levels, and insulin dose decreased from 0.4 ±0.2 U/Kg/day to 0.31±0.1 U/Kg/day ($p<0.05$) only in type II diabetic patients. Waist and hip (WHR) perimeters decreased from 83.2± 12 cm and 99.7±7 cm to 81.4±11 cm and 96.9±6.5 cm respectively ($p<0.05$), while WHR and BMI remained unchanged. HDL cholesterol levels increased significantly in the whole group of patients (1.37±0.42 to 1.49±0.6; $p<0.05$) , while LDL cholesterol levels changed favourably only in the type II subset (3.41±1 to 3.14±0.8; $p<0.05$). Plasma homocysteine levels and systolic, diastolic and mean blood pressure were not modified after the exercise program. In conclusion, physical training reduces insulin requirements in type II diabetes and improves lipid profile, even in well-controlled normolipidemic diabetic patients (FIS 95/1252)

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CIRCULATING VASCULAR CELL ADHESION MOLECULE-1 AS A USEFUL MARKER FOR ATHEROSCLEROSIS IN NIDDM PATIENTS

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Vascular cell adhesion molecule-1 (VCAM-1) has been shown to be highly expressed in atherosclerotic lesions. Although soluble (s) VCAM-1 is detected in human sera, it remains unclear whether sVCAM-1 level is increased in atherosclerotic patients. In this study, sVCAM-1 concentrations were measured in sera from 65 patients with NIDDM. The mean (±SD) sVCAM-1 concentration was 908 ±132 ng/ml in 15 patients with symptomatic atherosclerotic vascular diseases, which was significantly higher than the age- and sex-matched patients without the atherosclerotic vascular disease (660±139 ng/ml, P<0.001). Among 50 patients without symptomatic atherosclerotic vascular disease, 22 patients (44 %) had early atherosclerosis of the carotid arteries, based on the evaluation by high-resolution B-mode ultrasonography. Their sVCAM-1 concentration was significantly higher than that in the patients without atherosclerosis of the carotid arteries (755±235 ng/ml vs. 617±122 ng/ml, P<0.02). In addition, there was a significant correlation between serum sVCAM-1 and the intimal plus medial thickness of the carotid arteries in the patients without symptomatic atherosclerotic vascular disease (r=0.544, p<0.001). These results indicate that circulating sVCAM-1 may be a useful marker to predict atherosclerotic lesions in NIDDM patients, even when the atherosclerosis is asymptomatic process.

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CIGARETTE-SMOKING AND THE PROGNOSIS OF DIABETIC PATIENTS. A 15-YEAR OBSERVATION OF NIDDM PATIENTS IN OSAKA, JAPAN.

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The prognostic effects of cigarette smoking on diabetic patients were examined utilizing a long-term follow-up study. The subjects studied were 1,700 NIDDM patients with age at entry ≧ 35 years and with known history of smoking, who were first seen during 1960-1979 at our hospital, and followed up until the end of 1993, with the mean follow-up period of 14.8 ± 6.5 years. Smoking rates were 64.8% for males and 13.1% for females. The mortality rates per 1,000 person-years in male subjects were 32.1 for non-smokers and 37.6 for smokers, while the O/E ratios were 1.26 and 1.84 for non-smokers and smokers, respectively, both indicating statistically significant differences. The odds ratio of smokers to non-smokers in males was 1.45, and a significantly increased risk of dying in smokers compared with non-smokers was confirmed in Japanese male diabetic patients. But no significant increase in mortality was observed in female smokers. The analysis of odds ratios by causes of death indicated a significant increase in malignant neoplasms, especially in lung cancer, but cancer of other sites, such as stomach and liver, was appreciably increased. Increases in cardiovascular disease, heart and cerebrovascular disease, and in renal disease was also observed. In addition, a marked increase in pneumonia and bronchitis was noted. The odds ratio by causes of death were further analyzed in male subjects with and without proteinuria at the baseline. In the subjects with proteinuria, increases in heart disease, cerebrovascular disease and renal disease were remarkable, but no increase in malignant neoplasms was observed. By contrast, in the subjects without proteinuria, increases in malignant neoplasms and pneumonia and bronchitis were notable. Thus, it was indicated that causes of death in the smoker largely differed by presence or absence of the proteinuria at entry to the study.

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ANY ASSOCIATION BETWEEN LOW SERUM DHEAS LEVELS AND ISCHAEMIC HEART DISEASE IN NIDDM IS NEGATED BY AGE

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Patients with ischaemic heart disease (IHD) are reported to have lower serum levels of dehydroepiandrosterone sulfate (DHEAS) than control subjects, and furthermore, DHEAS supplementation in animal models appears to be protective for atherogenesis. Moreover non-diabetic subjects with the insulin resistance syndrome (Syndrome X) also have lower serum levels of DHEAS. In this study we aimed to determine the association between DHEAS levels and IHD in a population of patients with non-insulin-dependent diabetes mellitus (NIDDM).

Eighty-two males (59.7 ± 7.4yr) and 70 females (60.8 ± 12.2yr), known duration of diabetes 6.8 ± 7.4 and 6.9 ± 6.8 yr respectively, had DHEAS levels measured as part of an annual review for diabetes complications. The presence of IHD was determined by a history of myocardial infarction, angina or revascularisation procedure. By these criteria, 17 males (21%) and 6 females (9%) had clinical evidence of IHD. In males with IHD, DHEAS levels tended to be lower than those without IHD (3.05 ± 2.33 vs 4.5 ± 4.01uM; p =0.09). The findings were similar in females (1.35 ± 0.46 vs 3.22 ± 2.94uM; p= 0.126), but in both groups the difference was of borderline significance. In fact on multiple regression analysis, the major determinant of DHEAS levels in both males and females was age.

These data may be interpreted to indicate that low serum DHEAS levels may be associated with IHD diagnosed using clinical criteria. Alternatively, low DHEAS levels may be an epiphenomenon, loosely correlating with IHD due to the association of DHEAS levels with age. Larger cross sectional studies, and prospective studies involving DHEAS supplementation in NIDDM subjects may more clearly define any association.

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THE RISKS RELATED TO ISCHEMIC HEART DISEASES IN NON-SMOKER DIABETICS

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In general population obesity, hypertension, hyperglycemia, hyperlipidemia and smoking are known risk factors for ischemic heart diseases (IHD). But it is not known how much of which risk factors contribute to IHD in non-smoking group of non-insulin-dependent diabetes mellitus (NIDDM) subjects. To estimate the risk, from a total of 3,583 NIDDM subjects, we investigated 572 (M=262, F=310) randomly selected non-smokers of age 30-60yr with mean (SD) duration of diabetes 13.6 (3.6) mo. Diagnosis of IHD was based on cardiogram or exercise tolerance test or both when equivocal. Compared with non-IHD group the subjects with IHD had significantly higher age (p<0.001), higher waist to hip ratio (WHR, p=0.005), higher systolic and diastolic blood pressure (SBP and DBP, p<0.001) and higher fasting blood glucose (FBG, p<0.05). There was no difference of IHD prevalence between male and female diabetics (17.6 vs 18.1%). Compared with the male subjects the female had significantly higher body mass index (BMI, [wt in kg/ht in m^2], p<0.001), higher SBP (p=0.02) and total plasma cholesterol (CHOL p<0.001); whereas, the male had significantly higher WHR (p=0.009) and DBP (p=0.051). Both FBG and 2h after glucose (2-hBG) did not differ between men and women. Adjusting for age, sex and obesity, glycemia showed no association with WHR, SBP, DBP and CHOL; whereas, it showed signficant negative correlation with BMI (p<0.001). In contrast, BMI showed significant positive correlation with SBP (p<0.001) and DBP (p<0.01). Though IHD was not associated with increasing BMI it was significantly associated with increasing age (X^2 =18.2,p<0.0001) and high WHR (X^2 =8.1, p<0.04). Taking BMI, WHR, SBP, DBP, FBG, 2-hBG, CHOL and family history of diabetes or hypertesion as the independent risk variables and IHD as a dependent one, further analysis by stepwise logistic regression showed that high WHR (<0.95 vs > 1.1: OR, 3.0 95% confidence interval (CI) 2.2-3.8) and high DBP (<85 vs >95mmHg: OR, 12.6 95% CI 11.7-13.5) were the risks for IHD. When the age was included in the model it confounded the effect of WHR but not that of DBP. Therefore, it appears that the highest level of risk for IHD in non-smoker diabetics was observed with high DBP and increasing age, and moderate risk with high WHR.

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MALE HYPERTENSIVE NIDDM PATIENTS HAVE HIGHER SERUM INSULIN AND LOWER SEX HORMONES AND BINDING GLOBULIN.
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Hyperinsulinemia and abnormalities of gonadal hormone concentrations have been implicated in the pathogenesis of cardiovascular disease in people with normal and abnormal glucose tolerance.We measured fasting serum insulin (IRI),sex hormone binding globulin (SHBG),free testosterone (FT) and total estradiol (E_2) concentrations in 95 non insulin dependent (NIDDM) male diabetic patients (mean age 62.06,range 39-75 years) on treatment either with diet or/and oral hypoglycaemic agents.57 patients were normotensive and 38 hypertensive.We applied multiple analysis of variance to detect differences between means and the LSD test for post-hoc comparisons.There was no difference in the glycaemic control between the groups as assessed by fasting plasma glucose and HbA1c levels. Body mass index was higher in the hypertensive group (28.7±0.6 versus 26.5±0.42),p=0.002,and was entered as covariate in the statistical model together with age and duration of disease although the latter ones did not differ significantly.We found that fasting insulin (Mean±SEM) was higher in the hypertensive compared to normotensive group (113.58±11.69 versus 79.85±6.02pmol/L),p=0.007,and SHBG was lower (34.15±2.18 versus 43.1±2.57 nmol/L),p=0.009.FT was also lower in the hypertensive group (49.26 ±2.35 versus 58.41±2.80 pmol/L),p=0.02 as was E_2 (104.9±8.03 versus 127.381±7.45 pmol/L),p=0.03.No difference was found in total,HDL cholesterol and triglycerides concentrations between the two groups.It is concluded that the male hypertensive NIDDM patients have higher insulin and lower SHBG levels,both indices of insulin resistance,and also lower free testosterone and total estradiol levels compared to normotensive patients.

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TOBACCO CONSUMPTION PATTERN IN DIABETIC POPULATION OF AN INDUSTRIAL UNIT

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Purpose: To estimate prevalence and characterise the pattern of tobacco consumption amongst diabetics in an industrial population of north India.

Design: Cross sectional survey using a standardised structured interviewer administered questionnaire (Response rate: 89%) in diabetics (includes known and diagnosed on OGTT).

Results: None of 21 (0%) female and 110 of 252 (43.7%) male diabetics were current tobacco consumers; 0 and 7.5% reported to have used tobacco in the past. Of the male diabetics 34.0% smoked and 14.1% chewed tobacco. The mean age of smokers was similar to consumers of non-smoking forms of tobacco and non-consumers of tobacco. Of the male diabetic smokers 60 (70.0%) smoked cigarettes, 35 (40.2%) smoked *beedi*. Majority of cigarette smokers used it for 6 years or more (6-9yrs: 16.4%; ≥10yrs: 72.1%); data for *beedi* was similar (22.9% and 68.6% respectively). Cigarettes smoked per day were less than 6 in 55.0%, 6-10 in 31.7%, 11-20 in 10.0% and >20 in 3.3%. The respective numbers for *beedi* were 20.0%, 37.1%, 31.4% and 11.4%. Of the male diabetic workers consuming non-smoking form of tobacco 17 (47.2%) chewed tobacco, 22 (61%) consumed it with *paan*, and 3 (0.8%) consumed it with *paan masala*. Majority of individuals chewing tobacco were using it for over 6 years (58.8%). The respective numbers for tobacco with paan was 72.7%. A graded relationship between prevalence of tobacco consumption and socio economic status of diabetics was observed. Thirty four, 43%, 38%, 62%, and 56% of professional, clerical, skilled, semiskilled, and unskilled diabetic workers respectively consumed tobacco.

Conclusions: Consumption of tobacco was widely prevalent amongst diabetics in the industrial population. Beedi consumption and chewing tobacco contributed significantly to total tobacco exposure. Higher tobacco exposure in lower socio-economic class was apparent.

Paan: Betel leaf and betel nut often taken with tobacco leaves; *Paan masala*: perfumed powder of paan; *beedi:* tobacco packed in Tendu leaves.

1734

CORONARY ARTERY DISEASE RISK FACTORS AMONG POST MENOPAUSAL WOMEN.
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Post menopausal women is notorious to have higher prevalence of coronary artery disease (CAD), especially among diabetics. Whether higher risk factors occur in our post menopausal women is evaluated in this study as a part of a population study done in Kayuputih Subdistrict, Jakarta. Among 34,645 total population, 5.0% of the adults (age > 15 years) were randomly recruited (stratified clustered random sampling) to join this survey. Altogether there were 1,019 eligible respondents, comprising 478 male and 541 female subjects. The prevalence of CAD (ECG, Minnesota code), diabetes mellitus (WHO criteria, 1985), hypertension (JNC V criteria), obesity (BMI ≥ 27 kg/m^2 for male, and ≥ 25 kg/m^2 for female), high TC (≥ 240 mg/dl), high TG (≥ 200 mg/dl), high LDL-C (≥ 160 mg/dl), and low HDL-C (≤ 35 mg/dl) among female respondents of ≥ 50 years old were 22.7, 10.0, 57.3, 34.5, 9.1, 8.2, 6.4, and 49.1% respectively; while among the younger counterpart (< 50 years old) the corresponding figures were 28.3, 3.0, 14.4, 21.3, 2.6, 1.6, and 47.3%. For the menopausal diabetics the corresponding figures were even higher, namely 36.4, 63.6, 27.3, 36.4, 9.1, and 45.5% respectively for the prevalence of CAD, hypertension, obesity, high TC, high TG, high LDL-C and low HDL-C. All the differences between the menopausal women and their younger counterpart were highly significant (p <.01), except for HDL-C. The fasting plasma insulin level among the ≥ 50 years old respondents were similar (3.7 mU/L) as compared to the younger respondents (4.07 mU/L). As a conclusion, in our studies population we found higher CAD risk factors among menopausal respondents which might explain the higher occurrence of CAD in this population subset.

1735

CARDIOVASCULAR DISEASE IN IDDM: A TRANSATLANTIC COMPARISON OF RATES AND RISK FACTORS
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The reasons behind the dramatically increased rates of cardiovascular disease (CVD) in IDDM subjects are poorly understood. To better understand the development of CVD in IDDM, data from the Epidemiology of Diabetes Complications (EDC) study in the USA were compared to data from the 31 centers in the European study EURODIAB. Comparable age (mean = 28 years both studies) and duration subsamples (EDC: N = 567, EURODIAB: N = 1215) were drawn from each study. CVD prevalence (myocardial infarction, angina, coronary bypass surgery, stroke, or ischemic ECG changes (Minnesota codes 1.1, 1.2, 1.3, 4.1, 4.2, 4.3, 5.1, 5.2, 5.3, and 7.1) rates were similar in both studies for men (EURODIAB 18.1% v EDC 20.1%) and for women (18.9% v 19.9% respectively), except for angina (more frequent in EDC women, p = < 0.001). In men, hypertension and triglycerides independently predicted CVD in EDC subjects, while age and HDL cholesterol were predictors in EURODIAB. In women, age was a predictor in both populations along with hypertension (EURODIAB) or renal disease (EDC). HbA_{1c} was negatively related to CVD in EURODIAB women but showed a weak positive relationship in EDC women. Renal disease only related to CVD in EDC. We conclude that while overall rates are similar, the risk factor correlates of CVD in IDDM vary considerably by continent. The previously reported strong association between renal disease and cardiovascular disease is less marked in these analyses, while any relationship with glycemic control is inconsistent.

1736

GENDER INFLUENCE OF MACROVASCULAR DISEASE IN DIABETES

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We have demonstrated that diabetic (DM) women below 55 y are at lower risk of developing ischaemic heart disease (IHD) than DM men, while above 55, there is no significant difference. The male-to-female age-specific relative risk (RR) of IHD followed the same pattern in both DM and ND population but the event rate appeared to have occurred 10 years earlier in DM. To investigate the influence of gender on cerebrovascular disease (CVD) rates in DM vs. ND, we used primary event rates in a diabetic population (5602 DM out of total of 402,798). The number of cases of CVD was assessed in both DM and ND male and female populations over 4 y (1991-1995). In the DM population below 55y, the RR was 1.05 (confidence intervals CI 0.53-2.08, insignificant). In ND population, RR below 55 was 1.11 (CI 0.88-1.39, insignificant). The age-specific event rate curve in DM confirmed that between 55 and 75y, both sexes had the same event rate. Above 75y, however, women were more prone to CVD, presumably reflecting the greater number of women reaching that age. Shifting the event rate curve of ND population 10 years backwards resulted in virtual superimposition of the event rate curve of the DM and ND populations in both sexes. We conclude that 1) DM women below 55y, are not at increased risk of CVD, compared to DM men. 2) Unlike IHD, CVD risk is similar in both DM men and women below 75y. 3) As in IHD, DM population have the CVD event rate occurring approximately 10 years earlier.

1737

IDENTIFICATION AND EFFICACY OF MULTIPLE RISK FACTOR CONTROL IN DIABETIC MACROVASCULAR DISEASES

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Using 899 subjects with NIDDM who were registered to MSDM (Multiclinical Study for Diabetic Macroangiopathy), the correlation between diabetic macroangiopathy (MA) and risk factors have been evaluated in a cross-sectional study. In addition, a prospective study for developing MA is performed. Hypertension, diabetic neuropathy and low HDL cholesterol in subjects with MA were significantly more frequent compared with MA(-) when the duration of diabetes was adjusted. Brinkman index, TG, hypertension in subjects with MA were significantly associated with MA. The following cut-off levels were determined, based on the discriminating value to differentiate MA(+) from MA(-) group. BMI:24, chol: 200, TG:120, ApoB:110, FBS:140, S-BP:140, D-BP:90. Those fulfilled more than 5 had significantly less MA. In the prospective study, the existence of MI was related to the more frequent appearance of MA for subjects who had no previous MA. In subjects with MA, MA progressed more frequently in those who failed to attain control criteria more than 4 out of total 6 risk factors excluding ApoB. These results indicate that an effort to control the multiple risk factors seems to be essential for the prevention of diabetic macroangiopathy.

1738

SERUM FOLATE AND CORONARY HEART DISEASE IN TYPE II DIABETES

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Mild to moderate hyperhomocysteinaemia has recently been recognised as a risk factor for coronary heart disease and may be associated with deficiencies of vitamin cofactors particularly folate. We assessed the risk of coronary heart disease, mortality and serum folate levels in patients with Type II diabetes. Since 1973, patients attending the Royal Perth Hospital Diabetic Clinic have been invited to participate in a two yearly survey of diabetic complications and risk factors. Survival status of survey participants was ascertained at 31 December 1993 by computer linkage to the Registrar Generals Death Indices for 1973 to 1993 for Western Australia. The cause of death was defined according to the International Classification of Diseases ninth revision (ICD9) code for the underlying cause of death. If the code was related to diabetes the cause was redefined according to the primary cause of death. Coronary heart disease, as defined by a history of angina or previous myocardial infarction, was present in 21% of 1391 subjects with Type II diabetes at the initial visit. Significant risk factors for coronary heart disease after age and sex were triglycerides ($p<0.001$), folate ($p=0.005$) and cholesterol ($p=0.016$). The most common cause of death was coronary heart disease, accounting for 60% of all 542 deaths recorded. The risk factors showing the most significant associations with death from coronary heart disease, after adjusting for age and sex, were history of coronary heart disease at the initial visit, cholesterol ($p<0.001$, hazard ratio=2.2), plasma glucose ($p=0.007$), diastolic blood pressure ($p=0.008$), and folate ($p=0.022$). Low folate was a risk factor (hazard ratio=1.6) in those with normal cholesterol but did not add to the risk in those with high cholesterol. It is concluded that low serum folate is a significant risk factor for both the presence of and subsequent mortality from coronary heart disease in Type II diabetes. This risk may be mediated by homocysteine.

1739

ABDOMINAL FAT DISTRIBUTION AND CARDIOVASCULAR RISK STATUS IN TYPE 2 DIABETIC PATIENTS

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One of the main objective in diabetes care is to reduce morbidity and mortality from coronary heart disease (CHD) by risk factor reduction. Many studies have suggested that abdominal distribution of adipose tissue (ADAT) may have higher predictive value for CHD. **Objective:** To assess the cardiovascular risk status (CVRS) in NIDDM patients, according to the values of a new parameter called abdominal index (AI), this represents waist/height ratio. **Method:** For the assessment of CVRS in medical practice European Task Forces on prevention of coronary heart disease have proposed a chart, that can predict the risk for coronary events in the next 10 years. In order to be more accurate in diabetes mellitus, we added other parameters and called this new chart: Eurochart '94. **Patients:** We have applied Eurochart '94 in 949 NIDDM patients (395 men): age 54.1±7.1 yrs.; diabetes duration 7.9±4.8 yrs. and 1082 control subjects.Waist/hip ratio and AI were also measured. **Results:** CVRS score (%) has been greater in NIDDM (21.07±1.1) than in control group (6.1±0.3). The prevalence of ADAT (AI>0.5) has been higher vs. non ADAT in both NIDDM (85.3%) and control subjects (58.1%), with CVRS increased accordingly. AI better described the ADAT that waist/hip ratio (96.6% vs. 58.0% in NIDDM patients and 87.5% vs. 40.1% in control subjects), while CVRS remained the same. **Conclusions**: ADAT is a common condition in NIDDM patients. CVRS is strongly influenced by NIDDM and ADAT. AI can better discriminate abdominal vs. non abdominal distribution of adipose tissue. AI is easier to be measured, as the errors which come from hip measurement are avoided.

1740

RISK FACTORS FOR ATHEROGENESIS IN TYPE 2 DIABETES AMONG VEGETARIANS AND NONVEGETARIANS

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The aim of the study was to study the differences in risk factors for atherogenesis among vegetarians and nonvegetarians.Total of 658 diabetic patients -395 men and 242 women were studied. They included 230 vegetarians and 428 nonvegetarians.They were studied for BMI ,waist hip ratio ,hypertension and dyslipidemia. Dietary patterns were assessed, with a food frequency questionnaire. There was no difference in cholesterol intake with reference to total calories prescribed to them. The patients were matched for other risk factors.23.5 % of vegetarians and 23.3% of non vegetarians had BMI of > 27 in men and 25 in women(p <0.01). W/H ratio of > 0.85 in women and 0.95 in men was present in 30.8% vegetarian and 41.6% non vegetarians ($p < 0.001$) hypertension was present in 22.% vegetarians and 26.6% in non vegetarians ($p < 0.05$) Cholesterol of > 200 mg/dl was present in 42.6% in vegetarians and 55% in non vegetarians ($p < 0.05$) Triglycerides of > 170 mg/dl was present in 47% vegetarians and 45.8% in non vegetarians ($p < 0.01$) HDL < 35 mg/dl was present in 23.5% in vegetarians and 26.6% in non vegetarians ($p < 0.05$). It can be concluded that non vegetarians had significantly more W/H ratio, hypertension high cholesterol and low HDL when compared to vegetarians. BMI and Triglycerides was not significantly different.

1741

OBESITY AND ITS RELATIONSHIP WITH RISK FACTOR IN SCHOOLERS OF QUITO (X SYNDROME)

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The index weist hip (ICC) is used in adults as an indicator of the distribution of fat tissue its relationship with the hyperinsulinism called X sindrome. Eventhough it is an index it has not been used or considered valid in children. We proposed to find out its validity in pediatric children taking the same cut points recomended for adults by the WHO.In a representative population of schoolars in Quito (n=678), anthropometric measurements were done, BMI was calculated and the ICC to relate then with eachother,also the arterial tension was determined and fasting blood was taken to value lipids (total cholesterol, LDL, HDL and TG). The results reveal that the 68% of the children have ICC at high risk and 21,4% al moderate risk. The relationship between the ICC with the BMI was significative (p=0,003), relating the risk determined by the ICC with risk factors we found that the 93% of the obese by BMI had an ICC at risk,the ICC at risk was present in a 100% of the children with high systolic and dyastolic pressure,in 94% with low HDL, in the 87% with high total cholesterol. Conclusion: the ICC in children has the same validity and significancy as the adult population and that the risk found in the studied population is high,having an important relationship with other risk factors.

1742

ALBUMINURIA AS A NEW MARKER OF DIABETIC PERIPHERAL VASCULAR DISEASE.

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Peripheral vascular disease is a common complication of diabetes. Recently, microalbuminuria is suggested as a new marker of diabetic macroangiopathy.

We studied the levels of abuminuria and other risk factors of atherosclerosis such as Lp(a), Plasminogen activator inhibitor-1, LDL-cholesterol, triglyceride, HDL-cholesterol, free fatty acid, Insulin, C-peptide, Hemoglobin A_1C in 196 diabetic patients with(102) or without diabetic peripheral vascular disease(94), which comfirmed by doppler flow velosity waveform analysis and angiography.

The prevalence of microalbuminuria and macroalbuminuria were 19.8% and 24.5% respectively. Risk factors for peripheral vascular disease in diabetic patients with macrovascular disease were evaluated by multivariate discriminant analysis and the following factors were identified by statistical significance of $P<0.05$: duration of diabetes, low density lipoprotein, high density lipoprotein, male, systolic blood pressure and amount of albuminura.

These datas suggest that the level of albuminuria in diabetic patients can be a new marker for the peripheral vascular disease.

1743

OBESITY AND BODY FAT DISTRIBUTION IN RELATION TO CHD RISK FACTORS

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Purpose: To investigate the relationship of CHD risk factors to over-weight (BMI ≥25kg/m²) and body fat distribution (Apple ≥0.85 in females ≥0.95 in males) in an industrial population.

Design: Analysis of data from a survey (N: 2420; ie. response: 84.7%) of an industrial population of North India. Categories of body fat distribution were: 1. Pear-Lean, 2. Pear-Overweight, 3. Apple-Lean, and 4. Apple Overweight.

Results	Female				Male			
	1	2	3	4	1	2	3	4
N [%]	71 [29]	44 [18]	42 [17]	87 [36]	691 [32]	40 [2]	731 [34]	714 [33]
Diastolic BP	74 (8)	73 (6)	75 (8)	78 (8)	80 (8)	84 (9)	83 (9)	87 (9)
Systolic BP	113(11)	110(9)	113(11)	118(15)	118(11)	122(12)	122(14)	126(14)
FP Glu*	94(21)	103(31)	102(29)	101(23)	98(30)	103(42)	101(34)	105(37)
HbA1 (%)	5.0(1.1)	5.4(1.7)	5.3(1.5)	5.7(1.8)	5.9(1.9)	5.6(2.0)	5.7(2.0)	6.1(2.2)
Cholesterol*	163(40)	160(38)	173(49)	172(37)	174(43)	164(45)	184(44)	186(45)
HDL chol*	46(13)	46(13)	42(12)	43(13)	39(12)	36(10)	36(11)	36(11)
C:H Ratio	3.7(1.1)	3.7(1.3)	4.4(1.9)	4.3(1.8)	4.8 (1.8)	4.8(1.5)	5.4(1.9)	5.7(2.0)
Triglyceride*	93(38)	99(53)	98(47)	124(70)	120(80)	153(67)	153(89)	173(109)
DM [%]	11.7	5.7	12.1	8.9	7.4	8.6	12.2	16.6
HT [%]	12.9	9.1	9.5	19.5	18.0	37.5	27.8	44.5

Data presented as Mean (SD) or percent [%]; FP Glu:Fasting Plasma glucose; chol: cholesterol; C:H: cholesterol :HDL cholesterol; HT: hypertension; DM: diabetes. *: mg/dL

Conclusions: Weight and body fat distribution is related to CHD risk factors in a graded manner; the maximum in apple shaped overweight and minimum in pear shaped lean individuals.

1744

RISK FACTORS FOR CORONARY HEART DISEASE IN NON INSULIN DEPENDENT DIABETICS WITH AND WITHOUTH DIABETIC NEPHROPATHY.

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We studied 140 type 2 diabetics(71 M,69 F,age 66.7±7.1 yr) of whom 54(38.6%) with coronary heart disease(CHD) and 86 (61.4%) withouth coronary heart disease.The 2 groups were strictly comparable by sex,age and duration of disease.On the whole 88 subjects(62.9%) showed normoalbuminuria(No) (<30 mg/24 h),46(32.9%) microalbuminuria(Mi)(30-300 mg/24h) and 6(4.3%) macroalbuminuria(Ma)(>300 mg/24 h).CHD was less frequent in No(17%) than in Mi(76%) and Ma(66%)(chi-square for trend=25.3,p<0.00001).Hypertension was more frequent in patients with CHD in Mi(76%) than in No(18.8%,p=0.00001) and Ma(60%,p=NS).Systolic blood pressure was higher in patients with CHD than withouth CHD in No(176.4±26.1 vs 161+ 20.1,p=0.01),but was similar in Mi and Ma.Diastolic blood pressure,total and HDL-cholesterol,triglycerides,body mass index,fibrinogen,smoke,duration of diabetes,fasting glicemia,glycosilated hemoglobin,insulin therapy and insulin daily dosage were similar in patients with and withouth CHD in No,Mi,and Ma.The study confirms a significant association between CHD and increased albuminuria.Among the considered variables only hypertension and systolic blood pressure seem to be related to different albuminuria levels in the developement of CHD.The study suggests that their role could be less important when renal damage is established.

1745

ASSOCIATION OF CENTRAL OBESITY WITH CORONARY HEART DISEASE, BUT NOT WITH RETINOPATHY IN NIDDM

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Obesity and especially abdominal (central) obesity is often associated with NIDDM and the complications of this disease. In a group of 55 consecutive non-selected NIDDM patients, 27 of 55 (49%) are severely obese (BMI > 30) and 40 of 55 (72%) have a waist-hip ratio (WHR) ≥ 0.9 (mean of the group 0,95 ; range 0,75-1,10). Some characteristics of the group are summarized : mean age 61,3 ± 1,37 years, known diabetes duration : 9,64 ± 0,9 years, BMI : 29,74 ± 0,62 kg/m^2, HbA_1c : 7,79 ± 0,25 %, C-Peptide : 3,27 ± 0,28 ng/ml and fasting insulinemia : 11,97 ± 0,9 mU/l. 31% of the patients have coronary heart disease (CHD), 24% have background or proliferative retinopathy and 33% have micro- or macroalbuminuria. CHD is statistically significantly correlated to WHR. Men present more CHD (32% vs 29%) in the same BMI, systolic and diastolic blood pressure levels in spite of having lower fasting insulinemias than women. There is no statistically significant correlation between WHR and retinopathy or nephropathy.

In conclusion : These data suggest an association of central obesity and CHD but no association with retinopathy or nephropathy in NIDDM patients especially male patients.

1746

MICROALBUMINURIA IS ASSOCIATED WITH PERIPHERAL ARTERIAL DISEASE IN DIABETIC SUBJECTS ONLY IF HYPERTENSION IS PRESENT

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Microalbuminuria (MA) is a risk indicator for coronary heart disease in diabetic and in non-diabetic subjects. Whether this is also the case for peripheral arterial disease (PAD) is hardly investigated. Our aim was to study the associations between MA and PAD in the presence of hypertension and/or diabetes mellitus (DM). We investigated an age-, sex- and glucose tolerance stratified sample from a 50-74 year-old general Caucasian population (n=631) from the Hoorn Study. The urinary albumin concentration was measured in a first morning urine upon arising by radioimmunodiffusion, with a threshold of 6.2 mg/l. A valid albumin/creatinine ratio could be obtained from 580 subjects. The 53 non-valid ratios were excluded from further analyses. MA was defined as a ratio above 2.0 mg/mmol. Doppler-assisted systolic blood pressures were performed from brachial and posterior tibial arteries to calculate the ankle-brachial pressure index (ABPI). If the highest ABPI for either leg was less than 0.90, the subject was classified as having PAD (n=68). The prevalence of PAD was 7 and 19% for normo- and hypertensive subjects, and 8 and 15% for non-diabetic and diabetic subjects, respectively. Analyses were performed by multiple logistic regression. The table shows age- and sex adjusted OR (95% CI) of PAD in diabetic and/or hypertensive subjects compared to a control group in the absence or presence of MA.

		MA absent (n=482)	MA present (n=68)
control	(n=318)	1.0	0.7 (0.09 - 5.9)
DM only	(n=91)	1.4 (0.6 - 3.4)	2.1 (0.4 - 10.5)
HT only	(n=108)	0.8 (0.3 - 2.2)	3.6 (1.1 - 11.7)*
DM and HT	(n=64)	3.8 (1.6 - 9.3)*	6.3 (2.2 - 18.3)*

* $p < 0.05$ Wald-test

We conclude that in this Caucasian population peripheral arterial disease is not significantly associated with microalbuminuria in diabetic subjects. However in the co-presence of hypertension a pronounced association between microalbuminuria and peripheral arterial disease was observed.

1747

SYNDROME 'X' - MORE COMMON IN MEN OR WOMEN

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The Aim of the study was to see the association of Obesity, Hypertension, Lipid abnormalities & IHD among NIDDM Men & Women. The data of 13672 patients randomly selected from 2 different Hospitals were studied with the help of a computer aided statistical programme. Results showed Obesity among 44% of Women (BMI > 25) & 14% of Men (BMI > 27). Hypercholestrolaemia (> 200 mg/dl) was present in 14% of Women & 12% of Men. Hypertriglyceridaemia (> 170 mg/dl) was present in 12% of Women & 7% of Men. Hypertension was present in 32% of Women & 24% of Men. (Obesity was 52% among Women & 19% in Men in this sub group). IHD was present in 13% of Women & 12% of Men (Obesity was 45% among Women & 15% in Men in this sub group). Hypertension with IHD was 7% in Women & 5% in Men. (Obesity was 50% among Women & 18% in Men in this sub group). To Conclude, Obesity perse & its association with Hypertension & IHD is more among Women than Men. Hypertriglyceridaemia is significantly higher among Women than Men but, Prevelence of Hypercholestrolaemia shows no increased risk. Therefore, SYNDROME 'X' appears to be more common among South Indian Women than Men.

1748

MICROALBUMINURIA –AN INDEPENDENT NOVEL RISK FACTOR FOR IHD IN NON–DIABETICINDIVIDUALS.

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Background: Previous, cross–sectional studies suggest that "micro–albuminuria" is associated with CVD, dyslipidaemia and increased blood pressure. **Aim:** To analyze whether microalbuminuria predicts subsequent development of IHD in a non–diabetic population based cohort. **Material:** In the first Danish MONICA–studyin 1983 we collected morning urine samples in 2,094 consecutive participants without renal disease, urinary tract infection, diabetes or IHD at entry. We measured urinary creatinine and albumin (ELISA–technique). All participants were followed until death, emigration or Dec.31.1993 and IHD was defined as a hospital discharge diagnosis or cause of death including the diagnoses: ICD–8; 410–414). **Results:** 80 individuals developed IHD during the follow–up period, and they were characterized by male preponderance, higher age, higher BMI, BP, cholesterol and proportion of current smokers. Microalbuminuria (MA) was defined as the 90th centile of the urinary alb./creatinine ratio. When adjusted for other risk factors, the RR of IHD in cases with microalbuminuria was 2.3 compared to the controls, and the 10 year disease free survival was 91.5% and 97%. respectively ($p<0.003$). Interaction between the effect of microalbuminuria and other well known risk factors was observed. RR's for the combined effects: MA+current smoking: 4.9, MA+Syst. hypertension: 6.3, MA+low LDL: 5.3 and MA+hypercholest: 7,1. **Conclusion:** Microalbuminuria is an independent predictor of IHD. Generalized vascular hyperpermeability (as demonstrated trough microalbuminuria) may explain the increased susceptibility to smoking, hypertension and dyslipidaemia in patients with microalbuminuria.

1749

THE COMPLICATIONS UNDER THE ICEBERG: HOW DANGEROUS IS IMPAIRED GLUCOSE TOLERANCE ?

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Persons with impaired glucose tolerance (IGT) have an increased risk for developing type 2 diabetes when compared to persons with normal glucose tolerance. Although it is known that long-term diabetic complications can be rarely seen during IGT, it has not yet been demonstrated whether IGT period has any influence on the incidence of complications. According to WHO's criteria (75 gr standard OGTT) 68 patients (40 female/28 male; mean age 53.3±10.2 years) were defined to have IGT and followed for an average period of 47 months before developing clinical diabetes. They were all obese (BMI: 29.3±4.7 kg/m2), %60 of them had family history for diabetes. During follow up period the sequence and prevalence of macro and microvascular complications were assessed by physical examinations and relevant laboratory tests. Hypertension developed in 29 (%42) cases, hyperlipidemia was found in 27 (%39) of them. Although all had normal visual acuity and normal fundus findings, they were studied by Farnworth-Munsell 10 hue test for colour vision and Arden's contrast gratings and found that they have significantly lower contrast sensitivity and colour vision functions than normal persons. There was no case with microalbuminuria by micral test. 7 patients (%10) were identified to have subclinical polyneuropathy by EMG. As a conclusion it is probable to say that factors other than hyperglycemia have influence on developing long-term diabetic complications.

1750

CORRELATION OF MICROALBUMINURIA WITH LEFT VENTRICULAR DIASTOLIC FUNCTION IN DIABETES MELLITUS

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Microalbuminuria (MA), an index of nephropathy, is associated with increased Coronary Artery Disease (CAD) mortality and morbility in both types of diabetes. Echocardiographic alterations in Left Ventricular Diastolic Function (LVDF) are considered as an indicator of the presence of early CAD, in individuals without hypertension or cardiomyopathy. To investigate the relationship between these two indirect indices of CAD (MA and echocardiographic alterations in LVDF) in 64 normotensive and without cardiomyopathy diabetics were studied: 23 type 1 DM aged (m±SD) 29.3±8.9 years with disease duration (m±SD) 11.5±6.5 years and 41 type 2 DM aged (m±SD) 55.7±8.6 years with disease duration (m±SD) 11.7±8.2 years. No patient had clinical or ECG evidence of CAD. The diabetics of both types were divided according the presence of MA into groups: 16 MA normal/7 MA elevated in type 1 DM and 25 MA normal/17 MA elevated in type 2 DM. MA was measured in an overnight 8-hour urine collection by RIA while LVDF was determined as the ratio E/A waves (normal values >1) and the isovolumic relaxation time (IRT) (normal value 45-75 mm/sec) both indirect indices of CAD, on the two dimension echocardiography. No statistical difference was observed between the two groups in both types of DM for the ratio E/A 1.40±0.30 vs 1.21±0.20 type 1 DM, 0.94±0.29 vs 0.92±0.26 type 2 DM) or the IRT (71.0±16.6 vs 70.7±18.3 type 1 DM, 90.5±16.1 vs 82.2±11.9 type 2 DM). No correlation was found between MA and E/A or IRT in either type of DM. In conclusion these findings suggest that in both types of DM in the absence of hypertension or cardiomyopathy, MA is not related to echocardiographic alterations of LVDF which are considered indirect indices of CAD.

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CORRELATION OF CORONARY ARTERY DISEASE WITH MARKERS OF OBESITY IN DIABETES MELLITUS

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The increased risk of type 2 diabetics to the development of Coronary Artery Disease (CAD) is well known but few data are available regarding the relationship between CAD and specific markers of obesity as the Body Mass Index (BMI) or central fat distribution expressed as the waist to hip ratio (W/H). To investigate this relationship 225 type 2 diabetics, 119 males and 106 females, aged (m±SD) 57.6±10.3 years and with disease duration (m±SD) 9.9±7.1 years were studied. The BMI was calculated in all the diabetics and in 112 of them the W/H was also measured. Normal values were considered for the BMI <27 and for the W/H <0.95 for males and <0.85 for females. In all the patients the presence of CAD was established by the past history of cardiac infarct/ischemia, ECG findings (WHO criteria) or after maximal treadmill stress test (Bruce classification). It was found that CAD was present in the BMI overweight group in 56.8% (29/51) of males and in 48.8% (22/45) of females whereas according to the W/H in 50.0% (13/26) and 57.1% (12/21) respectively, without statistical difference of the CAD incidence between normal and overweight diabetics using either marker of obesity. Comparing the incidence of CAD in the patients in whom both markers of obesity were available, this was again not statistically different between the two markers ($x^2=2.17$). In conclusion, obesity expressed by either the BMI or the W/H ratio does not seem to differentiate, at least at the moment of the study, type 2 diabetics with CAD.

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RELATIONSHIPS BETWEEN MACROVASCULAR DISEASES AND MICROALBUMINURIA IN TUNISIAN DIABETIC OUTPATIENTS
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The aim of this study was to estimate the prevalence rates of macrovascular diseases and of microalbuminuria in tunisian diabetic outpatients. This study is related to a sample including 249 diabetic patients,(83 IDDM and 166 NIDDM),sex ratio 1.02,mean age 45.1 +/- 6.6 years,duration of diabetes 8.9 +/- 7 years. Cardiovascular questionnaire responses (Rose method) and Minnesota code for ECG were used to identify coronary heart disease and leg vascular disease.Microalbuminuria was determined using turbidimetric method. Global prevalence of macrovascular diseases was 24.5% including coronary heart disease 14.5%, leg vascular disease 12.4% and stroke 1.6% Prevalence of hypertension was 20.5%. Positive microalbuminuria (from 30 to 300 mcg/mn)was found in 25.3% . Macrovascular diseases except hypertension were more frequent in men than in women. Macrovascular diseases were slightly but not significantly more frequent in microalbuminuric patients (27% vs 23%). Prevalence rates of macrovascular disease and microalbuminuria increase with age and duration of diabetes. Cardiovascular risk factors associated with microalbuminuria were obesity, hypertension and high cholesterol levels. We conclude that microalbuminuria and macrovascular diseases are frequent in tunisian diabetic patients. Microalbuminuria is associated with cardiovascular disease risk factors.

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INCREMENTAL FASTING PLASMA GLUCOSE LEVELS IN MALE SUBJECTS IS ASSOCIATED WITH INCREASING PREVALENCE OF CHD RISK FACTORS.

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Purpose: To estimate CHD risk factors in relation to fasting plasma glucose and OGTT status.

Design: Analysis of data from a survey (N: 2043 i.e. response: 79.2%) of an industrial population of North India. Based on fasting plasma glucose levels and plasma glucose response to OGTT subjects were divided in to 6 groups.

Results:

	fasting plasma glucose						
[mean (SD)]	≤80	80 to 99	100 to 119	120 to 139	IGT	fresh DM	known DM
N	334	629	430	88	81	363	101
Age (years)	41 (6)	41 (7)	41 (7)	41 (6)	42 (6)	43 (5)	45 (5)
BMI (kg/m²)	23.0(3.3)	23.3(3.5)	23.4(3.1)	24.2(3.6)	24.9(3.5)	24.2(3.0)	24.9(3.5)
Waist hip ratio	0.97 (0.06)	0.97 (0.06)	0.97 (0.06)	0.97 (0.06)	0.99 (0.06)	0.98 (0.05)	0.99 (0.06)
Diastolic BP (mm Hg)	83 (9.9)	82 (9.1)	85 (9.6)	84 (9.5)	86 (8.9)	87(10.2)	84 (9.3)
Systolic BP (mm Hg)	120 (14)	119 (12)	123 (12)	124 (12)	125 (13)	128 (17)	127 (16)
HbA1 (%)	5.6 (1.7)	5.6 (1.7)	6.0 (2.2)	5.9 (2.2)	6.1 (2.2)	6.2 (2.2)	7.3 (3.4)
Triglycerides*	137 (78)	140 (79)	142 (70)	153(155)	155 (82)	187(117)	235(197)
Cholesterol *	178 (48)	182 (44)	182 (42)	181 (36)	182 (45)	186 (48)	181 (44)
HDL cholesterol *	35.8(12)	36.9(11)	37.4(11)	37.5(10)	37.6(12)	32.8 (8)	36.1(11)
CH ratio	5.4 (2.0)	5.3 (2.0)	5.2 (1.8)	5.1 (1.5)	5.2 (1.9)	6.0 (2.1)	5.4 (2.1)

CH ratio: cholesterol :HDL cholesterol ratio; *:mg/dL

Conclusions: Increasing fasting plasma glucose levels considered as "non-diabetic" correlate with increasing coronary heart disease risk factors esp. triglycerides, and BP despite comparable waist to hip ratio.

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THE ROLE OF SYNDROME-27 ON DIABETIC ANGIOPATHY.

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Background: Askandar Tjokroprawiro (1996) has firstly coined the term "Syndrome-27" or **GIGULOCHIPS-SAF$_3$ARIL-PAC$_3$GE.** This syndrome described about 27 determinant factors responsible for the quality of blood vessels, and it stands for *Genetic, Insulin Resistance, Glucose control, Uric acid, Lipid triad, Obesity, Cigarette, Hypertension, Inactivity, Platelet hyperaggregation, Stress, Sex, Age, Fibrinogen, Factor VIIIc & VII, Free Radicals, Alcohol, Race, Inhibitors, Left Ventricular Hypertrophy, Platelet Activating Factors, Androgen, Cytokines, Corticosteroid, Catecholamines, Growth hormone, and Estrogen.* These 27 risk factors might constitute a coronary risk syndrome that could be called "Metabolic Cardiovascular Syndrome". ***Objective:*** To determine the independent risk factors, which have dominant contribution to the development of macro and microangiopathy (Diabetic Angiopathy=DA) in NIDDM. ***Patients and Methods:*** one hundred and twenty five NIDDM patients with DA and 125 NIDDM without DA were recruited for case control study. Multiple logistic regression analysis were used to determine the dominant and significant risk factors for DA. ***Results:*** The dominant factor that significantly contribute to the development of DA was Lipid Triad (OR:3.7 95% CI:1.9-7.3), followed respectively by Age (OR:3.4 95% CI:1.3-8.4),Genetic (OR:3.3 95% CI:1.3-7.8),Platelet Hyperaggregation (OR:2.6 95% CI:1.4-4.9),Hypertension (OR:2.4 95% CI:1.2-5.1),Hyperuricemia (OR:2.1 95% CI: 1.0-4.0), and Insulin Resistance (OR:2.0 95% CI:1.0-4.0). Factor analysis revealed joint effect of Lipid Triad, Age, and Inactivity increased the risk of DA (OR:10.0 95% CI: 1.2-81.0). ***Conclusion:*** Lipid Triad abnormalities was the most dominant independent risk factor for the development of DA.

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GLYCEMIC CONTROL AND CARDIOVASCULAR RISK FACTORS IN NIDDM ATTENDED IN PRIMARY CARE: A MULTICENTRIC STUDY

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Aims: To assess glycemic control and cardiovascular risk factors (Total cholesterol, blood pressure, obesity and smoking) in NIDDM patients attended in Primary Health Centres (PHC) before an intervention programme. **Methods:** Cross-sectional study carried out in 1993-94 in 76 PHC (34.1% rural, 65.9 urban), by systematic sampling from 31.050 registered NIDDM and a total of 875.571 adult clinical records. The targets for control were the same used by the European NIDDM Policy Group. **Results:** The final sample included 2595 NIDDM patients (43.9% male). The mean age was 66,6 +/-10 years and the average evolution time was 8.1+/-5.9 years. Glycosilated Hemoglobin was registered in 73 % of patients, Total Cholesterol in 78 %, Blood Pressure in 95%, BMI in78%, and interrogated smoking in 92% of patients. There was a good/acceptable glycemic control in 50% of patients. Total cholesterol was lower than 250 mg/dl. in 72% and blood Pressure showed a bad control in 40% of patients. BMI was lower than 30 in 63% of cases. Smoking was present in a 13%, raising the prevalence to 29% in men and up to 42.4% in men younger than 60. There were 26.1% of patients younger than sixty with three or more cardiovascular risk factors with a poor control . This percentage was 15.6% in the patients older than sixty. There were no differences between genders. **Conclusions:** There was a acceptable glycemic control in more than a half of the diabetic patients, and the percentage of patients with acceptable cholesterol was satisfactory. The Blood Pressure control, presence of smoking and rate of patients with three or more risk factors with a bad control (specially those younger than sixty) can be widely improved.

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DIABETES COMPLICATIONS AND CARDIOVASCULAR RISK FACTORS IN A REPRESENTATIVE POPULATION SAMPLE OF DIABETES SUFFERERS

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A sample of adults (n = 191), aged 40 years or older, with non-insulin dependent diabetes were recruited to the South Australian Diabetes Study from a representative popultion health interview survey. They were assessed for macro and micro-vasculr complications using well documented medical criteria and tests. 63 percent were found to have at least one micro-vascular problem and 62 percent at least one micro-vascular problem, despite the fact that the mean fasting HbA_{1c} level was 7.8 percent. The prevalence of macro-vascular disease was similar in both males and females however significantly more males suffered a micro-vascular problem ($p=0.04$). 52 Percent of the sample were hypertensive, 76 percent obese, 16 percent had elevated cholesterol and 15 percent were smokers. The prevalence of these risk factors was little changed according to the presence of diabetes complications.

Interestingly, this study also showed that the frequency of advice given by doctors about cardiovascular risk factors increased when complications were present. Patient rating of general practitioner advice was good, however, these results suggests that more education/intervention on cardiovascular risk factors can be done at an earlier stage of diabetes.

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HYPERTENSION, HYPERINSULINAEMIA AND OBESITY IN ADULT FINNS

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The aim of the study is to analyse the data obtained from a 2-year follow-up study of the middle-aged Finnish subjects (n=183) with a previous history of impaired glucose tolerance (IGT) in order to elucidate the longitudinal relationship between hypertension, fasting hyperinsulinaemia and obesity. Hypertension was defined as either systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 95 mmHg or being on antihypertensive drug treatment regardless of the blood pressure value. Multiple logistic regression analyses shows that the odds ratios of elevated fasting insulin concentration at baseline were 1.14 (95% confidence interval 1.02-1.28) and 1.22 (95% confidence interval 1.01-1.47), respectively, for the prevalence and for the 2-year incidence of hypertension in subjects with IGT at baseline but reverting to normal glucose tolerance at follow-up. The association was independent of body mass index at baseline and weight gain during the two years. Body mass index and weight gain were not independently associated with the development of hypertension, but were the independent risk predictors for fasting hyperinsulinaemia (≥ 13 mU/l) at follow-up. The odds ratio of weight gain was 1.43 (95% confidence interval 1.03-1.98) for hyperinsulinaemia in lean (body mass index < 27 kg/m^2) and normoglycaemic subjects. It is concluded that elevated fasting insulin concentration preceded the onset of hypertension independent of body mass index and glucose intolerance, and may be a link between hypertension and obesity.

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THIS ABSTRACT HAS BEEN WITHDRAWN BY THE AUTHOR.

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HYPERINSULINEMIA AND DYSLIPIDEMIA IN PATIENTS WITH PERIPHERAL VASCULAR DISEASE

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In the pathogenesis of cardiovascular (cv) disease, the metabolic syndrome (MS) seems to play an important role. While epidemiological studies have identified hyperinsulinemia (HI) as a risk factor for coronary heart disease, there are virtually no studies looking at the association between peripheral vascular disease (pVD) and the MS. We investigated 59 elderly patients (mean age 69,4 years) with different degrees of peripheral arteriosclerosis as indicated by the tibio-brachial doppler-index (DI). According to DI, 3 subgroups were formed: while a DI>1,0 indicates no pathology (=Healthy), a 1,0>DI<0,8 suggests macroangiopathy (=at Risk) and DI <0,8 denotes marked pVD (=pVD). In all participants an oral glucose tolerance test (100g) with measurement of insulin levels was performed. Also, lipid profiles were determined in the fasted state. Glc and Ins sums were calculated by adding the values of the different time points (0+ 30+60 +120min). All groups were comparable in age, body-mass-index, waist-to hip-ratio and Total-Cholesterol.

	Sys.BP	Chol	LDL	HDL	TG	Glc-Sum	Ins-Sum
H	153±4.3	227±6.2	142±7.6	61±2.6	137±14	565±32	248±32
R	150±5.8	235±8.8	145±7.2	50±3.6*	172±22	581±29	317±47*
pVD	173±5.4*	247±9.4	163±8.8*	51±3.2*	192±31	586±20	324±44*

Although glc tolerance was similar in all groups, ins levels were 30% higher in R and pVD (*p<0,05, Wilcoxon), indicating that insulin resistance is compensated by HI. R and pVD also showed lower HDL and higher triglycerides (TG). LDL was elevated in pVD. Patients with pVD and those at risk display the cv risk pattern found in MS with dyslipidemia and HI. Therefore, in both groups intensive intervention, aimed at improving insulin sensitivity, will also improve atherogenic risk profile, as has already been shown in coronary heart disease.

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CHARACTERISTICS OF METABOLIC X-SYNDROME IN NIDDM PATIENTS IN PRIMARY HEALTH CARE IN HUNGARY

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In order to evaluate the characteristics of x-syndrome a screening procedure was carried out in NIDDM patients registered within primary health care. Using clinical classification a total of 804 NIDDM patients was identified from 20 affiliated GPs within Budapest. A coexisting history of hypertension was found in 571 (71.0%) patients. Abnormal body mass index (>27 kg/m^2 in men, >26 kg/m^2 in women) values were recorded in 520 (64.7%) patients. Abnormal casual systolic (>160 mmHg) and diastolic (>95 mmHg) blood pressure was found in 292 (36.3%) and 140 (17.4%) patients, respectively. Elevated serum total cholesterol (>6.5 mmol/l) and triglycerides (>2.2 mmol/l) were observed in 212 (26.4%) and 325 (40.4%) patients, respectively. Abnormal values of HDL-cholesterol (<0.90 mmol/l) were documented in 102 (12.7%) patients. The waist-hip ratio was abnormal (>1.0) in 105 (13.1%) patients. Clustering of abnormal clinical and laboratory findings was found to be more prominent with women than with men. Within this study the frequency of microalbuminuria was 25.0 % while that of macroalbuminuria was 8.6% with a significant male predominance. More appropriate treatment should be provided at the primary health care level to decrease the risk of macrovascular complications in NIDDM patients with clustering of components of metabolic x-syndrome in Hungary.

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INCREASED INSULIN SECRETION AND SYMPATHETIC ACTIVITY IN NORMAL GLUCOSE TOLERANCE OFFSPRINGS OF TYPE II DIABETICS.

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Recent papers have shown that first degree relatives of patients with type II diabetes (NIDDM) display insulin resistance, although with normal tolerance. This observation raises the possibility that some of the typical metabolic and/or hemodynamic abnormalities of NIDDM may already be present in such subjects. The aim of our paper was, therefore, to investigate insulin secretion behaviour and its relationship to hemodynamic parameters in offspring of NIDDM with normal glucose tolerance. We studied 9 offspring of NIDDM (P), compared with 18 subjects without family history of diabetes (C), all with normal glucose tolerance. The two groups were comparable for age (C: 24.6±0.9 vs P: 25.1±2.3 years), sex, BMI (C: 23.6±0.5 vs P: 24.3±1.1 kg/m^2). All subjects underwent a 180 min oral glucose (75 g) tolerance test (OGTT). Continuous blood pressure monitoring was performed every 3 min by oscillometric method, throughout the study; a continuous ECG recording (Holter) was performed in order to calculate the heart rate variability, by a fast Fourier algorithm. The derived LF/HF (low/high frequency) ratio is an index of the sympatho-vagal balance. Before glucose ingestion, plasma insulin concentration (IRI) was similar in the two groups. From time 30 min and throughout the study, IRI was significantly increased in P (57±5.7) when compared to C (29.6±4.2 μU/ml), p<0.005. Blood glucose (BG), basally similar in the two groups, was also significantly increased in P (103.1±4.5) vs C (91.6±4.9 mg/dl), p<0.05, from time 120 min on. BG/IRI ratio was reduced in P vs C, thus suggesting the presence of hyperinsulinemia in the group of offspring. Diastolic blood pressure significantly decreased in P, but not in C during the first hour of the study (basal: 72.1±3.9 vs 63±2.8, p<0.002). Finally, LF/HF ratio was significantly increased in P (2.5±0.4 vs C: 1.7±0.2) from time 15 min. In conclusion, offspring of NIDDM with normal glucose tolerance display an increased insulin secretion; however they are not resistant to the hemodynamic effects of insulin, as suggested by the reduction of diastolic blood pressure, probably due to a direct effect of vasodilatation. This, in turn, may determine a chronic sympathetic activation, which could be involved in the pathogenesis of type II diabetes.

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CAN SYNDROME X LEAD TO ENLARGEMENT OF VARIOUS VISCERAL ORGANS ?

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Syndrom X are characterized by glucose intolerance, obesity, insulin resistance, dislipidemia and hypertension. Insulin is also a growth factor in various tissues to determine whether insulin resistance leads to enlargement of visceral organs, we measured fasting and postglucose 1- hour insulinemia, cholesterol , triglyceride, HDL-cho, LDL-cho, VLDL-cho and the length of liver, spleen and kidneys by ulrasonographic investigations in 30 patients with Syndrome X. (Age:57.3±8.7 yrs., Body mass index: 32±4.4 kg/m², Waist-Hip-Ratio:0.88±0.6) liver and renal function tests are normal in study and healthy groups. Levels of fasting and postglucose 1 - hour insulin were 13.6±6.9 and 64±48 in study group, 8.8±3.6 and 44±28 (IU/ml in healthy group (p<0.05). The length of liver was significantly increased according to healthy subjects in Syndrome X (p<0.001). The length of kidneys were increased insignificantly (p>0.05). The length of spleen was not found different each two groups (p>0.05). Fasting and postglucose insulin levels were not correlated with length of liver and body mass index. The levels of triglyceride was found increased in Syndrome X (p<0.01) but not correlated with the length of liver. As a conclusion the liver is the only organ enlarged in Syndrome X . This enlargement was not result of hepatosteatosis and is not correlated with body mass index and levels of insulin. It is suggested that hyperinsulinemia may stimulate also extra-vascular growth factors in organs. The cause of hepatomegaly may due to growth factors.

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HYPERINSULINEMIA AS A RISK FACTOR FOR CORONARY ARTERY DISEASE:
A CASE-CONTROL STUDY IN COLOMBIAN SUBJECTS

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Insulin Resistance syndrome (IRS) is a novel predictor for coronary Artery Disease (CAD). A case-control study was designed, looking for association in both traditional risk factors and hyperinsulinemia, related to CAD. Inclusion criteria for New incident cases, (n=77): Subjects of both sexes (M/F 60/17; mean age 52.7±9.5 SD, interval 32 to 68 years), with CAD documented by angiography (obstruction > 70% in at least one major epicardial coronary vessel). Exclusion criteria: Presence of NIDDM, secondary hyperlipidemias, disease and drugs with interference on carbohydrate and lipid metabolism (diuretics, β-Blockers, lipid-lowering drugs). Controls healthy subjects (n=74) was chosen at random from outpatient room, (M/F 52/22; mean age 50.7±9.9 SD, interval 32 to 74 years); without NIDDM nor CAD, with normal Thorax X-rays, normal EKG, normal exercise tolerance and someones (n=7) normal angiography. Information about demographic variables, self-reporting physical activity degree (working and recreational), clinical and familiar background, physical examination, anthropometric variables, fasting plasma lipids concentration, serum uric acid and fasting blood glucose and insulin as well as 30, 60 and 120 minutes postglucose load (75 gr); was available for analysis. All patients were living in Bogota DC City. There was no differences in physical activity degree in Cases vs Controls. Smoke was more prominent in Cases than in Controls. Hyperinsulinemia was defined as a value greater than percentile 75 in normal controls subjects for insulin levels 2 Hs. post-glucose load (30 uUI / mL); as well as a value greater than percentile 75 in normal controls subjects for Homeostasis Model measure (HOMA-IR) (1.50). Mean values ±SD and t-test P values for measured parameters in Cases vs Controls are shown. A Logistic Regression Analysis model (forward stepwise-conditional) for CAD were calculated as we can see in the next table:

Main Predictors for Coronary Artery Disease

Variable	ß (SE)	Odds Ratio (CI 95%)	Sig
Insulin 2hs (> 30 uUI/mL)	2.53 (0.5336)	12.58 (4.39 , 36.86)	0.0000
Smoke	1.86 (0.5229)	6.43 (2.30 , 17.90)	0.0004
Diastolic BP (> 80 mm Hg)	2.13 (0.8719)	8.46 (1.52 , 46.47)	0.0143
HDL-Cholest. (< 45 mg/dL)	1.18 (0.5088)	3.27 (1.20 , 8.82)	0.0197
Glucose 2 Hs (> 140 mg/dL)	1.56 (0.7275)	4.76 (1.14 , 19.80)	0.0319
Variables not in the equation: Sex, BMI (> 25 K/m²), Systolic BP (> 120 mm Hg), Total Cholesterol (>220 mg/dL), Triglycerides (>200 mg/dL), and HOMA-IR (> 1.50).			

We conclude that in subjects without NIDDM, hyperinsulinemia 2hs post glucose load, as well as Impaired Glucose Tolerance have a fundamental role as risks factors for CAD in addition to others known factors such as smoke, diastolic hypertension and HDL-Cholesterol.

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Coronary artery disease prevalence in diabetic persons: the effect of socio-economic status.

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Coronary artery disease (CAD) accounts for approximately 50 % of diabetes related mortality, which is not all accounted for by the major coronary risk factors. In Western countries coronary artery disease is more prevalent in persons of low socio-economic status. We studied the impact of socio-economic status on the prevalence of coronary risk factors and CAD in persons with diabetes. A profile of CAD risk factors was established for 1,553 individuals with diabetes. Socio-economic scores were calculated from four measures of material wealth. The presence of CAD was based on the Rose angina questionnaire, past medical history and resting 12 lead ECG. Smoking was less common in the most affluent group compared to the most deprived 19.9% v. 41.1% respectively ($p<0.001$). The mean body mass index was lower in the most affluent group compared with the most deprived 28.8 $kg.m^{-2}$ v. 30.4 $kg.m^{-2}$ ($p<0.002$). For individuals under 70 years of age the prevalence of CAD in the most affluent group was 17.2 % and in the most deprived group 31.4% (chi squared for linear trend, $p<0.0002$). A multiple logistic regression model was constructed including smoking, obesity, hypertension, hyperlipidaemia and hyperglycaemia. The calculated odds ratio for the prevalence of CAD in the most deprived category relative to the most affluent was 1.72 ($p<0.02$). Coronary artery bypass graft procedures were undertaken more commonly in the most affluent group 19.2% compared to the most deprived 7.4% ($p<0.03$). CAD is more common among persons with diabetes of low socio-economic status after accounting for major risk factors.

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The Level of Serum Insulin in Japanease Coronary Artery Disease.

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In 224 patients with coronary artery disease(CAD:183 males and 41 females, 34-72 years old) who underwent coronary angiography, plasma glucose(PG) and insulin(IRI) were concomitantly measured at 75g OGTT. Subjects were divided into three groups as follows: normal(Nor;36 cases), impaired glucose tolerance(IGT;136 cases) and NIDDM(52 cases) groups. Furthermore, NIDDM was divided into two groups: 34 patients who were newly diagnosed by OGTT (ND-DM) and 18 patients who were already established (known DM). The severity of coronary atherosclerosis was evaluated as a coronary index(CI), calculated according to Balcon's method. Although, there were no differences both of age and body mass index, serum fasting IRI(FIRI), ΣIRI(0,30,60,120 min), triglyceride and systolic blood pressure became higher acording to the severity of glucose intolerance. That is to say, fasting IRI were 5.4±3.8 μm/mℓ in Nor, 6.5±4.6 μm/mℓin IGT, 7.6±6.4 μm/mℓ in ND-DM and 6.2±6.4 μm/mℓ in known DM. ΣIRI were 124.1±80.7 μm/mℓ, 184.9±112.5 μm/mℓ, 155.3 ±74.7 μm/mℓ, 90.9±80.7 μm/mℓ, triglyceride were 141.2±65.5 mg /dℓ, 151.8±66.4 mg/dℓ, 161.8±63.0 mg/dℓ, and systolic blood pressure were 123±17 mmHg, 128±17 mmHg, 133±22 mmHg, respectively. However, HDL cholesterol showed no differences among them. In ND-DM, FIRI and ΣIRI were highest but they decreased in known DM. In the former three groups whose insulinogenic index(ΔIRI/ΔPG from 0 up to 30 min) was ≧0.4, the severity of CAD was significantly correlated to fasting IRI. We conclude that X syndrome could be seen in Japanese CAD.

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FREQUENCY, CARACTERISTICS AND EVOLUTION OF HYPERTENSION IN DIABETIC PATIENTS

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The aim of this prospective study concerned 318 diabetics observed during 1993-1994 is to evaluate the frequency, caracteristics and evolution of the highblood pressure (HBP) in diabetic patients. The results show that the frequency of the HBP is 23 % predominant in female patients (15,5 % VS 7,5 %). HBPis hyghly common with NIDDM 73,2 %, insulinotherapie require in 30 % Diabetes duration is 7,8 ± 5,8 ys. HBP is common at the same time with diabetics in 7,8 % of patients, before it in 3,6 % and after diabetes onset in 67,8 % in the majority it's a systolic high blood pressure. The mean systolic presssure is 151 ± 2,3 and the mean diastolic is 80 ± 3,2 mmHg.

1767

CORONARY HEART DISEASE (CHD) RISK FACTORS IN PERSONS WITH ENDOGENOUS HYPERINSULINAEMIA

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The major goal of the research was finding out the risk factors frequency for obtaining CHD. The research enclosed 21 subject with endogenous hyperinsulinism (HI) according following criterion: Diabetes mellitus type II positive family history in the first and second degree relatives and exclude the presence of CHD (negative ergo-test). According to the insulinaemia movements during OGTT, subject with HI were registred (Group A), as well as subjects without HI (Group B). Next, each subject was exposed to the number of anthropological and biochemical examination, and the results were compared among itself, as well as against entire population. The results emphasize the importance of the following factors: android type of obesity (increased waist to hip ratio) (Group A vs B:0.90+/-0.04 vs 0.86 +/- 0.12; $p<0.01$); impaired glucose tolerance (Glicaemia 120 minute: Group A vs B: 8.24+/-1.33 vs 6.4+/-1.38mmol/l, $p<0.01$); diastolic arterial tension (Group A vs B: 92+/-10 vs 83+/-13 mmHg, $p<0.05$), atheroidic lipid profile- low HDL-cholesterole and high triglyceride ($p<0.05$) and acidum uricum (Group A vs B: 353+/-77 vs 309+/-76 nmol/l, $p<0.05$). It was concluded that persons with HI (Group A) had significaly more risk factors for CHD compared with Group B.

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Risk for death from cardiovascular diseases among Arab diabetic patients in the United Arab Emirates

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We studied the prevalence of cardiovascular diseases and mortality in diabetic and in non-diabetic subjects using medical records of expired patients from 1993 - 1996 in Tawam Hospital run by Ministry of Health.The diagnoses were based on previous history, medication, laboratory findings and ECG-changes.Altogether 734 charts were examined.Only nationals of Arab origin aged 15 yrs or more from the UAE,Oman,Yemen and Saudi-Arabia were included.The final study population comprised 260 men and 153 women.Of men 65 (26.0 %) and of women 54 (35.3%) were diabetic.Of diabetic patients 37 men (56.9%) and 36 women (66.6%),and of non-diabetic patients 37 (20.0%) men and 16 (16.8%) women died from a cardiovascular vascular disease.The risk for cardiovascular death was 2.8 fold for diabetic men and 3.9 fold for diabetic women.In diabetic subjects cerebrovascular disease was found in 18 (27.7%) men and in 18 (33.3%) women,and in non-diabetic subjetcs in 20 (10.8%) men and in 8 (8.4%) women.In diabetic subjects coronary heart disease was found in 34 (52.3%) men and in 31 (57%) women and in non-diabetic subjects in 40 (21.6%) men and 14 (14.7%) women.In diabetic subjects myocardial infarction was diagnosed in 19 (29.2%) men and in 16 (29.6%) women, and in non-diabetic subjects in 22 (11.9%) men and in 5 (5.3%) women.In diabetic subjects gangrene/amputation was diagnosed in 7 (10.8%) men and in 3 (5.5%) women,and in non-diabetic subjects in 9 (4.9%) men but in none of non-diabetic women.

1769

ESTIMATES OF INSULIN SENSITIVITY AND BETA-CELL MASS AS PREDICTORS OF CARDIOVASCULAR DISEASE IN ELDERLY MEN

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The role of hyperinsulinaemia and insulin resistance as independent risk factors for cardiovascular disease remains debated. We previously reported that the prevalence of coronary heart disease is highest in subjects with the highest fasting insulin levels. These results were derived from data assembled in 1990 on a cohort of men born between 1900-1920. Recently the morbidity and mortality follow-up of the cohort was completed and associations with estimates of insulin sensitivity (IS) and beta-cell mass (BCM) as assessed by the HOMA-model (made available by prof. Turner, Oxford, calculations based on fasting glucose and C-peptide) were determined. Men with known diabetes were excluded from the analyses, leaving 446 subjects. Eleven percent of the men had impaired glucose tolerance and 9% had newly diagnosed diabetes. IS and BCM were inversely correlated (r=-0.58). In addition, BCM was significantly positively correlated with body mass index, fasting triglycerides, resting heart rate, serum creatinine, and inversely correlated with HDL-cholesterol. IS was positively correlated with HDL-cholesterol, and inversely associated with body mass index, triglycerides, systolic blood pressure, resting heart rate, and serum creatinine. During follow-up 106 men died, of which 55 had a cardiovascular cause of death. CVD mortality was elevated in men with IS below the median (16.2% versus 9.0%, p=0.02). Regarding BCM, CVD mortality was highest in men with levels above the median (17.4% versus 7.7%, p=0.002). After adjustment for age, other risk factors and the presence of CVD at baseline, the effects of IS (RR=0.54, 95% CI 0.30-0.98) and BCM (RR=2.26, 95% CI 1.25-4.07) remained significant. When IS and BCM were combined in one model, the effect of BCM was the strongest. The highest CVD mortality was seen in men with low IS and high BCM. These results indicate that hyperinsulinaemia is an independent risk factor for CVD and supports the hypothesis that the insulin resistance syndrome plays a role in the etiology of cardiovascular disease.

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COMPARATIVE EPIDEMIOLOGY OF ACUTE MYOCARDIAL INFARCTION IN DEFINED GENERAL AND DIABETIC POPULATIONS.

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The study was aimed at epidemiological, comparative assessment of the incidence and mortality due to acute myocardial infarction (AMI) in the strictly demographically defined, natural population of the District of Bródno (Warsaw) in the period between 1993 and 1995 (3 years). The incidence of AMI in general population under study was in average 0,4% that is 335 cases. Among them were 66 cases of AMI in diabetic persons. Since the total number of diabetics living in the District of Bródno (Warsaw) was 2320 the incidence of AMI characterizing diabetics was 2,8% that is 7 times more than in general population from the same enviromental conditions. The general hospital mortality due to AMI related to the general population was 21% (72 cases) and in the diabetic group 41% (27 cases), that is 1,9 times higher. The mortality in diabetic women (49%) was higher than in diabetic men (30%). These comparative observations are analysed in the relation to the structure of the AMI symptoms at clinical presentation, additional, cardiovascular morbidity in both groups and chronic vascular complications in the diabetic groups. The group of AMI patients without diabetes mellitus was characterised by lower incidence of heart and conductivity disturbances and by lower morbidity due to involvment of other arterial system beside the coronary. Conclusion: results point to the very high risk of total AMI in diabetics living in the same defined enviroment in spite of decreasing indexes of AMI incidence and mortality for general population. It shows the necessity of separate for diabetics, more active preventive and therapeutic programs. The results obtained in the study may be applied as the base for such development in different localities and defined population.

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IMPORTANCE OF CARDIOVASCULAR RISK ASSESSMENT IN DIABETES CARE

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One of the main objective of the St. Vincent Declaration is to reduce morbidity and mortality from coronary heart disease (CHD) in the diabetic by risk factor reduction. **Objective :** To assess the global cardiovascular risk status (CVRS) in diabetes mellitus (DM) with a method that could be further recommended for practical purposes. **Method :** In 1994, European Task Forces on prevention of coronary heart disease have proposed a chart that can predict risk level % change of coronary events in the next 10 years. In order to be more useful in diabetes mellitus we added other parameters: body mass index, waist/hip ratio, HDL-cholesterol, triglycerides, retinopathy, microalbuminuria, personal and family history of CHD. This new chart has been called Eurochart '94.**Patients :** We have applied this Eurochart '94 in 949 NIDDM subjects, 341 IDDM subjects and 1082 control subjects. **Results :** CVRS score (%) has been greater in NIDDM insulin treated group (21.07±1.1) and NIDDM non insulin treated group (19.9±0.6) than in IDDM group (10.8±0.6) and control group (6.1±0.3). CVRS has been strong correlated with age (8.03±0.3 if age≤40 yrs and 17.22±0.4 if age > 40 yrs) and with diabetes duration (18.5±0.5 at onset and 23.8±0.59 after more than 10 yrs duration of diabetes). All the differences we found are statistically significant. **Conclusions** The assessment of CVRS is mandatory in prevention of CHD in DM. The Eurochart '94 is a useful and accessible tool, which can be recommended for all those involved in diabetes care.

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ASIAN INDIAN WOMEN WITH DIABETES ARE MORE PRONE FOR CEREBROVASCULAR DISEASE.

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Indian Council of Medical Research (ICMR) study of diabetes morbidity between 1984 and 1990 on 4637 subjects (M 2783, F 1854) with NIDDM from 9 teaching hospitals examined by standardized methodology, reported that while coronary artery disease (M 8.91, F 5.11 %), peripheral vascular disease (M 0.72, F 0.37%), nephropathy (M 15.42, F 13.27%) and retinopathy (M 16.25, 14.29%) were more frequent in men, cerebrovascular disease (M 1.63, F 1.75%) was ascertained in more women. To identify gender differences in diabetic vasculopathy at death, consecutive death records of 453 subjects (M 292, F 161) diagnosed as diabetic between 35 and 64 years age (type 2) and treated at a large University Hospital in South India were studied. Predominant morbidity factors (and prevalence %) at death in these diabetics were coronary heart disease (M 45.2, F 42.9%), nephropathy (M 37.0, F 30.4%), infections (M 30.8, F 34.2%), cerebral vessel disease (M 27.7, F 30.4%), ketosis (M 6.5, F 12.4%), neoplasms (M 4.8, F 6.8%) and cirrhosis (M 5.5, F 3.1%). The unpublished ICMR study on diabetes morbidity and the mortality data as presented here, suggest that Asian Indian women with diabetes (NIDDM, type 2) are more prone for cerebrovascular disease.

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RISK FACTORS AND CHRONIC COMPLICATIONS IN NON-INSULIN DEPENDENT DIABETES MELLITUS (NIDDM)

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Our study was aimed at investigating the relation between risk factors and long term complications in NIDDM. Analysis included 3364 patients with NIDDM (and 159 insulin dependent diabetics as controls). History, demographic and clinical data, mean arterial pressure (MAP), urinary albumin concentration in spot morning urines, HbA1c and body mass index (BMI) were recorded. Micro- and macro-albuminuria were defined as spot morning urine albumin concentration 20-200 μg/ml and >200 μg/ml, respectively. Retinopathy, neuropathy, ischemic heart disease (IHD), and peripheral artery disease (PAD) were diagnosed by standard procedures. Results of multivariate correlations between risk factors and complications in NIDDM patients are in the table.

	Micro	Macro	IHD	PAD	Retinopathy	Neuropathy
Prevalence (%)	24.6	14.4	19.8	20.5	20.5	12.1
Age	N.S.	N.S.	**0.0001**	**0.0009**	N.S.	N.S.
Sex	N.S.	**0.003**	N.S.	**0.0003**	N.S.	N.S.
Diabetes duration	**0.03**	**0.0001**	**0.002**	**0.0003**	**0.0001**	**0.0004**
Hypertension duration	N.S.	**0.01**	**0.0001**	**0.003**	N.S.	N.S.
HbA1c	**0.002**	**0.0001**	N.S.	N.S.	N.S.	N.S.
Mean arterial pressure	N.S.	**0.0008**	N.S.	N.S.	**0.01**	N.S.
Body mass index	**0.02**	**0.004**	**0.05**	N.S.	N.S.	**0.02**

N.S.: not significant, Micro: microalbuminuria; Macro: macroalbuminuria.

Retinopathy was the only complication associated with the type of diabetes (higher prevalence in IDDM). Albuminuria and retinopathy were associated with enhanced risk for all the other complications. Albuminuria and fundus evaluations may help identifying NIDDM patients at risk of renal and extra-renal complications in large population surveys.

1774

PREVALENCE OF ISCHAEMIC HEART DISEASE & PERIPHERAL VASCULAR DISEASE IN AN URBAN SOUTH INDIAN POPULATION

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In migrant Asian Indians high prevalence rates of ischaemic heart disease (IHD) have been recorded. There are few data from the Indian sub-continent and particularly from South India. The prevalence of ischaemic heart disease (IHD) and peripheral vascular disease (PVD) in Madras city was assessed by a house to house survey. A total of 613 individuals were tested which included 144 diabetics (DM), 98 with Impaired Glucose Tolerance (IGT) and 371 with Normal Glucose Tolerance (NGT). Rose angina questionaire was used for IHD and the WHO Multinational Study questionaire for PVD. Resting 12 lead ECGs were done and coded using Minnesota Coding system. Peripheral vascular doppler studies were done and an Ankle/Brachial index was <0.8 considered diagnostic of PVD. 42/144 with DM (29.1%), 13/98 with IGT (13.2%) and 28/371 (7.5%) with NGT had angina. Using ECG criteria, 125/613 (20.3%) had evidence of IHD The prevalence of PVD was low - overall 1.4% (9/613) and among diabetics 2.1% (3/144). The reasons for the discordance between prevalence rates of IHD and PVD in S.Indians merits further study.

1775

HYPERCHOLESTEROLEMIA AND CORONARY HEART DISEASE RISK STATUS IN DIABETIC PATIENTS FROM ROMANIA

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The prevalence of hypercholesterolemia, according to the National Cholesterol Education Program has been determined in 866 diabetic patients and 900 people without diabetes. Rates of elevated total and LDL cholesterol in persons with diabetes are greater than in those without diabetes, after adjusting for age and sex (39.83% vs. 27.55% and 33.14% vs. 29.33%, respectivelly). Moreover, the prevalence of all coronary heart disease (CHD) risk factors (except smoking) is significantly higher in diabetes. As far as 37.99% of diabetic patients and only 9.11% in the general population are at highest risk for future CHD events because of prior CHD or other atherosclerotic diseases. A high risk because of hypercholesterolemia together with at least 2 other CHD risk factors is present in 16.57% of diabetic subjects and 3.78% of non-diabetic people. No more than 2.28% of diabetic patients as compared to 17.11% of people without diabetes have a low risk because of high blood cholesterol but less than 2 other risk factors. Overall, CHD risk status is twice as much in diabetes (56.84%) as compared to the general population (30%).

1776

REMNANT LIPOPROTEIN AND MACROVASCULAR DISEASE IN DIABETIC PATIENTS

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OBJECTIVE - Several reports suggested that remnant like lipoproteins (RLP) can promote atherogenesis. Recently, a new method has been developed to measure these lipoproteins. The aim of this study is to evaluate diagnostic advantage of RLP as a clinical marker of atherosclerosis in diabetic patients.
METHODS - 144 NIDDM patients (Male 77, Female 67) were measured carotid intima-media thickness (IMT) by B-mode ultrasonogram (SSA-270A, TOSHIBA Co.). Also we measured serum RLP-cholesterol (RLPc) by immunoseparation method using monoclonal anti-apoA1and anti-apoB (JIMRO Co.). Patients were divided in three groups according to their RLPc level and analyzed for glycemic control, average IMT and clinical events of macrovascular disease (i.e. cerebral vascular disease (CVD), coronary heart disease (CHD) and peripheral vascular disease (PVD)).
RESULTS - 48 patients as Group L (RLPc<2.5mg/dl) , 77 patients as Group N(2.5≦RLPc≦7.5) and Group H (RLPc>7.5) 19 were divided. Incidence of the macrovascular diseases (CVD/CHD/PVD) were as below. Group L 6.3%(1/1/1), Group N 16.9% (7/6/0), Group H 36.8% (1/5/1). Significantly higher incidence of macrovascular diseases was shown in the group of high RLPc (Average 16.7 ± 10.6mg/dl).
No significant differences of carotid IMT revealed in these 3 groups. Although IMT of the 23 cases which has macrovascular disease revealed greater than 121 non-macrovascular cases.
DISCUSSION - Assay for remnant lipoproteins could become an additional marker for high risk group screening of macrovascular disease in diabetic patients.

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Macrovascular Disease and Atherosclerosis – Clinical Picture

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THE INFLUENCE OF INSULIN RESISTANCE ON CAROTID ARTERIAL WALL THICKNESS IN JAPANESE NIDDM PATIENTS.

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Insulin resistance and its associated compensatory hyperinsulinemia are considered to be etiologically related to the onset of NIDDM and diabetic pathophysiological states such as hypertension, obesity and atherosclerosis. The present study was designed to investigate the relationship between carotid arterial wall thickness measured ultrasonically and the degree of insulin resistance measured by the minimal model analysis in Japanese NIDDM patients.

We demonstrate that the advance of carotid arterial wall thickness (intimal plus medial thickness; IMT) of NIDDM patients was intense compared to that of non-diabetic group. Furthermore, negative correlation was significantly recognized between ΔIMT (progression of IMT per year) and SI (insulin sensitivity index) ($r=-0.489$, $p<0.05$). The systolic blood pressure in patients with highly advanced IMT was significantly ($p<0.05$) elevated than that in patients with low advanced IMT (141±3 vs 130±3 mmHg). These results suggest that the advance of IMT is influenced by the degree of insulin resistance. The improvement of not only hypertension but also insulin resistance are necessary for the protection of the advance in IMT.

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EARLY CARDIAC STRUCTURAL AND FUNCTIONAL ALTERATIONS IN IDDM

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IDDM is a major risk factor for congestive heart failure and ischemic heart disease. How early abnormalities of cardiac structure and function appear in the course of this condition in not yet clear, however. We have studied 60 normotensive IDDM patients (age 32 ± 1, duration of diabetes 11 ± 1 ys, means ± SE) with no clinical evidence of diabetic complications, and 61 age- and sex-matched healthy subjects (C). At the echocardiographic evaluation Left Ventricular Mass Index was similar in IDDM and C (107 ± 4 and 106 ± 3 g/m^2 in IDDM and C respectively) while the relative wall thickness (H/R = SWTd +LVPWTd / LVEDD) ratio (0.41 ± 0.08 and 0.37 ± 0.08) and the E/A (Doppler Mitral Flow peak E/A) ratio (1.3 ± 0.05 and 1.5 ± 0.05) were respectively greater and lower in the former than in the latter group ($p < 0.01$). All echocardiographic values were only slightly more abnormal in a third group of 40 IDDM patients with retinopathy or microalbuminuria (age 34 ± 2, duration of diabetes 15 ± 1 ys). Thus in uncomplicated IDDM patients there is already evidence of ventricular remodeling and abnormalities in diastolic function as in complicated IDDM patients. This suggests that alterations in cardiac structure and function are an early phenomenon in IDDM.

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RELATIONSHIP BETWEEN CAROTID ARTERIAL WALL THICKNESS AND INSULIN RESISTANCE IN NIDDM

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To investigate whether insulin resistance play a major role in the pathogenesis of diabetic macroangiopathy, we measured IMT (the intimal medial thickness of carotid arteries measured with ultrasound high-resolution B-mode imaging) as the index of atherosclerosis and evaluated its correlation with both insulin resistance of peripheral and splanchnic tissues in 53 NIDDM patients (36 males and 17 females, 53 ± 10 years old, duration of diabetes 11.4 ± 7.5 years, and average BMI 22.9 ± 3.2). Patients were divided to the three groups according to the grade of their leisure-time physical activity by the questionnaire. GIR (average glucose infusion rate) calculated from euglycemic hyperinsulinemic clamp was used as index of insulin resistance of peripheral tissue. Following to the glucose clamp as above, oral glucose was taken under the continuation of the clamp to measure HGU (hepatic glucose uptake) as index of insulin resistance in liver. GIR ($r=-0.32$, $p<0.05$) but not HGU ($r=0.139$) is significantly inversely related with IMT. Stepwise multi-variant analysis revealed that GIR remains as an independent risk factor for IMT (F value 3.6) following age, total cholesterol, and diastolic blood pressure (F value 19.0, 17.2, 7.4 respectively). On the other hand, FBS and HbA1c, the index of diabetes control, did not show statistically significant F value. According to the degree of habitual exercise, GIR was increased from 6.19 ± 1.02 to 6.38 ± 1.38 to 7.44 ± 1.80 mg/kg/min, in low, moderate and high active group, respectively, while IMT was decreased from 1.34 ± 0 33 to 1.20 ± 0.31 to 1.12 ± 0.29 mm respectively, in male NIDDM. These data suggest that insulin resistance in peripheral tissue per se is one of independent risk factors of carotid atherosclerosis and habitual exercise might reduce insulin resistance leading to attenuation of atherosclerosis.

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DIABETES AND MACROVASCULAR COMPLICATIONS. ODD RATIO FROM HOSPITAL DISCHARGE AS POPULATION INDICATORS.

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It is well known that diabetes mellitus and macrovascular diseases have strong association; also that the selection bias introduced on admission to a highly specialized cardiovascular center might alter the association as expected from community. The aim of the study was to measure the magnitude of association at hospital discharge with recent data and see whether it could be used as change indicators for surveillance. It is assumed that optimal metabolic control of individuals decrease complications on the population and hospital information should follow the change. Method: information was obtained from 35,269 cases (2,970 deaths) from 1987 to 1995 on a discharge registry. Rates and proportions were calculated, stratification analysis was made among persons with diabetes (3,670) and non-diabetic (31,599). The coexistent diseases taken as macrovascular complications were: acute myocardial infarction (AMI) 4,223, ischemic heart disease (IHD) 12,458 or hypertension (HT) 6,878. Diseases taken as control for possible confounding variable interaction were drawn from 20-59 years old congenital heart disease (C) and rheumatic heart disease (R). As morbidity in male the odd ratio (O.R.) ± 95% Cornfield confidence interval (CI) revealed for AMI= 1.87 (1.66-2.09), IHD= 4.59 (4.1-5.13) and HT= 5.81 (4.04-8.35); in female AMI= 4.63 (3.88-5.51), IHD= 6.15 (5.46-6.95) HT= 4.99 (4.43-5.62). As mortality in male for AMI= 2.35 (1.67-3.27), IHD= 5.47 (3.83-8.12), HT= 5.26 (3.67-7.54); in female AMI= 4.07 (2.82-5.88), IHD= 7.39 (5.09-10.75), HT= 3.19 (2.88-3.54). Selection of 20 to 60 year of age revealed even higher figures, while the O.R. of C and R were below one. Therefore, the association remains and can be measured. The selection bias from hospital admission did not alter the known association. Hence, the indicators might work to asses long-term induced changes on diabetic macrovascular complications of community and to evaluate the impact of community interventions, such as the reinforcement of preventive measures and metabolic control.

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The participation of coagulo-fibrinolytic system in the progress of carotid atherosclerosis in subjects with BLDM and DM

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AIM of Study: Whether coagulo-fibrinolytic abnormality observed in diabetics is primary or secondary to diabetic macroangiopathy remains to be clarified. We examined whether coagulo-fibrinolytic abnormality is responsible to diabetes itself or to advanced atherosclerosis in diabetics.

METHODS: The profiles of coagulo-fibrinolytic system and intimal plus medial arterial wall thickness of the carotid artery (IMT) were examined on 163 diabetic patients without any symptoms of diabetic macroangiopathy (DM; m/f: 114/26), 26 mild hyperglycemic subjects (BLDM; m/f: 20/6) and 6 normal subjects (N/C; m/f: 1/5) using ultrasound high resolution B-mode imaging.

RESULTS: PAI-1 antigen, tPA-PAI complex, and factor VII activity were significantly higher in BLDM with (IMT≥1.1mm) and without advanced caroitd atherosclerosis (IMT<1.1mm) than N/C. Fibrinogen concentration, vWF activity and PIC were significantly higher in DM with and without carotid atherosclerosis. Multivariant analysis disclosed that age, high total-cholesterol, duration of diabetes, smoking and high PAI-1 antigen were independent risk factors for carotid atherosclerosis in subjects with BLDM and DM.

CONCLUSION: These data showed that the coagulo-fibrinolytic abnormality in BLDM is quite different from that in DM irrespective of presence of the advanced carotid atherosclerosis. The high level of PAI-1 antigen might be an independent risk factor for progression of carotid atherosclerosis in subjects with mild hyperglycemia

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SILENT ISCHEMIC HEART DISEASE IN INSULIN-DEPENDENT DIABETES MELLITUS

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Aim: To study the prevalence of silent ischemic heart disease (SIHD) among patients with insulin-dependent diabetes mellitus (IDDM) and to identify factors influencing the developement of SIHD. **Methods**: We studied 32 patients (16 women, age 35.5 ± 10.1 years, duration of diabetes 20.5 ± 10.1 years); of these, 16 had diabetic nephropathy (seven with microalbuminuria and nine with overt diabetic nephropathy). Patients with history of any cardiac disease were excluded (clinical or electrocardiographic signs). Dipyridamole plus exercise thallium myocardial perfusion scintigraphy was performed on all patients. A general blood analysis was drawn and the presence of hypertension, smoking, treatment, years of IDDM, diabetic complications and family history (diabetes, hypertension, cardiovascular disease) were recorded. **Results**: We found seven (21.8%) patients with thallium studies suggesting SIHD. There were no differences among patients with and without nephropathy. Those with a family history of diabetes had a higher prevalence of SIHD (6.2% vs. 37.5%, $p < 0.05$). There were no differences in any other analyzed variable. **Conclusions**: There is a high prevalence of SIHD using thallium-dipiridamole scintigraphy in IDDM patients. The only factor associated to this finding is the family history of diabetes mellitus, probably as an inherited marker of cardiovascular risc. Long-term prognostic value of these findings are not know and follow-up of these patients will be needed. The absence of relationship with other diabetic complications is surprising, although the group of patients is still small; an alternative explanation would be a different pathogenesis between microvascular and macrovascular diabetic complications.

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PROGRESSION OF EARLY CAROTID ATHEROSCLEROSIS IN YOUNG IDDM PATIENTS

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By high-resolution ultrasound, the intima-media thickness (IMT) of the carotid artery can be exactly determined, an increased intima-media complex is a sign of early atherosclerosis. The IMT of the carotid artery also reflects the extent of general and coronary atherosclerosis. In former studies, we found intima-media thickening even in young IDDM patients. In this follow-up study we evaluated the development (progression/regression) of the IMT over a longer period. 106 IDDM patients (41 men, 65 women) who were not older than 40 years at the time of the first ultrasound reading of the carotid artery, were re-studied after two to three years (mean 28.8 ± 7.7 months; age 30.8 ± 6.3 y; diabetes duration [dd] 15.5 ± 8.8 y). We measured the IMT of the common carotid artery 1 to 1.5 cm proximal of the bulb with a high-resolution wide-frequency probe (5-10 MHz) and checked the patient's state of complications (albuminuria, retinopathy, limited joint mobility, hypercholesterolemia, hypertension). The IMT was significantly ($p<0.001$) greater at the time of the second investigation (mean of all patients: 0.64 ± 0.17 vs. 0.56 ± 0.14 mm). In 40 patients (37.7%, age 29.0 ± 6.6 y, dd 14.3 ± 8.7 y) the IMT did not increase or even decreased (mean 0.54 ± 0.09 mm, - 0.03 mm), in 66 patients (62.3%, age 32.0 ± 5.8 y, dd 16.3 ± 8.8 y) we observed an increase of the IMT (mean 0.70 ± 0.18 mm, + 0.14 mm). Patients with a progression of the IMT significantly more often had diabetic complications than patients with stable IMT (one or more of the above mentioned: 88% vs. 66% at the time of the second investigation, $p<0.05$) and more of them had developped new complications during the observation period (15% vs. 5%). Multiple regression analysis showed that, besides age, hypertension and albuminuria were independent variables contributing to the IMT ($p<0.01$), while there was no correlation for the other complications or diabetes duration, HbA1c and sex. The majority of young IDDM patients shows a progression of early carotid artery atherosclerosis over a two-to-three year period. Several diabetic complications accelerate intima-media thickening, especially patients with albuminuria and hypertension are at high risk.

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CARDIOVASCULAR PROGNOSIS FOR DIABETIC SUBJECTS WITH SILENT MYOCARDIAL ISCHEMIA

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The predictive value of silent myocardial ischemia (SMI) for diabetic subjects has not yet been established. The aim was here to compare the cardiovascular course of diabetic patients with SMI (SMI+) with those without SMI (SMI-). 96 patients, 79 NIDD's and 17 IDD's, with ≥ 1 additional risk factors but without end-stage renal failure were followed for from 4 to 7 years. Thirty patients were SMI+ according to the results of an exercise test, a thallium dipyridamole myocardial scintigraphy and a 48-hour continuous ECG : 11 had significant stenoses found on coronarography (group 1), 14 had a normal coronary angiography (group 2) and 5 refused the coronarography (group 3). A cardiovascular event (myocardial infarction, heart rate abnormalities, heart failure, death of cardiac origin, amputation or stroke) occurred in 12 patients, 8 of whom had a cardiac event of which 3 died. Four of them were in group 1, 1 in group 2, 2 in group 3, and 5 were SMI-. Eight of them had retinopathy, 3 nephropathy, 3 peripheral neuropathy and 5 cardiac autonomic neuropathy (out of the 8 patients tested). The number of SMI+ patients who had a cardiovascular event (7/30 = 23.3%) was significantly higher than that for SMI- patients (5/66 = 7.6%) ($p < 0.01$). The 11 SMI+ patients with coronary stenoses were treated by vasodilative drugs or had an aorto-coronary bypass (1 case). Only one SMI+ patient complained of angor pectoris whereas 7 SMI- patients complained of this. This study suggests that SMI has a predictive value for a poor medium-term cardiovascular prognosis, and should be looked for in diabetic patients with cardiovascular risks and justifies adapting active therapeutic measures.

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IMPAIRED GLUCOSE TOLERANCE IS ASSOCIATED WITH INCREASED CARDIOVASCULAR RISK AND CAROTID INTIMA-MEDIA THICKNESS

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Impaired glucose tolerance (IGT) is known to be associated with increased incidence of cardiovascular disease (CVD), though the nature of this relationship remains not completely understood. Intima-media thickness (IMT) of the carotid artery was shown to be an indicator of CVD. Therefore, we decided to examine risk parameters and carotid IMT in age and weight matched first degree relatives of NIDDM patients with IGT or with normal glucose tolerance (NGT). Standard oral glucose tolerance test with 75 g glucose was conducted in 470 first degree relatives of index cases with NIDDM, aged 40-70 years and with BMI of 25-40 kg/m². Plasma glucose, real insulin (EIA) and proinsulin (EIA) were measured in a fasting state and 30′,60′,90′ and 120′ postprandially. Fasting plasma triglycerides, total and HDL cholesterol, PAI, tPA and v.Willebrand factor were measured by conventional methods. IMT of the common carotid artery was examined by B-mode duplex-ultrasound. Results: IGT was established in 30% of the examined subjects, whereas 59% had NGT. The IGT subjects did not differ significantly for BMI (28.8±4.6 vs. 27.2±4.6 kg/m²), WHR (0.94±0.09 vs. 0.92±0.09) and age (53.8±9.0 vs. 51.5±9.8). Nevertheless, these subjects showed increased carotid IMT (0.82±0.1 mm vs. 0.62±0.1 mm). Besides, IGT was characterized by a more expressed hyperinsulinemia (0′ 107±70; 30′ 572 ±445; 60′ 902±571; 90′ 943±589; 120′ 838±568 pmol/l) and hyperproinsulinemia (0′ 3.5±2.9; 30′ 6.5±4.4; 60′ 12.1±7.8; 90′ 16.3±10.1; 120′ 18.6±11.9 pmol/l) in comparison to the NGT group (insulin: 0′ 83±76; 30′ 553±476; 60′ 697±574; 90′ 550±454; 120′ 396±436 pmol/l and proinsulin: 0′ 2.4±2.9; 30′ 5.3±5.7; 60′ 8.5±7.2; 90′ 9.7±7.7; 120′ 9.1±6.4 pmol/l;; values in healthy controls: insulin: 0′ 65, 120′ 316 pmol/l; proinsulin 0′ 1.5, 120′ 6.9 pmol/l). 44% of the IGT group had hypertension (vs. 16% in the NGT group). PAI was slightly higher in the IGT subjects (43±26 vs. 35±31 ng/ml), whereas tPA (10.3±2.5 vs. 10.1±3.9 ng/ml) and v.Willebrand factor (101±32 vs. 100±45 %) did not differ. Triglycerides were moderately increased in IGT (2.81±2.71 vs. 1.59±0.94 mmol/l), total cholesterol (5.83±1.20 vs. 5.63±1.10 mmol/l) and HDL cholesterol (1.35±0.36 vs. 1.46±0.39 mmol/l) were similar. In conclusion: IGT subjects display an increased cardiovascular risk and carotid IMT than age and weight matched NIDDM relatives with normal glucose tolerance.

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DEVELOPMENT OF ECG-ABNORMALITIES IN PATIENTS WITH IMPAIRED GLUCOSE TOLERANCE

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Macrovascular lesions seem to develop in diabetics with rather mild metabolic derangement compaired with microvascular lesions. It has been reported thrombotic and/or obstructive carotid artery lesions were frequently detectable in echogram at almost the same rate in both impaired glucose tolerance (IGT) and non-insulin dependent diabetes mellitus (NIDDM). We calculated the rate of cumulative ECG-abnormalities for 20 years retrospectively and found the rate was almost the same in both IGT (23%,27% at 10,20 years) and NIDDM (21%,29% at 10,20 years). We divided 82 IGT into 16 IGT-Proper whose OGTT result stayed persistently in IGT pattern and 66 IGT/NIDDM whose OGTT result shifted transiently to NIDDM pattern though they were judged to be IGT retrospectively. High cumulative rate in IGT was explained with IGT/NIDDM (26%,30% at 10,20 years) vs IGT-Proper (13%,12% at 10,20 years). Analysis of baseline characteristics (IGT-Proper 16, IGT/NIDDM 66, and NIDDM 75) revealed serum total cholesterol ($P <.05$) and systolic blood pressure ($P <.005$) were significantly higher in both IGT/NIDDM and NIDDM than in IGT-Proper. To prevent the development of ECG-abnormalities in IGT, we emphasize to control serum total cholesterol and systolic blood pressure as well as blood glucose as antiathrogenic management in IGT.

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Clinical Characteristics of Intima-media Thickness in Carotid, Femoral and Popliteal Arteries using B- mode Ultrasound Imaging in NIDDM.

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Aim: In order to evaluate atherosclerotic changes in common carotid arteries (CA), femoral arteries (FA), and popliteal arteries (PA) in NIDDM, we measured intima-media thickness (IMT) in the far wall of respective arteries using B-mode ultrasound imaging in 32 NIDDM patients. Results: IMTs of FA were significantly higher than those of CA and PA in NIDDM. (FA; 1.13±0.08 mm、PA; 0.88±0.05 mm、CCA; 0.84±0.04 mm). Percent stenoses of FA and PA were significantly higher than those of CA. (FA; 27%, PA; 28%, CA 22%). Positive correlation was evidenced between two groups in these arteries. Simple regression analysis showed that IMTs of FA and PA were positively correlated with age, diabetic duration, or systolic and diastolic pressure. On the other hand, IMT of CA was positively correlated with age and systolic pressure. Thickening degrees of IMTs of FA and PA, which were correlated with DM duration and blood pressure, were higher than that of CA. IMTs of PA in NIDDM patients with diabetic retinopathy and neuropathy were higher than those in NIDDM without any complications, respectively. IMTs of FA and PA in NIDDM patients complicated with nephropathy were higher than those in NIDDM without complication. IMTs of FA and PA in diabetes with more than 5 years duration were positively correlated with urinary C peptide contents. Conclusion: These data indicated that progression of atherosclerotic changes in lower extremities (FA and PA) were more prominent than that of carotid artery in NIDDM while evaluation of IMTs of FA and PA using B-mode ultrasound imaging was proved useful for estimation of severities of atherosclerosis in NIDDM.

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INSULIN AND STRESS HORMONES IN CORONARY ARTERY DISEASE.

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The study was performed to find out the relation of insulin and hormones to coronary artery disease. Twenty non obese, non diabetic,normotensive men with CAD were studied [10 subjects with acute myocardial infarction (group la) and 10 with old ischemia (group 1b)]. Ten age matched men served as controls (groupII). Blood glucose was measured at 0,"0, 60,90 and 120 min after 75g oral glucose. Fasting serum insulin, GH, cortisol, T3 and T4, and plasma glucagon were estimated by RIA. Serum FFA, cholesterol, HDL and LDL cholesterol and triglycerides, and 24 hours urinary epinephrine (E) and norepinephrine (NE) were determined. Fasting serum insulin and blood glucose were increased in group la compared to those in groups Ib and III (30.7 ± 20.9 uU/ml and 5.5 ± 1.3 mmol /L versus 15.2 ± 5.9 uU/ml and 5 ± 0.8 mmol/L, and 12.9 ± 2.9 uU and 4.3 ± 0.4 mmol/L, p<0.001 and p<0.05 respectively. The 120 min blood glucose in group Ia was higher than that in groups Ib and II (p<0.01). Fasting serum insulin correlated directly with fasting and 120 min blood glucose as well as with serum LDL cholesterol in group I patients. Mean serum GH, T3 and T4, and plasma glucagon levels were comparable in the 3 groups; only mean serum cortisol and 24 hours urine E and NE were elevated in groups Ib and II, both correlated directly with serum FFA, p<0.001). No relation was detected between serum insulin and other hormones. We conclude that hyperinsulinemia with increased cortisol. E and NE secretion occurs in acute myocardial infarction but not in chronic coronary ischemia. The increase in the latter two hormones is responsible for the increase in serum FFA which creates an insulin resistant state.

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RELATIOMSHIP BETWEEN THE APPEARANCE OF MIDBAND BY PAGE AND CAROTID ATHEROSCLEROSIS

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<Aim> We study to elucidate the relationship between the appearance of midband and Lp(a) levels, which are risk factors for atherosclerosis, and atherosclerosis of common carotid artery (CCA) measured by ultrasonography in diabetic patients. < Subjects and Methods > Subjects were ninety-five patients (53 men and 42 women). Carotid intima-media thickness (IMT) on approximately 2cm proximal to the bifurcation of CCA was measured by ultrasonography, and classified one to four groups according to Salonen's categories. Category 1 is no atherosclerotic group. More category 2was regarded as an indicator of atherosclerosis.The midband in polyacrylamid gel electrophoresis (PAGE) is showed abnormal band between the bands of LDL and VLDL. <Result>(1) The appearance ratio of midband : Category 1 24.0%, Category 2 79.6%, Category 3 73.7%, Category 4 50.0%. (2) High Lp(a) levels were associated with midband positive group.

<Conclusion> The appearance ratio of midband increased with progression of carotid atherosclerosis. We concluded that a positive correlation between appearance of midband and IMT, which is used for screening of an indicator of atherosclerosis .

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SIGNIFICANT GREATER FREQUENCY OF MAJOR STENOSIS IN THE DISTAL CORONARY ARTERY SEGMENTS IN DIABETES

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To compare angiographically-determined coronary disease in diabetics with controls, 2.761 people coming to cardic catheterization were reviewed respectively. In this study, we excluded people who had myocardial infarction, totally-occuluded vessels, intervention-history, and over 60 years age. We found 31 diabetics (NIDDM) (15 males, 16 females) and 36 controls (25 males, 11 females). There are no significant difference between diabetic and control groups in sex, age, and risk factors (hypercholesterolemia, hypertension, smoking). The localization of significant coronary artery stenosis was assessed using comparison between proximal and distal segments. The following results were obtained. The Gensini-score reflecting the severity of coronary heart disease for diabetics(DM) was 19±15 compared with 18±16 for controls(N.S.). 8 of 31 DM (26%) were diseased only in proximal segments compared with 19 of 36 (53%) for controls (P<0.03). 15 of 31 DM (48%) were diseased only in distal segments compared with 5 of 36 (14%) for controls (P<0.01). In males, 6 of 15 DM (40%) only in proximal segments, 13 of 25 (52%) controls (P<0.05). 6 of 15 DM (40%) only in distal segments, 3 of 25 (12%) controls (P<0.05). In females, 2 of 16 DM (13%) only in proximal segments, 5 of 11 (45%) controls (P<0.03). 9 of 16 DM (56%) only in distal segments, 2 of 11 (18%) controls (P<0.06). The cardiac index for DM was 4.1±1.1 l/min/m^2 compared with 3.8±0.7 for controls(N.S.). We conclude that stenosis of the distal coronary artery segments is more prevalent, and cardiac index isn't decreased in diabetes.

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ULTRASONOGRAPHICALLY ASSESSED CAROTID MORPHOLOGY IN DIABETICS

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The aim of this study is to evaluate the relationship between ultrasonographically findings of common carotid artery(CCA) and obesity, the diabetic duration, the glycemic control and lipids in diabetics. We evaluated the intima-media thickness(IMT) of CCA used with ultrasonography in ninety diabetics. According to Salonen's classification, we divided ninety patients into 4 categories. The category 1 is nonstenotic CCA group, the category 2 is intimal-medial thickning group, the category 3 is the group with nonstenotic plaque and the category 4 is large stenotic plaque. We compared that IMT with obesity, diabetic duration, HbA1c, serum cholesterol, triglyceride, lipoproteins, apoproteins, Lp(a), RLP-cholesterol and aortic pulse wave velocity. Of the 90 patients examined, the ratio of category 1 was 40%, the category 2 was 18%, the category 3 was 30%, the category 4 was 12%. The stenotic group of IMT were associated with the history of obesity. There were no correlation between IMT and diabetic duration or HbA1c. The levels of serum cholesterol and triglyceride of category 4 in male were lower than category 1 and 2. These results suggested that category 4 had different process in arteriosclerosis compared with another categories.

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EVALUATION OF LEFT VENTRICLE DIASTOLIC FUNCTIONS WITH DOPPLER ECHOCARDIOGRAPHY IN YOUNG DIABETIC PATIENTS

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In this study, left ventricle diastolic functions of patients with no cardiac symptoms and its diagnostic value were investigated. It comprises 25 diabetic patients (mean age 31.5± 5.5 yrs., BMI 24.2±4.0 kg/m^2, heart rate 77.0±9.9 /min., mean dur. of diabetes mellitus (DM) 2.42± 2.25 yrs., Type 1 /Type 2 DM:15/10) and 14 healthy control (mean age 30.4±4.4 yr., BMI 24.4±2.2 kg/m^2, heart rate 72.9 ±9.2 /min). Two groups were matched regarding these parameters, with using two dimensional and M mode echocardiography, the systolic functions and heart valves were found to be normal. Doppler evaluation was performed to the patients from the level of mitral valve. Isovolumic relaxation time (IVRT) (ms), peak flow velocity of E and A waves (peak E, peak A) (cm/s), peak E/ peak A, integral of E and A waves (E_i, A_i) (cm), E_i/A_i, deceleration time of E wave (E_{DT}) (ms) were measured. The results were given below for diabetics vs controls. IVRT 84.0±15.7 ms; 73.5±7.6 ms ($p<0.02$), peak E 70.5±11.6 cm/s ; 72.8±12.0 cm/s (NS), peak A 55.2±8.7 cm/s; 48.2±5.5 cm/s ($p<0.001$), peak E/peak A 1.3±0.2 ; 1.5±0.2 ($p<0.001$), E_i 10.5±2.1 cm; 10.3±2.0 cm (NS), A_i 6.0±1.3 cm; 4.7±0.7cm ($p<0.001$), E_i/A_i 1.8±0.4; 2.2±0.3 ($p<0.01$), E_{DT} 182.2±18.2 ms; 163.2±13.8 ($p<0.001$). No significant difference in all parameters was detected regarding Type of DM. When diabetes regulation is considered the diastolic parameters between two groups was not significantly different (Group I: $HbA_{1C}\leq 8\%$ n:13; Group II: $HbA_{1C}>8\%$, n:12). As a conclusion, this study shows that in diabetic patients using Doppler echocardiography, a noninvasive method, it is possible to detect left ventricle dysfunctions in preclinical stage and this method can be widely used as a diagnostic test.

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ORGANIC AND FUNCTIONAL CHANGES OF ARTERIAL WALL IN NIDDM PATIENTS WITH CARDIOVASCULAR DISEASES

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The aim of this study is to assess the organic and functional changes of arterial wall in NIDDM patients with cardiovascular disease and to investigate the risk factors for these disease. The carotid intima-media thickness (IMT) was measured using B-mode ultrasonography and the stiffness of carotid artery wall was measured using echo-tracking system and aortic stiffness (PWV) was measured using pulse-wave velocimetry in 178 NIDDM patients. Carotid IMT was regarded as an index of organic change of arterial wall and carotid stiffness and aortic PWV were regarded as indices of functional change of arterial wall. The carotid IMT and stiffness, and aortic PWV were significantly higher in patients with CAD or PVD than in those without CAD or PVD ($p < 0.05$). The carotid stiffness and aortic PWV were significantly higher in patients with CVD than in those without CVD ($p < 0.05$), while there was no significantly difference in carotid IMT between patients with and without CVD. Multiple regression analysis showed that systolic blood pressure was an independent and common risk factor for CVD, increase in carotid stiffness and aortic PWV but not for CAD, PVD or increase in carotid IMT. The results suggests that CVD is related to the functional change of arterial wall rather than the organic change and that systolic blood pressure is an important factor for CVD and functional change of arterial wall.

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LEFT ATRIUM DILATION IN DIABETES MELLITUS : AN EARLY MARKER OF LEFT VENTRICULAR DYSFUNCTION ?

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One of left atrium dilation (LAD) causes is dysfunction of left ventricle (LV). Diabetes mellitus (DM) is associated with a specific cardiomyopathy. In this study, we investigate the prevalence of LAD in diabetic persons and its relation to LV dysfunction and/or other factors. We studied 27 patients (P) with DM (6 type I, 21 type II), 17 men & 10 women, age 31 - 70 yrs (x±SD : 54.3±10.6), with no other causes of LAD and without clinical, electrocardiographic or echocardiographic evidence of other cardiovascular diseases and without cardiac failure. Microangiopathy (MAG) was assessed by ophthalmoscopy. Long-term metabolic control (MC) was assessed by HbA1c (good MC : HbA1c≤7.2%). We screened LV function by color Doppler echocardiography study. A resting ejection fraction (EF) ≥50% characterized the normal systolic function of LV. We evaluated diastolic function by the transmitral flow indexes : isovolumic relaxation time (normal : 60-100 msec), deceleration time of E wave (normal : 160-240 msec), and ratio of peak E to atrial A wave velocity (normal : 1-2.4); 2 or more abnormal indexes characterized LV diastolic dysfunction (DD). LA dimension was estimated at the parasternal long axis (normal ≤ 4cm). *Results :* The duration (DU) of DM ranged from 0.7 to 32 yrs (9.6±3.9). 6 P had MAG. 10 P had good MC; HbA1c ranged from 5 to 13.7% (9.03±2.9). All P had normal systolic function; EF ranged from 53 to 70% (62.3±5.9). 15 P had DD. LA dimension ranged from 2.6 to 4.92 cm (3.9+0.57). 12 P had LAD (44.5%), 10 P out of whom had DD. 5 P out of the remaining 15 with normal LA had DD. LAD was not related to MC (p=0.2), DU (p=0.9), MAG (p=0.6) or age (p=0.09), but it was related to DD (x^2, p=0.03). *In conclusion :* Left atrium is often dilated in diabetic patients. LAD is related to subclinical diastolic dysfunction of LV, which it may indicate.

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The Cerebral Hemodynamic Changes Measured by Transcranial Doppler in Normotensive, Hypertensive and Diabetic Patients

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Objective: This study was designed to demonstrate cerebral hemodynamic changes as macrovascular complication related to diabetes mellitus and hypertension.

Method: We measured systolic velocities(Vs), diastolic velocities(Vd), mean velocities(Vm), and pulsatility indices (PI) of the middle cerebral artery (MCA) and the internal carotid artery (ICA) in stroke-free 37 normotensive diabetic(DM, M:F=15:22), 33 hypertensive diabetic(DMHT, M:F=16:17), 51 non-diabetic hypertensive(HT, M:F=25:26, duration ≥5 years) patients and 57 healthy controls(C, M:F=26:31), using a three-dimensional transcranial doppler sonography (Trans-scan, EME, Uberlingen, Germany).

Results: DM, DMHT and HT patients showed significantly higher PI of MCA, and DMHT patients also higher PI of ICA than C. PI of MCA in DMHT patients was more elevated than in DM.

(cm/sec)		C	DM	DMHT	HT
MCA	Vm	54.23±1.26	58.03±2.99	52.90±3.27	47.87±1.50*
	Vs	76.05±1.79	85.00±4.46*	79.71±4.94	70.19±2.33*
	Vd	37.87±0.98	38.63±2.16	32.85±2.30*	31.82±1.10*
	PI	0.71±015	0.81±0.21*	0.90±0.04*#	0.80±0.02*
ICA	Vm	25.07±0.63	25.37±1.15	24.00±0.97	23.70±0.71
	Vs	37.44±0.88	39.28±1.59	38.56±1.80	36.45±1.01
	Vd	17.43±0.53	17.44±0.87	15.61±0.64*	16.29±0.58
	PI	0.83±0.02	0.88±0.03	0.95±0.03*	0.88±0.03

*: $p<0.05$ compared with C, #: $p<0.05$ compared with DM

Conclusion: These results showed that the cerebral hemodynamic changes in diabetes mellitus and hypertension affect pulsatility of cerebral arteries, especially small arteries and arterioles. These atherosclerotic changes of cerebral arteries apppear to be more aggravated in diabetes mellitus combined with hypertension.

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DIASTOLIC DYSFUNCTION OF LEFT VENTRICLE IN DIABETIC PATIENTS WITH NORMAL SYSTOLIC FUNCTION

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It is known that diabetes mellitus (DM) is associated with a specific cardiomyopathy with primary & preclinical diastolic dysfunction (DD) of left ventricle (LV). In this study, we investigate the prevalence of DD in diabetic persons and the factors to which it is related. We studied 27 patients (P) with DM (6 type I, 21 type II), 17 men & 10 women, age 31 - 70 yrs (x±SD : 54.3±10.6), with no clinical, electrocardiographic or echocardiographic evidence of other cardiovascular diseases and without cardiac or renal failure. Microangiopathy (MAG) was assessed by ophthalmoscopy. Long-term metabolic control (MC) was assessed by HbA1c (good MC : HbA1c≤7.2%). All P were screened for cardiac autonomic neuropathy (CAN) with 5 stndard clinical tests; the responses were graded as normal (0), borderline (0.5) or abnormal (1), and a score of 1 or more was considered as indicating CAN. Doppler echocardiography study was performed in all P; a resting ejection fraction (EF) ≥50% characterized the normal systolic function of LV. By the transmitral flow indexes : isovolumic relaxation time (normal : 60-100 msec), deceleration time of E wave (normal : 160-240 msec), and ratio of peak E to atrial A wave velocity (normal : 1-2.4) we evaluated diastolic function; 2 or more abnormal indexes characterized DD. *Results :* The duration (DU) of DM ranged from 0.7 to 32 yrs (9.6±3.9). 6 P had MAG. 14 P had CAN. 10 P had good MC; HbA1c ranged from 5 to 13.7% (9.03±2.9). EF ranged from 53 to 70% (62.3±5.9). 15 P (55.5%) presented DD - 14 P the pattern of delayed relaxation (DLR) and 1 P that of increased stifness. Between P with normal diastolic function and those with DD, no significant difference was found for age, MAG, DU or MC, but it was found for CAN (x^2, Fisher : p=0.038). *In conclusion :* In diabetic patients with normal systolic function of LV, more than half demonstrated DD, mostly DLR, which is related to cardiac autonomic neuropathy. Furthermore, echo study has a pivotal role in the follow-up of diabetic persons.

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CENTRAL ARTERIAL PRESSURE AND ITS IMPLICATIONS IN DIABETES

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Atherosclerosis is more severe in diabetes. Whether diabetic subjects have increased arterial hardening, i.e. arteriosclerosis, is less clear. Arteriosclerosis increases pulse wave velocity, central arterial pressure and cardiac work load. We measured radial artery pressure waveform noninvasively by applanation tonometry (SphygmoCor™). Aortic waveform and pressure are derived using a transfer function developed from previous studies during cardiac catheterization. Radial pressure waveforms were obtained from 30 IDDM (Age 29.1 ± 7.8 yrs, HbA_{1c} 8.1 ± 2.1%, Duration 12.9 ± 9.4 yrs), 20 IDDM Controls (Age 32.5 ± 9.5 yrs), 48 NIDDM (Age 56.3 ± 9.6 yrs, HbA_{1c} 7.9 ± 2.0%, Duration 8.2 ± 6.3 yrs) and 15 NIDDM Controls (Age 59.6 ± 12.0 yrs). Aortic augmentation index (Aortic Aug %) is defined as {100 + (augmentation in systolic pressure expressed as a percentage of the pulse pressure)}. Subendocardial viability (SubE Viab) is defined as {(diastolic time x pressure)/(systolic time x pressure)}. Results shown as mean ± SD.

	Heart Rate (beats/min)	Aortic Systolic BP (mmHG)	Aortic Diastolic BP (mmHg)	Aortic Aug (%)	SubE Viab (%)
IDDM	74.2 ± 10.8	108.2 ± 18.5	75.5 ± 9.6	107 ± 17.9	136 ± 28.7
Control	65.4 ± 9.3[a]	99.6 ± 11.4[b]	71.5 ± 8.6	107 ± 19.2	162 ± 27.8[a]
NIDDM	72.1 ± 10.5	127.2 ± 17.1	84.2 ± 9.8	135 ± 18.0	137 ± 22.2
Control	70.2 ± 7.1	117.2 ± 14.9[c]	78.8 ± 8.6	132 ± 24.2	140 ± 18.4

[a] different to IDDM $p < 0.001$, [b] different to IDDM $p < 0.05$, [c] different to NIDDM $p < 0.05$ (unpaired t test)

Diabetic subjects have elevated central arterial pressure but no evidence of excessive augmentation. IDDM subjects do suffer from a relative rise in systolic work load in relation to diastolic filling time. Therefore, subtle changes in central cardiovascular parameters are present in diabetic subjects and could contribute to the cardiac disease in diabetes. (Supported by the Diabetes Australia Research Trust. SphygmoCor™ was a gift of Servier Laboratories).

1798

DIASTOLIC FUNCTION AND ARTERIAL COMPLIANCE ARE RELATED TO DURATION OF IDDM AND URINARY ALBUMIN EXCRETION.

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Indexes of left ventricular diastolic and systolic function (M-mode and Doppler echocardiography) and arterial compliance (volumen-oscillometric method) were studied in 55 consecutive IDDM patients, age 40.8±12.7 yrs, disease duration 16.8±12.9 yrs and HbA1c 8.4±1.0 %. Peak velocity of late diastolic filling (Peak-A) was gradually elevated with the severity of the stratification after urinary albumin excretion (UAE): normoalbuminuric (N=40): 0.62±0.20 m/sec, microalbuminuric (N=11): 0.77±0.14 m/sec and macroalbuminuric (N=4): 1.1±0.13 m/sec, $p<0.0001$. Peak-A was strongly correlated to IDDM duration, independent of the level of UAE ($r=0.64$, $p<0.00001$ for all patients and $r=0.69$, $p<0.00001$ for normoalbuminuric patients). Peak velocity of early diastolic filling, deceleration time, isovolumetric relaxation time, and left ventricular ejection fraction were not correlated to disease duration and no differences were seen between risk-groups. Like Peak-A, Compliance (C) of the peripheral arteries at the ankle level (at transmural pressure 0 and 100 mmHg) were related to disease duration and level of UAE: C^0: $r= -0.42$, $p<0.005$ and 21.3±6.6, 18.5±5.8, 13.0±7.4 µl/mmHg/10 cm, $p=0.06$, respectively. C^{100}: $r= -0.53$, $p<0.0001$ and 7.6±2.3, 6.1±1.7, 4.0±0.1 µl/mmHg/10 cm, $p<0.01$. Stepwise multiple regression analyses for the variation in Peak-A and compliance showed that age, HbA1c and blood pressure were of no importance. We conclude, that the prominent feature of diastolic dysfunction in IDDM is an increasing peak velocity of late diastolic filling. This might be due to an increasing stiffness of the heart, as it is seen in the major arteries.

1799

RENO VASCULAR HYPERTENSION IN NIDDM WITH SEVERE SYSTEMIC HYPERTENSION

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Renal atherosclerosis can lead to severe hypertension(HT) witch is deliterius to vessels and kidney. Does a renovascular HT(RVHT) must be searched in NIDDM with severe HT ? witch are patients with high risk of RVHT ?

70 consecutive NIDDM with severe HT (≥ 3 hypotensive drugs), 49 F/21 M (SR 2,3), mean age :65,6 ± 7,7 yrs, diabetic duration:11,1 ± 5 yrs have had metabolic, ABPM and renal investigations : renal US - n = 70, color dupleix scan (CDS): n = 53, and/or arteriography : n = 19.

14 (20 %) renal artery stenosis ≥ 70 % (RAS): 8 unilateral/6 bilateral were prouved by arteriography. We compared classic HT vs R.V.H.T. There was no difference for age (yrs) : 65,6 ± 8 vs 71 ± 6,3, HT duration (yrs) : 13 ± 7,5 vs 12,7 ± 6, B.M.I : 31,5 ± 6 vs 27,6 ± 3,3, HBA1C(%): 8,9 ± 2,2 vs 8,8 ± 0,9, CT (mmol/l): 5,7 ± 1,3 vs 5,5 ± 0,6. Significant difference ($p < 0,05$) was noticed for S.R (F/M) : 3 vs 1, diabetic duration (yrs) : 11,2 ± 5,1 vs 16,5 ± 8,8, frequency of retinopathy (%) : 30 vs 64, smoking (%): 14 vs 43, triglycerides (mmol/l) : 1,9 ± 1,1 vs 2,6 ± 1,1, and ($p < 0,01$) for blood pressure level (mmHg) (SBP : 139 ± 21 vs 154 ± 7, DBP : 80 ± 14 vs 86 ± 16, MBP : 102 ± 16 vs 110 ± 6), frequency (%) of HT escape (≥ 140/SBP, ≥ 90/DBP) on ABPM : 30 vs 75 and 24 vs 39,insulin requirance (%): 35 vs 71, macroangiopathy (%): 55 vs 100, micro/macro albuminuria (%): 34 vs 93, creatinaemia (mmol/l): 80 ± 23 vs 129 ± 47 + clearance (ml/mn): 66 ± 41 vs 39 ± 13. We tried to determine a RVHT score for investigations : renal US : 43 % - n = 6/14 (4 uni/1 bilateral hypotrophies),and for clinic : in the group (n = 36) with a poor control HT(SBP≥ 160 ± DBP≥ 90 ± MBP ≥ 120) and/or renal function decrease (albuminuria ≥ 100 mg/24 h ± creatinaemia ≥ 120 mmol/l), we have found all the RAS (n = 14) with positive diagnostic score : 39 %. In the group(n = 34) with well controled HT + normal renal function, there was no R.A.S.

Conclusion : in NIDDM with severe HT, reno vascular HT is frequent (20%). It must be evocated in unstable HT and/or renal injury with macro angiopathy, old NIDDM (> 15 yrs), requiring insulin .Renal U.S and colour duplex scan may lead to arteriography to confirm renal artery stenosis.

1800

ANGIOGRAPHIC CHARACTERISTICS OF CORONARY ARTERY DISEASE IN PATIENTS WITH TYPE 2 DIABETES

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The question of whether the severity or extent of coronary artery disease is different in diabetic patients compared with nondiabetic subjects is controversial. The aim of the present study was to compare the angiographic characteristics of coronary artery disease (CAD) utilizing quantitative coronary angiography (QCA) in a well-defined group of Type 2 diabetic patients (n=55) with those of individually matched nondiabetic control subjects. Both groups were undergoing clinically indicated elective coronary angiography. The QCA-derived parameters were incorporated into global perpatient indices describing severity and extent of CAD and overall atheroma burden. These indices were also calculated separately for different coronary segments, i.e. left main, proximal, mid, and distal segments and, also, for different vessel territories: left main, left anterior descending, left circumflex, and right coronary artery. No significant differences were found between the groups (global severity index, 51.5 ± 14.2 vs. 54.2 ± 13.2, p=NS; global extent index, 34.2 ± 13.2 vs. 32.2 ± 12.4, p=NS; global atheroma burden index, 26.8 ± 15.7 vs. 24.4 ± 11.9, p=NS). We also found no between-group differences in proximal, mid, or distal segments; in separate vessel territories; or in left ventricular function. In conclusion, we found no evidence to support the hypothesis that CAD would be more severe, extensive, or distal in Type 2 diabetic patients, compared with sex-, age, and BMI-matched control subjects, as evaluated by QCA.

1801

24 HOURS AMBULATORY BLOOD PRESSURE MONITORING IN NON-INSULIN DEPENDENT DIABETICS

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24 Hours ambulatory blood pressure monitoring (ABPM) was done in forty NIDDM subjects to study the diurnal blood pressure variation and to correlate them with cardiac autonomic neuropathy and left ventricular hypertrophy (LVH) Of them 21 were hypertensives and 19 normotensives. Male to female ratio was 25 : 15. Their mean age was 51.90 ± 7.12 years and the duration of diabetes ranged from 0 - 15 years. All patients underwent Cardiac autonomic function test and echo cardiography to assess left ventricular mass. Antihypertensive drugs were stopped 2 days prior to ABPM. Normally there is a 10 - 20% fall in mean systolic and diastolic blood pressure at night, these people are dippers. Those with less than 10% fall are non-dippers. In this study only 5 (16.5%) of the 40 subjects were dippers. Of them 2 were hypertensives and 3 were normotensives. All the 5 dippers had normal autonomic function. Of the 35 non-dippers 23 (65.72%) had evidence of cardiac autonomic neuropathy. All the 7 female non-dippers with hypertension and 3 out of 7 non-dipper female normotensives had LVH. Among males only 1 of the 12 non-dipper hypertensive and 1 of the 9 normotensive non-dipper had LVH. Of the 1 female and 4 male dippers 2 males had LVH. It is concluded that in this study (1) there is no significant difference in diurnal B.P. variation between hypertensive and normotensive diabetics. (2) 24 hrs. ABPM may help in early detection of Cardiac autonomic neuropathy (3) Female diabetics especially non-dippers are at increased risk of LVH.

1802

POOR GLYCAEMIC CONTROL PREDICTS CORONARY HEART DISEASE DEATH IN PATIENTS WITH INSULIN-DEPENDENT DIABETES

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The aim of our study was to examine the predictive value of cardiovascular risk factors with respect to coronary heart disease (CHD) death in patients with insulin-dependent diabetes mellitus (IDDM) without nephropathy. We examined a representative cohort of IDDM patients (113 men and 115 women), aged from 45 to 64 years, in eastern and western Finland in 1982-84. These patients were followed up to 7 years. Fifty-one patients (26 men, 25 women) with urinary excretion >300 mg/L and/or serum creatinine >110 umol/L in women and >120 umol/L in men were excluded. Altogether 20 IDDM patients (13 men (7.3 %) and 7 women (3.9 %) died of CHD during the follow-up. Univariate and multivariate Cox regression models were used to investigate the association of cardiovascular risk factors with the incidence of CHD death. In univariate analysis a previous history of myocardial infarction, glycated haemoglobin A_1 and the duration of diabetes were the only variables associated with the risk of CHD death ($p<0.001$). In multivariate analysis a previous history of myocardial infarction (Hazard ratio (HR) 8.0 (3.1-21.0, $p<0.001$), high glycated haemoglobin A_1 (>10.4 %, the highest tertile, HR 5.4 (1.4-20.4, $p=0.013$), and the duration of diabetes (>16 yrs, the highest tertile, HR 4.2 (1.3-12.9, $p<0.001$) were significantly associated with CHD death even after adjustment for other cardiovascular risk factors. In conclusion, our results indicate that poor glycaemic control is a strong predictor of CHD mortality in patients with IDDM without neuropathy.

1803

BLOOD PRESSURE AND TYPE I DIABETES COMPLICATIONS: DEFINITION OF HYPERTENSION CRITERIA USING 24 h-AMBULATORY BLOOD PRESSURE.

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Hypertension criteria recommanded by WHO appear too high for diabetic patients. The aim of this study was to evaluate by casual measurement and 24 hours-ambulatory monitoring (24-ABPM) the blood pressure level able to reduce the frequency of type 1 diabetes vascular complications. **Patients and methods:** 77 patients with type 1 diabetes mellitus were studied. Physical examination, BP measurement, smoking habits and serum lipids were realized at the beginning of the study (Y0) and two years later (Y2). In the same time, a statement of diabetes and its complications (nephropathy, retinopathy, coronary heart disease and arteriopathy) was performed. A 24-ABPM was also performed at Y0. These 77 patients were divided in two groups according to urinary albumin excretion (UAE) rate at Y0 either lower than 30 (group 1; N = 56) or upper than 30 mg/ 24 hours (group 2; N = 21). At Y2, patients of group 1 were divided in two groups according to the same criteria (group 1a: UAE<30: N = 44 and group 1b: UAE>30: N = 12). All parameters were compared between group 1 and 2 then between groups 1a and 1b. **Results:** No statistically significant difference was found between group 1 and 2 for age, sex-ratio, body mass index, smoking habits and serum lipids, diabetes duration, glycemic control as well as casual BP. The mean systolic and diastolic 24-AMBP values were significantly higher in group 2 versus group 1 (24 h-SBP: 128±15 vs 115±14 mmHg, p<0.001 and 24 h-DBP: 77±8 vs 73±7, p<0.05). Frequency of others complications was significantly lower in group 1. Blood pressure evaluated by clinical measure and 24-AMBP did not differ significantly between groups 1a and 1b. Patients of group 1b were older than those of group 1a (61±14 vs 51.7±15.8 years, p<0.05) and diabetes duration was higher (21±7 vs 15.6±10.1 years, p< 0.02). **Conclusion:** This study confirms the influence of BP level on the frequency of type 1 diabetes complications. Using 24h-AMBP and considering our results, a level of 24h-SBP lower than 115±14 mmHg and 24h-DBP lower than 73±7 should to be able to reduce this frequency.

1804

HYPERINSULINEMIA, CAROTID ATHEROSCLEROSIS AND LEFT VENTRICULAR HYPERTROPHY IN HYPERTENSIVES AND CONTROLS.

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High serum insulin levels have been associated with atherosclerotic vascular disease and incident hypertension in the multiple metabolic syndrome (MMS). To examine the prevalence of MMS and hyperinsulinemia in relation to left ventricular hypertrophy (LVH) and intima-media thickness (IMT) oral glucose tolerance test, bilateral carotid ultrasonography and echocardiography were performed in a random sample of middle-aged (40-59 yrs) hypertensive subjects (261 men (HM), 258 women (HW)), and in age-matched controls (259 men (CM) and 267 women (CW)). The prevalence of MMS was 3.8-35.3% in HM, 0.8-13.5% in CM, 8.5-21.7% in HW and 0.8-5.6% in CW. Insulin correlated significantly with LVH in HW and CW but the relation was confounded by body mass index in multivariate analysis. The mean IMT among all the 1031 subjects was 0.83 mm (SD 0.14) being 12% higher in men than in women (p<0.001). In HM the amount of plaques was 28% higher (p<0.003) than in CM. In HW there were 18% more plaques than in CW (ns). IMT was correlated significantly to age, LDL cholesterol (LDL), LVH, systolic blood pressure (SBP) and pack-years of smoking. In univariate analysis age-adjusted insulin parameters showed a negative correlation with IMT in HW (p<0.03) and in HM (p<0.06). In stepwise regression analysis after adjustment for age, BMI, LDL, and SBP only 2-hour insulin tended to be negatively correlated with IMT (p=0.055). We conclude that in contrast to conventional risk factors such as age, LDL, SBP and smoking, insulin had no independent contribution to the extent of carotid atherosclerosis and LVH in this cross-sectional population-based study.

1805

DUPUYTREN'S DISEASE IN DIABETIC PATIENTS: ASSOCIATION WITH ATHEROSCLEROTIC VASCULAR DISEASES

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Previous studies have shown an association between diabetes related microvascular complications and Dupuytren's disease (DD). This study aimed to clarify if DD was also associated with macrovascular diseases in diabetic patients. We examined 297 type 1 (age±SD 33.2±10.0 years) and 139 type 2 (61.3±12.3 years) diabetic patients. History of myocardial infarction (MI), coronary heart disease (CHD), cerebrovascular disease (CeVD) and peripheral vascular disease (PVD) were evaluated. DD was present in 14% of both type 1 and 2 diabetic subjects. Type 1 diabetic patients with DD were older and had had diabetes longer than those without DD, whereas in type 2 no association between DD and age or duration of diabetes was found. There was no association between control of diabetes (HbA_{1c}) and the presence of DD. Proportion (%) of atherosclerotic vascular diseases in type 1 and 2 diabetic subjects with and without DD are shown in the table.

Prevalence of atherosclerotic vascular disease (%)

	No DD	With DD		No DD	With DD
Type 1			**Type 2**		
MI	2	7*	MI	17	30
CHD	6	12	CHD	39	60
CeVD	2	7*	CeVD	20	40
PVD	12	30**	PVD	25	39

**p<0.05 and **p<0.01, compared to diabetic subjects without DD.*

Conclusions: Type 1 diabetic subjects with DD had a history of MI, CeVD and PVD more frequently than subjects without DD, but these associations were fully explained by the age of diabetic subjects (logistic regression analysis). No significant association between DD and atherosclerotic vascular diseases was found in type 2 diabetic subjects.

1806

INFLUENCE OF DIABETES ON DEVELOPMENT OF CARDIOGENIC SHOCK DURING ACUTE MYOCARDIAL INFARCTION.

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To clarify the relation between diabetes mellitus (DM) and cardiogenic shock (CS), clinical data of 458 consecutive pts hospitalized with acute myocardial infarction (AMI) were retrospectively analysed. Of 71 pts who developed CS, 52 had pre-hospital CS (PCS) and 19 in-hospital CS (ICS). Incidence of DM was not different between PCS, ICS, and non-CS (NCS) pts (32.2%, 31.6%, and 26.1%, respectively). However, a subgroup of ICS pts with aggravating pump failure (n=10) had significantly higher incidence of DM compared with another subgroup of ICS pts with re-infarction (n=9)(60.0% vs 0 %, P<0.05). Mean of maximum CPK excursion (max-CPK during re-infarction minus that on admission) in ICS pts with re-infarction was significantly greater than that in NCS pts with re-infarction (4986 ± 5826IU/l vs -737 ± 1626IU/l, P<0.001), indicating larger size of infarcts in the former group. Elapsed time after admission to CS in each individual of ICS with pump failure was less than 24 hours , whereas ICS pts with re-infarction developed CS in 3.2±1.9 hospital days. It is concluded that the influence of DM is more likely on cardiac performance than on cardiac vasculature in already infarcted heart, and that diabetic pts with AMI are at high risk of developing CS due to pump failure within 24 hours after admission.

1807

COMPUTERIZED EVALUATION OF PERIPHERAL VASCULAR DISEASE IN THE CLINICAL PRACTICE: THE AGREEMENT BETWEEN ANCKLE BRACHIAL INDEX (ABI) AND DOPPLER CONTINUOUS WAVE (DCW).

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The diagnosis of peripheral arterial disease is often made late in patients with diabetes. The consensus conference (Diabetes Care 16: 1199,1993) recommends to evaluate with DCW and/ or ABI all patients with more than 10 years of disease. These techniques can sometimes produce wrong diagnosis (NEJM 335:46,1996) above all in patients with diabetes where there are a lot of confounded factors (neuropathy, Monckeberg's sclerosis). We evaluated the agreement with K of Cohen between ABI, found with a new computerized system with three pletismographic probes, and DCW to verify if this new tool could useful to screen correctly peripheral arterial disease. 700 type2 diabetes patients were studied with ABI at rest and after exercise and DCW on leg arteries. K test was applied in 246 patients selected in four different groups: ABI very high (>1.20), normal (1-1.20) , borderline (0.80-0.99), small (<0.80) and with equal duration of disease. The agreement (κ) (5°–95° C.I.) was evaluated in these different clinical studies between ABI's and arterial DCW (normal or post occlusive signals). ABI's variability was 10-12%

N	age	Duration of Disease	ABI at the rest	κ (I.C 5°-95°)
40	69±7	9±3	0.66±.15	0.89 - 0.90
68	69±7	9±2	0.88±.06	0.74 - 0.85
96	65±6	9±2	1.06±.05	0.98 - 0.99
42	61±7	9±3	1.28±.08	1

In patients with diabetes ABI could produce wrong diagnosis (in 15-20%) of borderline situations respect DCW analysis. For screening peripheral arterial disease this new computerized tool produce a correct diagnosis without operator dependence problems of DCW. But borderline situation the specialized vascular assessment need.

1808

THROMBOLYTIC THERAPY IN DIABETICS WITH ACUTE MYOCARDIAL INFARCTIONS

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Thrombolysis of diabetic patients with acute myocardial infarctions (MI) saves 37 lives per 1000 treated but may be withheld inappropriately. We wished to see whether this occured in our unit. We examined the medical records of all patients admitted to this hospital with an acute MI, from 4/95 to 3/96, to see who did not receive thrombolytic therapy. Of the 244 patients records examined, 47 (19.3%) were known to have diabetes (8 receiving insulin, 37 oral agents, 2 diet alone) with 28% (13/47) receiving thrombolysis compared to 39% (76/197) of the non-diabetics. Only 1/3 of patients were thrombolysed within 30 minutes of admission. In the diabetic group reasons for withholding thrombolysis were: 57.4% late presentation (32% in non-DM), 6.4% non-Q wave infarct and 4.3% contraindications. Two patients (4.3%) in the diabetic group, and 4 (2%) of the non-diabetic group, should have received thrombolysis but did not. In our unit the majority of patients are appropriately thrombolysed but the door to needle time needs to be reduced. Diabetic patients present later with a median duration of symptoms prior to admission of 15 hours compared to 6 hours in the non-DM. Improved education of both doctors and patients is needed to ensure earlier presentation and more rapid initiation of therapy.

1809

ULTRASOUND WAVEFORM COMPLEXES IN DORSALIS PEDIS AND POSTERIOR TIBIAL ARTERIES IN DIABETIC PATIENTS ASYMPTOMATIC FOR PERIPHERAL VASCULAR DISEASE

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Occlusive peripheral vascular disease is responsible for the development of ischemic foot lesions in diabetic patients. Examination of waveform complexes obtained with doppler ultrasound, particularly of their phasic characteristics, may be useful in the assessment of the peripheral vascular system in diabetic patients. The identification of diabetic patients with asymptomatic peripheral vascular disease may allow the institution of early preventive measures to retard progression. In patients who already have foot ulcers, ultrasound assessment of the perpheral vessels is helpful in prognosticating disease outcome and directing clinical management. We compared the doppler ultrasound waveform complexes of a cohort of 11 diabetic patients versus a age-matched control group of 6 nondiabetic patients. Inclusion criteria for the study group were: duration of diabetes of at least five years, age range between 35 to 65 years, no cigarette smoking, and no previous history of intermittent claudication, rest pain or foot ulceration. The control group was matched for all these criteria apart from diabetic status. The mean HbA_{1c} in the diabetic group was 9.4% compared to 5.3% in the control group ($p<0.01$). The dorsalis pedis and posterior tibial arteries in both lower limbs were studied using a ES1000SPM doppler ultrasound (Hadeco, Japan). A trace of 5 to 6 waveform complexes was obtained and recorded for each of the four arteries in each subject. Using a calibrated rule, the height of the first deflection in the waveform complex (the A wave), corresponding to flow velocity in the vessel, was measured. The phasic pattern of the waveform, particularly of the succeeding waves (B and C waves) in the complex, was also analyzed. We found that the mean height of the A wave in both left and right posterior tibial arteries in the diabetic group was significantly reduced compared to the control group (left posterior tibial 14.9 vs 24.4 cm/s, $P<0.05$, right posterior tibial 16.3 vs 29.7 cm/s, $p<0.01$). There was no significant difference in this study in the A wave height of the dorsalis pedis arteries between study and control subjects. Diabetic patients had flatter succeeding B and C waves, suggesting a loss of elasticity and compressibility in the arterial walls. We conclude that asymptomatic diabetic patients may have significant stenosis in their foot vessels, and that ultrasound vascular assessment may be useful in risk stratification.

1810

CEREBROVASCULAR DISEASE IN DIABETICS: AN EXPERIENCE AT BIRDEM HOSPITAL

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From March '95 to August '96, Medical Unit I received 105 cases of NIDDM patients of acute onset cerebrovascular disease. Of them 71 (M:F = 64:7) under went CT scan of brain to determine the type of lesion. Factors, such as age, sex, duration of diabetes, hypertension, smoking habit, aspirin intake, cardiac lesion, plasma lipids etc. were studied to find their relation with the pattern of CT lesion and outcome. The study documented infarction is the main form of lesion, haemorrhage is the major killer, hypertension is a common feature in all type. Cardiac lesion is more prominent infarction group. Duration of diabetes mellitus was more in infarction group than in the rest. Mean age of different type of lesions were almost identical. Cerebrovascular disease is a major problem in which a diabetic is crippled in moments is being encountered at BIRDEM Hospital round the year in a large number. Considering this the present study was designed to see the pattern of lesion in our NIDDM cerebrovascular disease patients and also to evaluate the factors related to course and outcome in them. The patients were selected from the admitted patients of acute onset cerebrovascular complications of NIDDM.

1811

CARDIAC AUTONOMIC NEUROPATHY HAS A HIGHER PREDICTIVE VALUE FOR CARDIAC EVENTS THAN SILENT MYOCARDIAL ISCHEMIA

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The predictive value of silent myocardical ischemia (SMI) is not well known for diabetic subjects. The prognostic value of cardiac autonomic neuropathy (CAN) has been strongly suggested in the literature but has not yet been determined according to the presence or absence of ischemic heart disease. The aim of this study was to analyse the predictive value of these two complications of diabetes in a series of 63 patients, who had been followed for from 4 to 7 years, 52 NIDD's and 11 IDD's, with $\geqslant$ 2 additional cardiovascular risk factors without end-stage renal failure. Evidence of SMI was found in 18 of them during the initial assessment (exercise ECG, thallium dipyridamole myocardial scintigraphy and ECG recording for 48 hours) : 7 had significant stenoses on the coronary angiography, 9 had a normal coronary angiography and 2 refused this examination. Evidence of CAN was found in 26 of the 63 patients by means of 3 parasympathetic tests (Valsalva, deep-breathing, lying-to-standing). The frequency of CAN was not significantly different in the patients with SMI (5/18), in particular those with stenoses (2/7), and in the patients without SMI (21/45). A major cardiac event occurred in 4 patients (death, pulmonary edema, myocardial infarction), all of whom had CAN and two of whom had SMI. A noncardiac vascular event (stroke or amputation) occurred in 4 patients, one of whom had CAN and two SMI. This study does not provide positive proof of the involvement of cardiac neuropathy in the silent clinical aspect of the ischemic heart disease but suggests that CAN has a predictive value for a poor medium-term cardiac prognosis, independent of the presence of ischemic heart disease.

1812

CLINICAL EVALUATION OF CEREBROVASCULAR ACCIDENT WITH NIDDM.

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In patients with non-insulin-dependent diabetes mellitus(NIDDM), the risk of cerebrovascular accident(CVA) is known to be increased. Therefore, we evaluated the significances of variables with respect to the risk of CVA in NIDDM patients. We assessed retrospectively these variables in 160 subjects(82 men, 78 women), divided into 3 groups; 90 CVA with NIDDM patients(50 men, 40 women), 40 NIDDM patients(17 men, 23 women) and 30 CVA patients(15 men, 15 women). We found that the mean values of cholesterol(5.15±0.83 mmol/L), triglyceride(2.29 ±0.21 mmol/L) and blood pressure(systolic B.P 158.5±30.9 mmHg, diastolic B.P 97.5±17.3 mmHg) were higher in CVA with NIDDM patients than the others($p<0.05$). There were high incidences of lacunar infarcts in NIDDM patients(57.8%). But, there were no significances in the other variables($p>0.05$). Thus, we suggest that hypertension, hypercholesterolemia and hypertriglyceridemia in NIDDM patients could be associated with increase in the risk of CVA.

1813

QT INTERVAL LENGTH AND QT DISPERSION AS PREDICTORS OF MORTALITY IN NON-INSULIN-DEPENDENT DIABETES MELLITUS

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The aim of our prospective study was to evaluate the impact of the QT interval length and the QT dispersion on mortality in all (n=328) Caucasian NIDDM patients <65 years of age, attending our clinic in 1987. A resting ECG was recorded at baseline. Normoalbuminuria (urinary albumin excretion (UAE) <30 mg/24 h) was present in 191, microalbuminuria (UAE 30-299 mg/24 h) in 86, and macroalbuminuria (UAE ≥300 mg/24 h) in 51 patients at baseline. Mean age at entry was 54 ± 9 (SD) years. The minimum and the maximum QT intervals were measured in a 12-lead ECG and corrected for heart rate (QTc-min and QTc-max). QTc dispersion was defined as the difference between QTc-max and QTc-min. QTc-max was 450 (362-671) (median (range)) ms and QTc dispersion was 56 (0-268) ms. 61% (199/328) of the patients had prolonged QT interval defined as QTc-max ≥440 ms. During the 9-year follow-up period 100 (30%) patients had died; 44 patients from cardiac causes. 38% of patients with prolonged QT interval had died compared with 19% of patients with QTc-max within the normal range, $p<0.05$. Cox proportional hazard analyses revealed the following independent predictors of death:

	Mortality (relative risk (95% CI))	
	All-cause (N=65)	Cardiac (N=29)
Sex (males)	-	2.30 (1.15-4.49)
LogUAE (factor 10)	1.69 (1.33-2.14)	-
Age (1 yr)	1.08 (1.04-1.11)	1.09 (1.03-1.14)
HbA_{1c} (1%)	1.14 (1.03-1.26)	1.19 (1.02-1.38)
QTc-max (1 ms)	1.005 (1.000-1.010)	1.007 (0.999-1.014)*
s-cholesterol (1 mmol/l)	1.12 (1.00-1.24)	1.25 (1.08-1.45)
Systolic BP (1 mm Hg)	1.009 (1.000-1.018)	1.01 (1.00-1.03)
Duration of diabetes (1 yr)	-	1.05 (1.01-1.09)
		*P=0.07

We conclude that longer QT interval, but not increased QT dispersion indicate an increased mortality risk in NIDDM patients.

1814

ROLE OF FIBRINOGEN ON PATHOGENESIS OF THE STROKE IN NIDDM PATIENTS.

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In order to examine the role of the fibrinogen (FB) on the pathogenesis of the stroke in NIDDM patients we studied 32 consecutive patients with previous isquemic stroke confirmed with cranial CT, that have been examined at 48 hours of the stroke. We analized plasma glucose (PG), cholesterol (CH), triglycerides (TG), HDL-, LDL-, VLDL-cholesterol (VLDL), apolipoprotein (apo) AI and apo B, and FB [all by standard methods] levels, matched for age, sex, body mass index and arterial pressure in two groups, diabetics (n=16) and non-diabetics (n=16). The results obtained have been processed by the mean and standard deviation and the correlation between all them. The results were: PG 12.2±4.6 vs 5.5±0.6 mmol/l ($p<0.01$); CH 5.3±1.0 vs 5.1±1.2 mmol/l; TG 3.0±0.6 vs 2.9±0.8 mmol/l; HDL 0.9±0.2 vs 1.1±0.3 mmol/l; LDL 3.8±0.9 vs 3.4±1.1 mmol/l; VLDL 0.6±0.1 vs 0.6±0.2 mmol/l; apo AI 1.55 ±0.27 vs 1.51±0.27 g/l; apo B 1.31±0.23 vs 1.16±0.19 g/l, and FB 2.72±1.44 vs 5.34±1.49 g/l ($p<0.01$), in the diabetic and non-diabetic groups, respectively. We find a positive correlation ($r=0.7$; $p<0.01$; $n=16$) between FB and PG levels in the non-diabetic group, but not in the diabetic group ($r=0.1$). FB levels were lower in diabetic group than in non-diabetic group. The PG levels in acute phase of the stroke are correlated with FB levels only in non-diabetic group. Our results suggest a slight influence of the FB levels on the acute phase of the stroke in NIDDM patients.

1815

QTc INTERVAL AND QT DISPERSION PROLONGATION IN NIDDM PATIENTS WITH AUTONOMIC NEUROPATHY

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To clarify whether QTc interval and QT dispersion can be employed as objective and meaningful indicators for evaluating the severity of diabetic autonomic neuropathy, we investigated the relationship between NIDDM patients with diabetic autonomic neuropathy and QTc interval prolongation suggested to be induced by imbalance of sympathetic innervation on heart. Patients with NIDDM (n=60, age=61.18±10.4 yr), nondiabetic controls(n=50, age=59.3±7.36 yr)were included in this study. Performing standard cardiovascular autonomic function test (deep breathing test, lying to standing test, cough test, postural BP drop test), sixty NIDDM patients are divided into 3 groups (group I :normal n=3, II :borderline n=10, III :abnormal n=47)by the degree of diabetic autonomic neuropathy.We measured QT,QTc interval and QT dispersion in each diabetic group and nondiabetic control subjects. Statistically significant difference was observed in QTc interval and QT dispersion between diabetic and nondiabetic control group as followings ; Patients group QTc interval 435.7±37, controls 418.6±34 (msec) (p=0.015), QT dispersion 48.43±9.332, 39.41±6.277 (msec) (p=0.001) respectively. Furthermore, significant difference was observed in comparing three diabetic groups. These data provide further evidence of a relationship between the presence and severity of diabetic autonomic neuropathy and degree of QTc interval and QT dispersion prolongation.We suggest this QTc interval, QT dispersion prolongation may offer relatively prospective means of estimating diabetic autonomic neuropathy in patients with NIDDM.

1816

INSULIN RESISTANCE IN CORONARY ARTERY DISEASES AND CEREBRAL INFARCTION.

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In non-diabetic, non-obese, physically active patients with coronary artery diseases(CAD) or atherothrombotic cerebral infarction(A-CI), insulin sensitivity determined by SSPG method using Sandostatin was significantly reduced(40-50%). CAD includes both vasospastic(VS) and obstructive coronary diseases(OAP). The insulin resistance correlated with the carotid intima-media thickness determined ultrasonographically in VSAP and the degree of stenosis in OAP respectively. In A-CI, the resistance is correlated with low HDL-chol and low ratio of LDL-chol/LDL-ApoB, indicating the preponderance of small dense LDL. Compensatory hyperinsulinemia has been observed and subjects with these vascular lesions suffer from vicious cycle of potentialing multiple risk factor syndrome as well as tendency for developing diabetes. Efforts to maintain normal insulin sensitivity are particularly important to correct the vicious cycle and to prevent the initiation as well as progression of coronary and atherothrombotic cerebral diseases.

1817

HYPERGLYCEMIA, DIABETES AND AUTONOMIC CARDIAC DYSFUNCTION.

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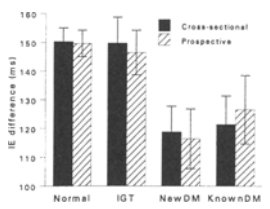
We studied the association between glucose and autonomic cardiac function in a sample of 774 participants of the Rotterdam Study, a population based prospective cohort study. An oral glucose tolerance test was performed at baseline and at the follow-up examination after 2.1 years (range 1.2-4.5). Cardiac autonomic function was assessed at follow-up by the heart-rate responses on deep breathing and standing. At baseline the mean age was 64.4 years (SD 5.2), and random serum glucose 7.1 mmol/l (2.7). Mean fasting glucose at follow-up was 6.2 mmol/l (1.7).

Glucose at baseline was associated with a lower response to deep breathing: the age-adjusted regression coefficient was -3.5 mmol/l per millisecond difference (95%CI -6.2; -0.8, $p<0.01$). The association with glucose at follow-up was -5.8 mmol/l per ms (-10.0; -1.5, $p<0.01$). The same trends were observed for HbA1c, while insulin level showed no association, nor at baseline or follow-up. The age-adjusted difference during deep breathing according to glucose tolerance (normal, impaired, diabetes) is given in the figure. In subjects with diabetes cardiac autonomic function was significantly impaired compared to normal subjects ($p<0.01$). This was more evident in the cross-sectional analyses. All associations remained after adjustment for gender, body mass index or blood pressure. When using the heart rate responses after standing essentially the same results were obtained.



These results indicate that cardiac autonomic dysfunction, while associated with hyperglycemia, is the same in newly diagnosed as treated diabetes patients, suggesting that it is related to concurrent glucose levels.

1818

ESTIMATED PREVALENCE OF DIABETES MELLITUS IN ACUTE CEREBROVASCULAR DISEASE PATIENTS: THE CAPTURE-RECAPTURE METHOD

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Diabetes Mellitus (DM) has a high prevalence and is an important risk and prognostic factor for Cerebrovascular disease (CbVD) in Salvador. It is widely underestimated in acute CbVD patients. On the other hand, CbVD is the main cause of death in Brazil and Salvador has the highest standardized incidence rate reported in western countries. The study aim was to estimate the "prevalence" of DM among incident cases of CbVD using the capture-recature (C-R) method. Data sources: lab/ glycemia results for CbV patients and; medical records of CbV patients for identification of self-reported history of DM. Criteria for DM lab-test: glycemia value ≥160mg/dl for patients with either negative, positive out of treatment or unknown reported history of DM and any glycemic value for those with self-reported DM with comproved past or present treatment. Chapmann C-R method and 95% C I were used. The medical records data were corrected by excluding 6.6% of cases (probability of a DM positive history in nondiabetic CbVD patient). The C-R method was applied to the 3 medical assistance sectors. Results and conclusions: Among 1227 CbVD patients there were 331 DM cases in both sources and for the 3 assistance sectors. This figure corresponds to 70% of ascertainment of the estimated uncorrected value (n=470, CI: 406-538). The corrected identifyed value was 324 and the estimated = 454, CI= 399-509. The uncorrected and corrected "prevalences" were 27% and 26,4% and its estimated correspondents = 38.3%(CI: 33-44) and 37%(CI: 32.5-41.5). The lowest ascertainment probability was found in the public assistance sector Since the frequencies of questioning for DM and Lab-tests for glycemia were lower than those expected (≅ 100%), the C-R method was of great value to showing a) the expected "prevalence" of DM among CbVD patients; b) the quality of medical assistance for CbVD/DM inpatients; c) and to allert to possible unnecessary death in unrecognized and untreated diabetic patients with CbVD. As prevalence of DM and incidence of CbVD increase with age, this may explain the high "prevalence" of DM among CbV patients.These results may be used to the improvement of DM and cardiovascular disease programs and the quality of medical assistance.

1819

FACTORS THAT INFLUENCE THE DEVELOPMENT OF AUTONOMIC NEUROPATHY IN DIABETES MELLITUS: A HEART RATE VARIABILITY STUDY

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One of the effect of diabetes on cardiovascular system with implications on prognosis is its potential to create autonomic neuropathy. For this reason 24 hour Holter recordings were examined for heart rate variability, a method gaining acceptance in the diagnosis of autonomic neuropathy. The study group comprising of 8 Type 1 and 19 Type 2 diabetic, 13 male and 14 female, with a mean age of 47.5± 10.9 (Range 30-70) (Group 1) was compared to 19 healty controls, 7 male, 12 female with a mean age of 47.8± 10.4 (Range 20-60)(Group 2). Age is not significantly different between groups. The heart rate variability parameters of the groups and their significance are as follows;

	Group 1	Group 2	p value
Mean heart rate	78.96 ± 8.80	77.16 ± 6.60	0.45
Mean NN	762.11 ± 86.1	786.16 ± 67.7	0.31
SDNN	102.81 ± 45.3	152.10 ± 42.9	0.001
SDANN	94.22 ± 41.96	138.36 ± 42.24	0.001
SD	35.44 ± 16.70	61.42 ± 15.93	0.0001
rMSSD	18.44 ± 8.68	35.42 ± 14.08	0.0001
pNN50	3.08 ± 4.42	11.19 ± 8.61	0.001

This results show that spectral analysis parameters of sympathic and parasympatic nervous system activity is decreased in diabetic patients compared to controls. There is no correlation between glycaemic control as assessed by HbA1c and heart rate variability parameters. When diabetic patients were grouped according to presence or absence of diabetic complications, patients with diabetic retinopathy and to a lesser extent patients with diabetic nephropathy and neuropathy formed a subgroup of diabetics with more severely affected heart rate variability parameters. We concluded that heart rate variability parameters for symphatic and parasymphatic nervous system activity were impaired in diabetics and this impairment was most excessive in the subgroup of diabetic patients with retinopathy.

1820

RELATIONSHIP BETWEEN CAROTID ARTERY PLAQUE AND CEREBRAL INFARCTION IN PATIENTS WITH NIDDM

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The aim of this study was to investigate the relationships between extracranial carotid artery plaque and cerebral infarction using non-invasive B-mode ultrasonography brain computed tomography and MRI in patients with NIDDM. Ultrasound high resolution B-mode imaging of carotid arteries was conducted on cerebral infarction patients with NIDDM and without NIDDM, to define the plaque of carotid artery. Concurrently, total cholesterol, HDL cholesterol, serum triglyceride and HbA_{1c} were measured by standard laboratory technique. The incidence of cerebral infarction was increased in relation to extracranial carotid artery plaque existence. The frequency of carotid artery plaque was higher than in NIDDM patients with cerebral infarction(63% vs 35%, $p<0.05$). There was no significant difference in existence of carotid plaque in NIDDM patients and non diabetic patients with CVD. Multiple regression analysis showed that development of cerebral infarction in NIDDM patients was positively related to hypertension, hypercholesterolemia and carotid plaque. Existence of carotid plaque is closely related to cerebral infarction. So early detection of extracranial carotid plaque by B-mode ultrasonography is very useful to predict cerebral infarction in NIDDM patients.

1821

JUST TO FIND THE BEST NON-INVASIVE METHOD WHILE COMPARING CARDIAC STATUS IN NIDDM AND IDDM!

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The aim of the study is to find the best non-invasive method to compare cardiac status in diabetics.15 patients with IDDM and 14 patients with NIDDM (without hypertension or any other cardiac problem)are compared according to following parameters:Age,duration of DM, HbA1C, albuminuria, lipid profile; Echocardiographically obtained Left Ventricular Mass Index (LVMI reflects LV hypertrophy),Ejection Fraction (EF reflects systolic function),E/A ratio (reflects diastolic function obtained from doppler flow velocities of the mitral valve,where E=peak LV inflow in early diastole and A=flow in late diastole with atrial filling), Late potentials (including t-QRS, RMS, HFLA parameters obtained from Signal Averaged ECG which is a strong predictor of arrhythmia) and QT-dispersion (QTd) in ECG that is claimed to be related to autonom neuropathy and reflect high risk of sudden death.(QTd=QTmax- QTmin).

	age ** (year)	LVMI (N<90 g/m2)	E/A * (N>1)	QTd (N<40ms)	Albuminuria(RIA) (N<20mic/min)
NIDDM	57.1∓7.8	126∓34	0.87∓0.27	37.8∓23.3	176.4∓30.6
IDDM	27.6∓3.7	118.9∓47.9	1.25∓0.32	50∓27.08	288∓367

	Late potentials (LP) t-QRS (N<114 ms)	RMS voltage (N>20microvolt)	HFLA (N<38ms)
NIDDM	98.4∓8.9	76.5∓29.2	25.7∓8.6
IDDM	101.3∓6.7	91∓17.2	24∓5.9

****=p<0.001 *=p<0.05**

Although both groups had increased LVMI, there was no difference between the parameters except for advanced age and diastolic dysfunction (E/A <1) in NIDDM.Albuminuria was correlated to LVMI(p<0.05) and LP in both groups and to QTd in IDDM.So,NIDDM carries more risk for diastolic heart failure and both groups hold the same risk for arrhytmia and sudden death which are correlated to albuminuria.Serial follow up by echocardiography and albuminuria especially in NIDDM and by QTd mainly in IDDM will be convenient.

1822

CAROTID ARTERY STIFFNESS IN MICROALBUMINURIC IDDM.

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In IDDM microalbuminuria is a risk factor for the development of atherosclerotic disease. We investigated arterial distensibility, as a marker of functional or structural vessel wall properties, of the common carotid artery in 24 microalbuminuric (MA) and 53 normoalbuminuric (NA) IDDM patients, and in 54 healthy control subjects (C) comparable for gender, age, number of smokers and lipids. The IDDM patients were all normotensive and had no signs of clinical vascular disease. Arterial distensibility was measured with an echo-doppler method (Wall Track System). The distensibility coefficient (DC) was different between the groups (MA: 21.6[SD7.5], NA: 24.8[5.9], C: 25.9[5.7] 10^{-3}/kPa; P=0.018), based on lower values in MA as compared to both other groups. In addition a difference in body mass-index (MA:24.4[2.6], NA:23.8[2.7], C:22.1[2.1]kg/m^2; P=0.0001), HbA1c (MA:8.8[1.3], NA:7.9[1.2], C:4.8[0.3]%; P<0.0001), mean arterial pressure (MAP) (MA:93.5[7.9], NA:87.0[7.9], C:83.9[7.3]mmHg; P<0.0001) and end-diastolic diameter (D) (MA:6.54[0.86], NA:6.31[0.69], C:6.03[0.43]mm; P=0.0037) was observed. Multiple regression analysis showed that only MAP, age and gender were significant determinants of the DC. After taking these variables into account, there was no difference left between the DC of the three groups.

Conclusions: The distensibility of the common carotid artery is decreased in microalbuminuric, normotensive IDDM patients, as compared to normoalbuminuric IDDM patients and healthy control subjects . Because the groups were comparable for the significant determinants of DC with exception of the MAP, it is suggested that the higher blood pressure in the microalbuminuric patients might be the main cause of the difference in DC, which seems not to be caused by a change in vascular wall properties itself.

1823

METFORMIN INCREASES TOTAL SERUM HOMOCYSTEINE LEVELS IN NON-DIABETIC MALE PATIENTS WITH CORONARY HEART DISEASE.

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Background: The metabolism of the CVD risk factor homocysteine (Hcy) depends on the vitamins B_6, B_{12} and folate. The peroral antidiabetic drug metformin reduces serum vitamin B_{12} levels, and possibly also serum folate. **Aim of the study:** To investigate whether metformin treatment affects serum total Hcy (tHcy) levels. **Design:** 60 non-diabetic male patients with cardiovascular disease were included in an open, prospective, randomised study. During a four week run-in period, patients were given diet and lifestyle advice, and treated with lovastatin 40 mg/day. At week 0 metformin in doses up to 2000 mg/day was given to half the subjects. Lovastatin treatment was continued throughout the study in both the metformin group (M) and the control group (C). Total Hcy (tHcy), vitamin B_{12}, folate and methylmalonic acid (MMA) were measured. **Results:** Values given as mean ± SEM:

Variable	Treatment group	Week 0	Change week 0 to 12	Metformin effect (%)	p	Change week 0 to 40	Metformin effect (%)	p
tHcy (µmol/L)	C	12.8 ± 0.5	-0.4 ± 0.3			-1.6 ± 0.3		
	M	12.5 ± 0.5	0.5 ± 0.3	7.2	0.02	0.0 ± 0.5	13.8	0.02
Vit B_{12} (pmol/L)	C	362 ± 16	6 ± 7			32 ± 8		
	M	380 ± 16	-45 ± 10	-13.4	0.0001	-34 ± 16	-17.7	0.000
Folate (nmol/L)	C	8.30 ± 0.54	0.40 ± 0.43			1.14 ± 0.58		
	M	8.66 ± 0.57	0.24 ± 0.44	-2.0	ns.	0.50 ± 0.33	-8.0	0.06
MMA (nmol/L)	C	112 ± 12				1 ± 11		
	M	122 ± 13				1 ± 14	0.0	ns.

Conclusion: Metformin treatment increases total homocysteine levels. Since MMA levels are unaffected, it remains an open question whether the increase in tHcy levels is secondary to reduced vitamin B_{12} levels, folate levels, or a combination of both.

1824

CAROTID INTIMA-MEDIA THICKNESS IN SUBJECTS WITH IMPAIRED GLUCOSE TOLERANCE

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Cardiovascular mortality was significantly higher in subjects with impaired glucose tolerance, although it has not been adequately explained by known risk factors. Since abnormal vascular wall structure could be a factor, we studied the carotid intima-media thickness (CIMT) which has shown to have an association with the grade of atherosclerosis, in 23 non-smoking glucose intolerant and 29 non-smoking normal subjects. Both glucose intolerant and normal subjects were similar in age (44±9 vs 39±9), body-mass index (29±4.5 vs 27±5), LDL (122±19 vs 115±22 mg/dL), HDL-cholesterol (41±5 vs 42±4 mg/dL), triglyceride (129±50 vs 127±45 mg/dL), and blood pressure (118±15 vs 121±16 mmHg systolic, 74±6 vs 77±8 mmHg diastolic). There were no significant statistical differences between groups. Left and right carotid arteries were scanned by a high resolution B-mode ultrasonography using a 7.5 MHz transducer. Three measurements of the intima media complex were taken from the far wall of the right and left common carotid artery, two cm proximal to the bulb. The average of the six measurements were evaluated. We found that impaired glucose tolerant subjects had significantly higher carotid intima-media thickness than the normal subjects (0.76±0.08 and 0.68±0.08 mm, respectively, $p<0.05$). According to our data, although both groups were similar for the risk factors of atherosclerosis, CIMT was significantly increased in glucose intolerant subjects. In conclusion, it can be suggested that increased CIMT may be an early evidence of the future macrovascular disease even in glucose intolerant subjects.

1825

ANTITHROMOGENIC DRUG RETARED PROGRESSION OF SILENT CEREBRAL INFARCTION (SCI) AND CAROTID ATHEROSCLEROSIS

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AIM: Diabetics and IGT subjects showed advanced atherosclerosis and higher incidence of cardiovascular diseases. However, there were no medication reported to be effective in attenuating atherosclerosis in diabetics.

METHODS: A controlled randamized open trial was done on 53 subjects with IGT and NIDDM. After baseline examination of carotid arterial wall thickness (IMT) and number of T2-enhanced high intensity area (HIA) (diameter $\geq$ 5mm) diagnosed by a radiologist on MRI brain imaging, an antithrombogenic drug, cilostazol (100mg /day), was given for following 2 years on 26 subjects (cilostazol group). The remaining 27 subjects were not given with any anti-thrombogenic drugs (non-treatment group). After 2 years, IMT and number of HIA were re-examined by the same physician.

RESULTS: At baseline examination, both groups showed no significant differences in fasting plasma glucose level (111 ± 25 vs 125 ± 37 mg/dl), HbA1c (6.4 ± 1.5 vs 7.0 ± 1.8 %), fasting plasma insulin concentration, systolic and diastolic blood pressure, serum lipid profiles, number of smokers, IMT, and number of HIA. During follow-up period, annual increase rate of IMT was significantly suppressed in cilostazol group, compared with non-treatment group (0.016 ± 0.020 vs 0.070 ± 0.040 mm/yr, $p<0.05$). In non-treatment group, averaged number of HIA increased from 0.34 ± 0.11 to 0.91 ± 0.22. In cilostazol group, increase in number of HIA significantly ($p<0.001$) was suppressed (from 0.76 ± 0.28 to 0.91 ± 0.32).

CONCLUSION: Two-year administration of cilostazol effectively retarded progression of carotid wall thickness and SCI in diabetics and IGT subjects. IMT and number of HIA are shown to be clinically useful parameters for evaluating the effectiveness of medication on diabetic macroangiopathy.

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Diabetic Foot – Pathogenesis, Risk Factors and Prevention

1826

PREDICTORS OF THE VIBRATION PERCEPTION THRESHOLD RISE TO LEVELS AT RISK FOR DIABETIC FOOT ULCERATION.

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Our aim was to assess predictive factors associated to a rise in vibration perception threshold (VPT) to levels at risk for foot ulceration (VPT≥30V,Biothesiometer). A three years follow up, 325 diabetic patients (76% women, age 62±11 y, diabetes duration 13.7±6 y) was made. After 3 years, VPT increased to values ≥30 in fifty out of 188 patients who had VPT values <30 at the initial examination. Patients who shifted into VPT≥30, were older (63.6 ±8.5 vs 59±11.3 y; p<0.05), had longer diabetes duration (15.5±2.5 vs 13.9±6.5 y; p<0.01), and had previous lower ankle/braquial doppler index (ABI) (1.07±0.9 vs 1.13±0.16; p<0.05), higher VPT (19.5±2.4 vs 18.2± 4.4; p<0.05), more clinical ischemia (ischemic pain and pulses) (46 vs 25.7%; p<0.01) and more clinical neuropathy (symptoms and Rydel tuning fork test) (44 vs 28.5%; p<0.05) than those whose VPT remained <30 V. No differences were found in sex, height, HbA1c and creatinine levels. In a multiple logistic regression analysis, only clinical ischemia, ABI and clinical neuropathy remained as independent factors associated to a VPT rise. We conclude that about 30% of diabetic patients experience a short-medium term VPT clinically significative change, being peripheral vascular disease an important predictive factor for VPT deterioration.

1827

NITRIC OXIDE IN THE PATHOGENESIS OF DIABETIC FOOT ULCERS

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Diabetic foot ulcers are a major clinical concern both in terms of cost and debility to the patient and have been estimated to affect approximately 2% of the diabetic population. The condition is more prevalent in males than females (2:1) and in the most severe cases amputation may be necessary. In view of the large numbers of the population affected by this condition an understanding of the basic mechanisms involved in the pathogenesis of the disease is vital. Nitric oxide (NO) is a free radical involved in host defence and vasodilatation of vascular smooth muscle. Recent evidence has implicated NO in the tissue destruction of a number of diseases. In this study we have therefore investigated the role of NO in diabetic foot ulcers. Age matched (40-60yrs) control (n=12), diabetic (n=22) and diabetic foot ulcer patients (n=11) were used in this study. Patients with evidence of predominant peripheral vascular disease, severe uncontrolled hypertension and nephropathy were excluded from the study. 6ml blood samples were taken and plasma nitrite levels determined by the Griess reaction. Results showed a significant (P<0.0001) decrease in plasma nitrite levels in diabetics (4.5±0.7μM) compared to controls (12.7±1.7μM). In contrast, diabetic foot ulcer patients had a significantly (P<0.05) elevated plasma nitrite in comnparison to diabetics. (P<0.05). Furthermore, there was a significant difference (P<0.01) between male diabetic patients (6.4±0.75μM nitrite) and females (1.8±1.7μM nitrite). The finding of raised plasma nitrite in ulcer patients may implicate NO in the pathogenesis of diabetic foot ulcers. Furthermore, males were found to have higher nitrite levels than females, which may help to explain the higher incidence of diabetic foot ulcers in men.

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DIABETICS WITH FOOT LESIONS AND AMPUTATIONS IN REGION OF „HORNÝ ŽITNÝ OSTROV" IN YEARS 1993-95.

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We evaluated in a population based prospective study the incidence and prevalence of diabetics with foot lesions and amputations after establishment of Internal department specialized in diabetes and podiatric clinic in our region in a year 1992. The number of registered diabetics (RD) was 910, 1098,and 1205 (3,0, 3,6, and 3,9%) between 1993 and 1995 out of 30 592 total population (TP).In the last three years prevalence of diabetics with foot lesions (Wagner grade 2-5) increased from 62 to 98 /100 000 of TP (from 2,0 to 2,5% of all RD) and incidence of diabetics with new lesions decreased from 49 to 26/100 000 of TP (from 1,6 to 0.6 % of all RD). The incidence of repeated lesions also decreased. The prevalence of patients with minor amputations(below Chopart) increased from 20 to 29 /100 000 of TP (from 0,7% to 0,7% of all RD) while the number of patients with major amputations (Chopart and above) decreased from 20 to 10/ 100 000 of TP (from 0,7 to 0,2% of all RD). The incidence of patients with minor amputations increased from 3 to 10 /100 000 of TP (from 0,1 to 0,2% of all RD) while the number of patients with major amputations decreased from 7 to 0 /100 000 of TP (from 0,2to 0% of all RD). In conclusion the better educational and foot care in our region substantialy decreased the number of patients with new foot lesions and also a number of patients with major amputations.

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AUTOREGULATION OF THE SKIN MICROCIRCULATION IN HEALTHY SUBJECTS AND DIABETIC PATIENTS WITHOUT AND WITH VASCULAR COMPLICATIONS.

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The skin microvascular autoregulatory capacity was investigated in 14 healthy subjects, 21 diabetics without vascular complications and 29 with vascular complications. To assess this capacity, changes in skin blood flux (SBF) were recorded : ▪ on the pulp of the 2nd finger during a 30-second venous stasis induced by inflating a cuff at the base of the same finger to 50 mmHg ; ▪ from the dorsal surfaces of the foot and big toe during a 3-minute standing ; ▪ from the dorsal and plantar surfaces of the big toe during a passive lowering of either leg from the hip, so that the foot was 50 cm below the midaxillary line. SBF was measured at skin temperature, after acclimatization in 23±1°C, by laser doppler fluxmetry (PF4 Perimed). In healthy subjects, the same degree of microvascular vasoconstriction, i.e.same reduction in SBF, was observed irrespective of the site of measurement and the procedure used. In diabetic patients, the vasoconstriction elicited, on the pulp of the finger by venous stasis was normal By contrast, the vasoconstrictor responses to standing or lowering of the leg were impaired. In fact, there was in some patients, an increased SBF instead of a decreased one during standing or lowering of the leg. On average, a significant loss of the skin microvascular vasoconstriction during standing or lowering the leg was observed even in patients without complications. The loss was, however, more marked in patients with complications. These findings indicate that an early alteration of autoregulation is present in the skin microvasculature of diabetic feet, and is related to the diabetic disease rather than to late diabetic complications. The inability of diabetic skin microvasculature to respond normally to postural changes may be an important factor initiating the development of the foot complications.

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EVALUATION AND CLASIFICATION OF THE DIABETIC FOOT IN A RURAL COMUNITY OF COSTA RICA

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Introduction: The diabetic foot is a public health problem, we know little about this problem in the rural areas. This study analyze this problem in the rural community of Costa Rica, Buenos Aires de Palmares.

Materials and Methods: From the data collected by EBAIS (Basic Equipment for Integrated Health Care), the diabetic feet were examined with the PATON-A scale. The metabolic control was assessed by the fasting glycemia media.

Results: 77 patients (154 feet) were examined, we found 74 feet (48%) to be green (low risk), 72 (46.8%) were yellow (moderate risk) and 8 (5.2%) were red (high risk). In 58 patients both feet were classified in the same category, in 30 both feet wew green, 13 had one green and the other yellow. The 8 red feet: 2 patient had both red, 5 had the other foot yellow and 1 had it green.

Table 1.

Feet Classifications and years of evolution of the Diabetes disease

Years of Decease	No. of feet	Feet classification		
		Green	Yellow	Red
0-4	62	42	20	0
5-9	42	16	21	1
10-14	28	12	12	4
15	20	2	15	3

All patients with feet had glycemia under 140 mg/dl and all with red feet were over 140 mg/dl.

Conclusions: The more severe the classification of the diabetic foot the worse glucemic control and the longer evolution of the disease.

1831

THE ACUTE AND LONG-TERM EFFECTS OF ANTIPLATELET AGENTS ON TRANSCUTANEOUS OXYGEN TENSION IN DIABETIC PATIENTS

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To examine the acute and long-term effects of two antiplatelet agents, aspirin and ticlopidine, on transcutaneous oxygen tension ($PtcO_2$) in non-insulin-dependent diabetic patients, 20 subjects (11 wonen and 9 men, aged 65.8±9.5 years) with baseline $PtcO_2$ below 60 mmHg measured on the pulp of the right big toes, were recruited. The acute effects of both drugs were tested by measuring $PtcO_2$ before and continuously for 2 hours after taking either aspirin 100 mg or ticlopidine 250 mg; and then crossed over to the other drugs 2 weeks later. Long-term effects were evaluated after randomly dividing the subjects into 2 groups taking either aspirin 100 mg qd or ticlopidine 250 mg bid for 3 months. The results are shown in Table 1. This study showed beneficial effects of both aspirin and ticlopidine on $PtcO_2$ in diabetic patients after long-term usage, but no acute effects were observed.

Table 1. Acute and long-term effects of antiplatelet agents on transcutaneous oxygen tension

Effects evaluated		Antiplatelet agents	
		Aspirin	Ticlopidine
Acute	Before	27.9±6.9(n=17)	29.2±13.2(n=20)
	After	27.7±7.7	28.9±12.6
	p	NS	NS
Long-term	Before	28.9±8.0(n=8)	28.7±8.6(n=10)
	After	40.0±14.3	39.3±12.0
	p	< 0.05	< 0.05

Data expressed as mean ± SD and analyzed by paired t-test.
NS= non-significant

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CLINICAL OBSERVATION OF THE DISTRIBUTION OF FOOT ULCERATIONS IN ASIAN PATIENTS.

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In June 1996 the Podiatry Clinic at Tan Tock Seng Hospital began to collate information on new NIDDM patients with foot ulcerations. Between June and November 1996, 62 patients were registered. Of the 62 patients 14 had more than one foot ulcer, 15 had an amputation and four had neuropathic osteoarthropathy. The aim of this study was to identify ulceration distribution of the foot and link ulcer formation with footwear and other cultural aspects. Of the 62 patients screened 38 (61.3%) were females and 24 (38.7%) male. Of the patients 47 (75.8%) Chinese, 11 (17.7%) Indian and 4 (6.4%) Malay's registered. The mean age was 57.7 years (range 42-85). In total 76 ulcers were noted, 18 (23.7%) over the plantar metatarsal area, 14 (18.4%) on the plantar surface of the hallux, 12 (15.8%) under the first metatarsal head, 10 (13.2%) on the apex of the digits and 8 (10.5%) on the dorsum of the digits. The other 14 (18.4%) were distributed over the remainder of the foot. A majority of the ulcers (71%) were located on the plantar aspect of the foot and forefoot region. Of the 62 patients 56 (90.3%) walked barefoot at home and at temple and 51 (82%) had been using incorrect footwear, such as slippers with interdigital thongs or shoes that are ill fitting, have insufficient cushioning or are worn out , adversely effecting patient foot biomechanics . From this observation it is felt that with better education and understanding of footcare and footwear many foot ulcerations are preventable in the Asian population.

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INCREASED LACTATE PRODUCTION IN CHRONIC DIABETIC WOUND FIBROBLASTS SHOWING DECREASED CELLULAR PROLIFERATION

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In non-insulin dependent diabetes mellitus (NIDDM), insulin resistance is caused by primary genetic and secondary metabolic factors. Hyperglycaemia on its own induces insulin resistance by decreasing the numbers of glucose transporters, but also by affecting the insulin receptor. There is a high risk of amputation due to chronic diabetic foot ulcers possibly explained by the lack of granulation tissue formation caused by impaired fibroblast activity. Hyperglycaemia increases nonoxidative glycolysis, leading to increased lactate release. Patients with physiologic hyperinsulinemia show increased lactate efflux peripherally. Fibroblasts derived from chronic wounds in NIDDM-patients have decreased proliferation as have normal fibroblasts grown in high glucose. In this study we wanted to further explore the possible mechanisms explaining these observations. Fibroblasts were derived from normal uninjured skin and chronic NIDDM wounds. Cellular proliferation was measured by a fluorometric method using Hoechst dye. After the addition of NIDDM fibroblast conditioned media to normal fibroblasts, it was found that their proliferation was significantly decreased. Conditioned nedia from NIDDM fibroblasts showed significantly higher lactate levels compared to conditioned media from normal fibroblasts. D- but not L-lactate caused a dose-dependent decrease in the proliferation of normal fibroblasts. There is an increase in lactate level in the medium when normal fibroblasts are cultured in high glucose. In conclusion, the decreased proliferation in fibroblasts derived from chronic NIDDM wounds and normal fibroblasts cultured in high glucose is associated with altered glucose utilisation, and more specifically increased non-oxidative metabolism. We suggest that excessive lactate production may be of importance for the impaired healing process seen in the chronic diabetic wound.

1834

EPIDEMIOLOGICAL SURVEY FOR THE DETECTION ON NON-TRAUMATIC LOWER EXTREMITY AMPUTATIONS IN DIABETIC PATIENTS IN THE UMBRIA REGION (ITALY)

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One of the five year targets of the Saint Vincent Declaration (SVD) is to reduce by 50% non-traumatic lower extremity amputations (LEAs) in diabetic patients. An epidemiological observatory has been created in our region and an epidemiological survey for the detection of all non-traumatic LEAs performed in Umbria was carried out by the Cooperative Study Group CSG) of the Progetto Umbria Diabete (PUD). The diabetic Centres of the region were involved in the study and an ad hoc protocol was developed to collect data; the registries of all surgical (n°23) and Orthopedics (n° 11) divisions were used to identify the subjects. Data were stored and analyzed by our Department. In 1991 196 LEAs (n° 106 in diabetics and n° 90 in normal subjects) were performed in Umbria region. The incidence of LEAs in diabetic subjects was 42.16/10000/year while in non diabetic was 1.77/10000/year. These results, quite similar to those reported in the literature, are based upon a previous epidemiological survey which indicated a prevalence of known diabetes mellitus of 3.1% in our region. According to the surgical records peripheral vascular disease was the major cause of LEA for both diabetic and non diabetic patient. However the infection of bones and/or soft tissue was present in at least 25% of LEAs carried out in diabetic patient, whereas this happened only in 11% of LEAs in non diabetic subjects. With the same methodology we collected information related to diabetic and non diabetic patients who underwent LEA in 1992 (n°=204), 1993 (n°=200) and 1994 (n°=147). These results indicate a decrease of LEAs by 25% with a similar decrease of incidence both in diabetics and non diabetics. These figures also indicate: a) the usefulness of ad hoc sensitization plans which were developed during the collection of the data, b) the possibility to reduce LEAs also in those areas which provides a baseline satisfactory quality of care, c) the effectiveness of tertiary intervention plans which non affected the glico-metabolic balance. The Cooperative Study Group of the PUD aims to a further reduction of the incidence of LEAs through primary and secondary intervention.

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BETA-1,3-D POLYGLUCOSE AND GROWTH FACTORS IMPROVE WOUND HEALING IN DIABETES MELLITUS.

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Healing of diabetic wounds is delayed not only by neuropathic changes and local ischemia, but also by way of altered cellular mechanisms in the healing wound. During the early phase of the healing process the macrophages infiltrate the wound and take part in the inflammatory response by producing cytokines [e.g. Tumor Necrosis Factor-α(TNF-α)] and growth factors [e.g. Platelet-Derived Growth Factor (PDGF) and Insulin-like Growth Factor-1(IGF-1)]. Monocyte-macrophage dysfunctions such as reduced chemotaxis and inappropriate cytokine activity have been described in diabetes mellitus. Beta-1,3-D polyglucose is known to improve macrophage chemotaxis and secretory activity.The present study was undertaken to see if [a] β-1,3-D polyglucose and [b] PDGF +IGF-1 would improve wound healing. Cutaneous wounds were established on the back of either diabetic(C57Bl-db/db)- or non-diabetic (C57Bl-db/+)-mice. Five groups were studied: 1 db/db-mice with placebo wound treatment (n=7, average blood glucose(BG) 24,2±3,2 mmol/l), 2 db/db-mice treated with insulin (n=8, average BG 14,4± 4,5 mmol/l), 3 db/db-mice treated locally with PDGF +IGF-1 (n=7, average BG 22,6±3,1mmol/l) 4 db/db-mice treated locally with β-1,3-D polyglucose (n=7, average BG 29,5±1,9mmol/l) and 5 non-diabetic control mice without treatment (n=10, average BG 8,6±0,7 mmol/l). Biopsies from untreated diabetic wounds showed decreased infiltration of macrophages. The LPS-stimulated release of TNF-α from isolated peritoneal macrophages was only 1/8 of that from non-diabetic mice. The percentage reduction in wound area was after 10 days for group 1-5: 17±4, 18±7, 35±3, 37±7 and 58±5 % (mean±SEM, p<0,05). The corresponding results after 16 days were:64±6, 62±5, 73±3, 71±7 and 96±1 % (p<0,05). Conclusion: Macrophage function as judged by TNF-α release, is impaired in diabetes. Topic applications of β-1,3-D polyglucose or PDGF+IGF-1 in diabetic mice improve wound healing significantly despite serious hyperglycemia.

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DIABETIC FOOT IN PRIMARY HEALTH CARE. A COMMUNITY BASED STUDY.

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Objectives: To asses prevalence of diabetic foot in Non-Insulin-Dependent Diabetes Mellitus (NIDDM) in Primary Health Care and to analyze its relationship with clinico-biological variables.**Patients and Methods**.- Cross-sectional study carried out during 1993-94 in 76 PHC Centres, attending 1.256.193 adults . 2.595 NIDDM were selected by systematic sampling from 31.050 registered NIDDM . Each patient contributed once (either with an ulcer/lesion or amputatión) to this study.Results.-Diabetic foot was reported in 260 of the 2595 patients (10% , CI 95%: 8.85-11.15).Fifty-five patients (2.1% , CI: 1.55-2.65) had undergone amputation in any moment of his life. During the study 205 patients reported ulcers/lesions (7.9%, CI : 6.86-8.94). We found significant differences in the mean age (4.53 years higher in the Diabetic Foot Group (DFG), CI , 3.32-5.74), average diabetes evolution(2.75 years longer in DFG , CI: 1.64-3.87), microvascular complications prevalence(32.1% higher in DFG, CI:24.6-39.6), macrovascular disease (15.9 % higher in DFG CI, 9.6-22.2) and current level of HbA1c (0.55 % higher in DFG, CI 0.22-0.87).The number of current smokers was higher in DFG (5.1% , CI: 0.7-9.5), specially in men older than fifty (9.7%, CI:0.3-19.1).**Conclusions**.- Diabetic foot is close related with long standing diabetes (>10 years), poor glycaemic control and presence of any kind of micro/macro vas cular complications. Our data suggest the need to carry out specific interventions on foot care in all patients with more than 10 years of diabetes evolution.It is urgent to stress anti tobacco counseling in diabetics, specially in those with previous foot lesions

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MULTICHANNEL LASER DOPPLER PERFUSIN MEASUREMENTS IN DIABETIC PATIENTS.

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The purpose of this study was to investigate the skin microcirculation in patients with IDDM. The study group comprised 67 patients divided into four subgroups: 13 women and 11 men were heathy and had no family history of diabetes; 19 women and 24 men had IDDM. The studied measurements included 7 parameters determined during rest and postocclusive reactive hyperaemia. They were performed with multichannel laser Doppler fluxmetry using 10 surface probes localized in distal part of the lower limb. The most significant data localized the probe on toe and probe under the knee. The maximum of hyperaemic response (MAX) was significantly lower in the diabetic patients as compared to the healthy subjects ($p<0.01$). The time of peak flow (TM) was higher ($p<0.01$) in diabetic patients as compared to the control subjects. The half time of hyperaemia (TH) was significant longer ($p<0.05$) for diabetic groups.
Conclusion: the laser Doppler method is helpful to identify patients with risk development diabetic foot complications. The most valuable data is MAX, TM and TH; the best localization of the probe seem to be the most distal point of foot.

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ANALYSIS OF PATIENTS WITH DIABETIC FOOT ULCERS

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Foot problems still remain one of the most common reasons for hospital admission among diabetic patients. The rate of lower limb amputations is 15 times higher in diabetic patients compared with non-diabetic patients. In the present study 62 patients (44 males and 18 females; mean age 61.8 years) with diabetic foot ulcers were analysed. The mean duration of diabetes was 14.1 years (1-35 years). Forthy-seven out of 62 patients were on oral antidiabetic therapy and 15 patients were on insulin therapy before admission to hospital. Physical and neurological examination of patients revealed that 90.3 % of patients have peripheric neuropathy, 51.6 % have nephropathy (>500 mg/gün proteinuria), 19.3 have chronic renal failure, 48.4 % have diabetic retinopathy, and 58.1 %have arterial hypertension. On admission to hospital, the mean fasting blood glucose level was 237.23 mg/dL (77-478 mg/dL), mean plasma cholesterol level was 191.13 mg/dL (115-274 mg/dL) and mean plasma triglyceride level was 136.55 mg/dL).Wagner classification of diabetic foot ulcers was as follows; 16 patients (30.6 %) were grade 2, 23 patients (37 %) were grade 3, 19 patients (29 %) were grade 4 and 2 patients were grade 5.The pathologic states that contribute to diabetic foot ulcers were neuropathy (34 %), vascular disease (15 %), and both of them (51 %). Gangrenous lesions occured 61 % on digits, 20 % on the heel, 12 % on the dorsa of feet, and 7 % at the metatarsal region.Infections of diabetic foot ulcers contained various types of bacteria.These were staphylococcus epidermidis, staphylococcus aureus. E coli, proteus species and group B streptococci Infection was present in 78 % of patients. In spite of infection, white blood cell count mildly elevated but the erythrocyte sedimantion rate was characteristically elevatad (mean 86.21 mm/h). Two- agent therapy with imipenem and metranidasole was initiated intravenously. The healing rates of ulcers was 78 % with antibiotic therapy and local wound care. Twenty-five out of (40 %) patients underwent amputation. Nine percent of these patients underwent second amputation in the following three years. This high rate of amputations may be due to later admission of patients to hospital. At the hospital staying time for the patients was mean 24.6 days (6-82 days). Diabetic foot ulcers are associated with a high degree of morbidity and mortality. It is possible to determine which patients are at risk for ulceration and to place them in education programs. When ulcers do occur, it is important to take a systematic approach to management.

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LASER DOPPLER EVALUATION OF MICROCIRCULATION IN THE EARLY DIABETIC FOOT SYNDROME

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Recognition of early microcirculatory disturbances in feet of diabetics may facilitate the pathogenic interpretation of the diabetic foot syndrome, selection the patients at risk of developing clinical problems and serve as the base for designing the preventive measures. This could be particularly true in diabetics with peripheral neuropathy.Therefore the study aimed at the assesment of functional parameters of the foot microcirculation in IDDM patients presenting signs of peripheral neuropathy but without any symptoms of the diabetic foot syndrome was undertaken. For comparision 20 IDDM subjects with the signs of peripherial neuropathy and 10 IDDM subjects without this complication were studied both clinically and metabolically. All of them were underwent the examination of microcirculation of the feet with the use of Laser Doppler Flowmeter. The parameters measured were: resting blood flow, post-occlusive , hyperemic response, flow change after heating to 44°C and the flow on dependency. In IDDM subjects with peripheral neuropathy the folowing functional microcirculatory abnormalities were found :delay and decrease in post-occlusive, hyperemic response (4.5 ± 1,8 s in neuropathic vs 8.5 ± 2,4 s in non-neuropathic IDDM), decrease of the peak flow (36 ± 7,6 PU in non neuropatic vs 18 ± 5,6 PU in neuropatic IDDM) and also impairment of the response of the skin floow to local heating peak flow at 44°C (48 ± 7,4 PU vs 12 ± 3,4 PU in non neuropatic IDDM). Also the veno-arteriolar reflex measured as the ratio of resting to standing flow in the feet skin was significantly decreased (80% in non-neuropathic versus 35% in neuropathic IDDM). Conclusion: Laser Doppler Flowmetry discovers the very early functional abnormalities in the microcirculation of the skin in the feet of IDDM with peripheral neuropathy, when none of the typical symptoms of the diabetic foot syndrom is present. It points to the significance of the relation between neuropathic and microcirculatory disturbances in the early pathogenesis of diabetic foot syndrom.

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PROFILE OF DIABETIC FOOT INFECTIONS IN A SEMI URBAN AREA IN A DEVELOPING COUNTRY.

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Aim of the study is to look at the incidence, risk factors, association of other complications and the outcome of foot infections in a Diabetic Clinic population. In India where bare foot walking is the norm, rat bites and ants are some causes for foot infection and people report quite late, a dedicated team approach has resulted in a excellent salvage of the limbs. In a restrospective analysis of 8000 people with Diabetes 5.17% presented with foot problems varying from trophic ulcers to Gangrene and all were between 30-60 years age group. Of them 64.2% were males and 35.8% were females. NIDDM contributed for 99% and IDDM 1%. Incidence of Neuropathy was 64%, Ischaemic Heart disease 33%, Nephropathy 19% and Retinopathy 14% Peripheral Vascular disease in 32%. Smoking, Hypertension and Lipid abnormalities as risk factors were seen in 26% 29% and 18% respectively. The healing rate was 89%, disarticulation of toes in 8% and Below Knee Ampulation was done in 3%. In conclusion the foot problems in Diabetics need a multidisciplinary approach alongwith aggressive glycaemic control, proper footcare and education to achieve higher success rates.

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HYDROSTATIC MEASUREMENT OF ARTERIAL BLOOD PRESSURE IN THE ISCHAEMIC DIABETIC LIMB

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Background: hydrostatic measurement of systolic arterial pressure in the big toe (hydrostatic toe pressure HTP) has recently proven useful for detection of critical limb ischaemia (CLI) in cross-sectional studies of diabetic patients. **Aim:** to further evaluate this new method in a clinical setting. **Patients and methods:** HTP was measured twice in 49 legs of 45 patients with CLI, before and after surgical(n=41) or angioplastic (n=8) arterial reconstruction (AR). A 8 MHz Dopplerprobe (Hadeco/Japan) was used for monitoring pulsations in a digital artery of the 1st toe for assessment of HTP (upper detection limit 60-70 mmHg, depending on the length of the leg). Simultaneously, the conventional ankle/brachial index (ABI) was assessed with sphygmomanometry and 8 MHz Dopplerprobe. **Results:** Before AR, HTP was 28 (SD 21) mmHg in 47, and > 70 mmHg in 2 legs. After AR, HTP rose to 50 (SD 8) mmHg in 17, and > 70 mmHg in 32 legs. ABI was 0.54 (SD 0.29) before and 0.94 (SD 0.20) after AR. **Conclusion:** Synchroneous increase of both, HTP and ABI, after successful AR confirms the accuracy of the HTP measurement.

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A TIME TREND ANALYSIS OF DIABETES (DM) RELATED LOWER EXTREMITY AMPUTATIONS (LEA) IN ONTARIO, CANADA

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As a proxy for the effectiveness of long term diabetes care we examined LEA rates in the province of Ontario (pop. 11.08 mil) from 1990 - 1995 using an inclusive database of all hospital discharge abstracts. The annual number of all cause amputations ranged from 2423 - 2551; however the proportion related to diabetes increased progressively from 49% in 1990 to 59% in 1995 ($p< .01$). The age adjusted LEA rate in DM males increased progressively from 39.4 in 1990 to 46.4 in 1995 (per 10^4 DM men) $p< .01$, while the rates in DM women remained constant at 18.8 in 1990 to 18.6 in 1995 (per 10^4 DM women). Over the study interval 19% of DM amputees had 2 amputations and 7.2% had 3 or more procedures. Diabetes-specific rates for toe, foot, below knee and above knee amputations increased progressively till age 79, then consistently decreased in both men and women. Analysis of variations in LEA rates amongst 7 health planning regions indicates a significant North-South gradient of 1.7; lower in southern urban communities vs. more remote and rural areas. Coincident with this apparent increase in DM-related LEA, our analysis of trends in antidiabetic drug treatment among all persons aged 65+ showed exponential($p<.01$) increases in the use of oral hypoglycemic agents (particularly glyburide and metformin) and insulin and combinations thereof. Conclusion: Despite increasing efforts to improve glycemic control there is no evident impact on limb preservation. LEA in DM is an increasing health care burden (men > women) and will require new, targetted and aggressive preventive strategies.

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REDUCED CUTANEOUS BLOOD FLOW IN RESPONSE TO A HYPERAEMIC INSULT IN DIABETIC SUBJECTS WITH EVIDENCE OF NEUROPATHY

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Abnormal vascular control mechanisms have been implicated in the pathogenesis of the diabetic neuropathic limb. To investigate this further, we used Laser Doppler flowmetry (LDF) to study changes in cutaneous blood flow (CBF) in response to an ischaemic event. CBF was measured in the pulp skin at the toe, before and after an ischaemic insult in 2 groups of diabetic subjects, 16 with peripheral and/or autonomic neuropathy and 18 without. All had palpable pedal pulses and ankle-brachial pressure indices of > 1.0. Subjects with and without neuropathy were matched for age and body mass index {(mean ± SD) 60.9 ± 10.4 Vs. 54.6 ± 15.2 years and 29.6 ± 4.3 Vs. 29.6 ± 7.6 kg m^{-2}, respectively}. The percentage change (ratio of the peak flux to the resting flux) in CBF after hyperaemic insult was reduced in subjects with neuropathy {407 ± 238 Vs. 552 ± 239%, $p<0.05$}, while the time to maximum flux after removal of the ischaemic insult was similar {27.1 ± 19.1 Vs. 29.9 ± 15 7 seconds}. These results demonstrate that following a hyperaemic insult, diabetic subjects with neuropathy have an impaired increase in blood flow to the limb. This abnormality may be implicated in the development of neuropathic complications.

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EVALUATING DIABETIC PATIENTS WITH FOOT ULCERATIONS IN TURKEY

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We recently reviewed diabetic patients with diabetic foot ulcers. Fortysix patients (48 % male, 52 % female - with median age of 50 ± 9 years) who were admitted to our department and hospitalized for diabetic foot problems between July 1995 and November 1996 were evaluated. Duration of diabetes was below 12 months in 10 % , 1- 5 years 10 % , 5 - 10 years 16 % and over 10 years 64 % of the patients. Thirty two percents had a previous diabetic foot history and 10 % experienced amputations before. On admission, 45 % were on insulin treatment while 55 % were receiving oral agents. Cigarette smoking prevalence was 42 %. Sixtyfour percent were hypercholesterolemic and 90 % had poor glycemic control (Hb A_{1c} > 7 %). On foot examination, 45 % had dry foot, 10 % callus formation, 32 % tinea pedis, 10 % hallux valgus deformity. Hospitalization for diabetic foot costs a lot for a developing country like Turkey. In another study of us we demonstrated the efficacy of education on improving glysemic control in diabetic patients. On questionnaire, most of our patients were found to have little knowledge about diabetes and diabetic foot care because they had never participated to an education program. Most of them also had low sociocultural - economic statues and poor hygienic conditions. They also didnot know the untoward effects of poor glycemic control on their disease progression. In conclusion, we think that in order to prevent diabetic foot ulcerations, uneventful amputations and unnecessary hospitalizations educational interventions, especially specific to diabetic foot care is imperative. To improve the quality of life and sociocultural conditions, routine and regular systemic foot examinations should be taken into consideration.

1845

PLANTAR CALLUS DENSITY IN DIABETIC NEUROPATHIC AND NEUROISCHAEMIC FEET MAY BE DEPENDANT ON BLOOD FLOW.

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Plantar ulceration is a common complication of the neuropathic foot but rarely seen in the neuro-ischaemic foot. An association has previously been suggested between dense plantar callus and subsequent neuropathic ulceration. This report demonstrates that plantar callus density is markedly increased in neuropathic feet compared with neuro-ischaemic feet even though similar degrees of neuropathy are present. We studied 50 diabetic patients with history of plantar ulcer and severe neuropathy. 25 patients(Group A) had palpable pedal pulses and 25(Group B)had absent pulses and low pressure index (mean ± SD): 0.6 ± 0.14. The two groups were matched for age (A=64.5yrs, B=7lyrs [*p=0.103*], duration of diabetes (A=I6.00yrs, B=22.5yrs [*p=0.625*], sex (A=Ml4 Fll, B=M13 Fl2], type (A=IDDM 8 NIDDM I7, B=IDDM 3 NIDDM 22). Plantar callus was removed from site of previous ulcer by sharp debridement by the podiatrist and dry weight determined. The removal area was traced on an acetate sheet and density of callus was calculated in g/cm^2. Time since previous callus removal was noted. There was no significant difference between the two groups regarding area of callus removed (A=2.25cm^2, B=2.92cm^2)[*p=0.907*], treatment interval (A=30.00days, B=26.00days) [*p=0.263*] or vibration perception threshold (A=43.6±I5.6 volts, B=45.7±7.31) but callus density was significantly increased in the neuropathic group with good blood flow and palpable pedal pulses (A=0.169g/cm^2, B=0.018) [*p<0.0001*].

1846

THE EPIDEMIOLOGY OF LOWER EXTREMITIES AMPUTATIONS IN THE CITY OF ROME IN THE PERIOD FROM 1984 TO 1993

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Since italian epidemiologic data on amputations in diabetic people are not available, the aim of our study was to investigate the number and the causes of amputations annually carried out in Rome, to detect the possible risk factors and to evaluate the mortality rate after three years from amputation. In Italy Hospitals have to bury amputated limbs in the cemeteries, where, in a dedicated register, the surname, the name, the hospital of origin and the date of amputation have to be indicated. Starting from these data the research has been completed by recovering case sheets in every hospital and with informations obtained directly from patients or their relatives.Three great hospital centers have been utilized to create the checking sample.From 1984 to 1995 3.953 amputations had been carried out in Rome, with an annual mean of 330±11; during this period the incidence did not vary significantly. The causes of amputations were as follows: 48.7% of cases diabetes, alone or associated to peripheral vascular disease (PVD); 35.7% peripheral vascular disease alone; the other 15.6% were trauma, neoplasia and burns.Since Rome resident population is about 2.800.000 and non-traumatic amputations observed in resident patients are 260/yr the incidence rate is about 9.3/100.000 inhabitants. Since the prevalence of diabetes in Rome is about 2.6% and the number of amputations in diabetic resident people is about 130/yr, the extimated incidence is 1.8/1.000 diabetics. The hospital admission period was 57±38 days for diabetic patients and 62+/-60 days for patients withPVD. The age at the time of amputation was significantly lower in diabetics (68.8±9.9) than in patients with PVD (75.5±10.3) ($p<0.01$). A good rehabilitation was possible in 46% of patients, while 43% were obliged to the use of wheel-chair and 11% to stay in bed. The mortality rate after one years from tha amputation was 28%, 40% at three years and 60% at five years.

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DIABETIC FOOT RISK FACTORS FOR CHILDREN AND ADOLESCENTS WITH DIABETES

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Objective: Little investigation has focused on the early screening of the risk factors and prevention of the diabetic foot formation in children and adolescents with diabetes. We examined 50 diabetic children. The age of the group was 14,5 ±3,0 years, the desease duration - 6,4 ±3,8 years (M±SD).

Methods: Neuropathy was assessed using Neuropathy Disability Score (NDS) and modified Autonomic Disability Score (ADS) according to Ewing. We used pedography (EMED system) for measuring the peak pressure (PP) in the 7 points of the right and left plantar surfaces: hallux - H, the first metatarsal head - MTH1, the third metatarsal head - MTH3, the fifth metatarsals head - MTH5, the middle of the plantar arch - M, the right and the left part of the heel RH and LH.

Results: The prevalence of the sensomotor (NDS>5) and the autonomic (the pathology of>1 cardiovascular test) neuropathy was 58% and 12,8% in correspondence. Unsatisfactory metabolic control (HbA level - 11,1±2,6) was determined in the group).

The NDS correlated positively with the age, the desease duration and the HbA level ($r_1=0,58$, $r_2=0,62$, $r_3=0,72$, $p<0,05$). The abnormal distribution of PP under the metatarsal heads was revealed: $MTH_1<MTH_3>MTH_5$ and "pes cavus" was diagnosed in 48% of patients. There was no ulcers or hyperkeratoses in the group. The linear regression analysis revealed the high correlation between NDS and PP MTH_1, MTH_3, MTH_5, RH, LH ($r_1=0,74$; $r_2=0,8$; $r_3=0,76$; $r_4=0,82$; $r_5=0,84$; $p<0,05$), HbA level and PP MTH1,3,5, RH and LH ($r_1=0,86$; $r_2=0,93$; $r_3=0,87$; $r_4=0,95$; $r_5=0,9$; $p<0,001$).

Conclusion: The data suggest that abnormal foot pressure in diabetic children and adolescents is related to the neuropathy and the HbA level and considered to be the predisposed factors to the developing of neuropathic ulcers.

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COMPARISON OF PREVALENCE OF LOWER EXTREMITIY MALFORMATIONS AND PRE-LESIONS IN DIABETIC SUBJECTS AND A CONTROL GROUP.

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A multicenter study was carried out on a randomized sample of 33.554 out-patients attending 21 diabetes centres in Lombardia Region. A high prevalence of malformations (36.2%) and pre-lesions (48.9%) was found out. To establish if there was an increased prevalence of such changes in diabetic subjects, we studied a sample of non diabetic subjects, matched by gender and age. Cases: 5813 diabetic patients (D), 2982 men and 2831 women, average age 63.3± 11.6. Controls: 587 non diabetic subjects (C), 287 men and 300 women, average age: 65.8±14.2. A complete examination of the lower extremities was performed in all patients, with exploration of Achilles tendon reflexes and vibration perception (by tuning fork). The comparison between D and C does not show statistically significant differences concerning malformations, but for the flat foot (8.9% vs 4.8% respectively, $p<0.05$). Significant differences were found either for cutaneous and ungueal changes and for pre-lesions. A significant reduction of peripheral pulses, reflexes and vibration perception was found.

	maceration %	fissure %	hyperkeratoses %	Total pre-lesions %
D	13.2	7.1	33.3	48.9
	p<0.001	p<0.001	p<0.001	p<0.001
C	3.4	1.9	22.8	26.9

	cutaneous lesions%	ungueal lesions%	pathological pulses%	pathological O-T Reflexes%	vibration perception %
D	33.3	21.7	39.6	57.5	57.1
	p<0.05	p<0.001	p<0.001	p<0.001	p<0.001
C	27.8	12.4	28.9	35.5	37.6

Our results stand the damage either of macro and microcircle or peripheral and vegetative nervous system in diabetic subjects. It would be possible to implement the primary prevention of lower extremity amputations in diabetic patients using simple and low cost procedures. foot clinical examination.

1849

RISK FACTORS FOR DIABETIC FOOT DEVELOPMENT IN TYPE II DIABETIC PATIENTS

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The aim of the study was to determine the prevalence of foot pathology and to examine possible risk factors associated with foot ulcers in NIDDM patients compared to age-matched controls. We examined 2 groups of NIDDM patients above 50 years of age: patients with diabetes and previous or present foot ulcers; patients with diabetes without foot ulcers, and control group without diabetes. In the foot diseased group, diabetes duration was longer (17±10 vs 9±7 years), insulin treatment was more common (84% vs 8%), fasting blood glucose and HbA_{1c} were significantly higher (11,7±4,1 mmol/l; 9,6%±1,8%) than in the diabetic group without foot ulcers (8,2±1,7 mmol/l; 7,5%±1,6%). Body mass index did not differ between the groups. Current or previous alcoholic problems were more common in the foot diseased group (27%) compared to the diabetic group without foot ulcers (11%) and the control non- diabetic group (6%). The foot diseased patients had ulcers (previous or present) of probably neuropathic origin in 54% and probably ischemic origin in 34%. In the 12% the ulcers were of mixed type. Callosities, dry skin, redness as well as hammer toes, hallus valgus, ingrowing nails, mycosis of nails and fissures were more common in the foot diseased patients. They emphasise the need of screening diabetic patients with regard to changes predisposing for diabetic foot disease. Our study shows that psychosocial problems, long standing diabetes duration and poor metabolic control seem to be importance for the development of diabetic foot ulcers.

1850

THE INCIDENCE OF LOWER LIMB AMPUTATION IN LEEDS, UK: SETTING A BASELINE FOR ST. VINCENT.

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Lower limb amputation (LLA) is an important diabetes outcome which is avoidable in many cases through provision of appropriate care. The St. Vincent Declaration aimed to halve diabetic LLAs within five years. At the end of 1993 a new diabetes service was introduced in Leeds (UK). To evaluate its effectiveness in addressing LLA outcome we collected data on incidence for 18 months preceding its introduction (July 1992-December 1993). From operating theatres, hospital discharge data and from the regional prosthetic centre we identified 353 LLAs, 112 carried out in 100 people with diabetes. This is an incidence of 32 $x10^{-5}$ in the general population and 510 $x10^{-5}$ in the diabetic population per annum (assumed 2% prevalence of diabetes). Patient and operation details were abstracted from case notes. Mean age (SD) at index amputation for patients with diabetes was 67.5(11.1) years with 75% carried out on males. Where smoking history was available (65%) 82% of operations were in smokers or ex-smokers. 41% of LLAs were classified as minor, 22% were below knee and 35% above knee. Mortality was 40% at 36 months. These baseline data will be used to assess progress towards St. Vincent targets in Leeds.

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RISK FACTORS FOR THE DEVELOPMENT OF DIABETIC FOOT: THE IMPORTANCE OF ITS EVALUATION

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Peripheral vascular disease (PVD) and diabetic neuropathy (DN) are frequent cause of morbidity in non-insulindependent diabetic patients (NIDD). Intermittent claudication and foot ulcer carries a major risk of lower extremity amputation and usually they are the consequence of many concurrent facilitating pathologic conditions which individually carries a risk for the limb. The aim of the study was to evaluate the potential accumulated risk for the development of diabetic foot in a population of NIDD patients. 28 NIDD patients; 13 male (mean age 51.4±10 years) and 15 female(mean age 50.7±10 years), range of age 38 to 60 years old, were studied. Diabetes duration was 4.7±5.4 for men and 10.5±7 for women. Peripheral vascular condition was determined using Doppler technic to establish the ankle/braquial ratio for the systolic blood pressure; a ratio <1.0 was taken as indicating of PVD and a ratio >1.5 established the presence of calcified arteries. Neurologic function tests to explore somatic and autonomic conditions were performed. The mean of two blood pressure was taken to establish the presence of hypertension as another risk factor. Serum lipids by enzimatic methods, and lipoproteins were also determined. A clinical questionnaire specifically about habits; smoking and sedentarism was also established. We found that 23% of men showed ankle/braquial criteria <1.0, diagnostic for PVD and another 23% had Doppler criteria for arterial calcification, 54% of them were current smokers and 52% showed neuropathic compromise. Hypertension was found in 38% and dyslipedemia in 31% of men population. Regard to female population; 13% showed criteria for arterial calcification, 20% a ankle/braquial ratio <1.0 and only 13% were smokers. Neuropathy was found in 33% of women, 46% were hypertensive and 60% showed dyslipedemia. Trying to establish parameters for accumulated risk for diabetic foot we gave an score of 1 to each major individual risk (arteriopathy, neuropathy and tabaquism) and 0.5 to each minor risk (hypertension, dyslipedemia ans sedentarism). Two categories was then stated: A) Those who scored ≥3. 5 were diagnosed as carrying major risk for the development of diabetic foot. B) Those who scored ≤3. 0 as with minor risk for dibetic foot. Then we found that 31% of men had an score 3. 5 or higher meanwhile only 13.3% of women reach that score. Therefore, men population although with less duration of diabetes presented higher accumulated risk for diabetic foot than diabetic women. Perhaps we can speculate that the smoking habits, more prevalent among men in our population, makes for such a difference. However, women were more frequently hypertensive and dyslipidemic than men, probably because they have been diabetic for a much longer period of time. Finally, we think that it is important to try to establish the potential risk for diabetic foot because it would allow us to educate and try to take some preventive measures.

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SAINT VINCENT DECLARATION AND DIABETIC FOOT- EXPERIENCE OF A MULTIDISCIPLINARY TEAM APPROCH.

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Aim: To evaluate the results of diabetic foot care in patients with foot problems. Patients and methods: Since 1991 a multidisciplinary team (endocrinologist, nurse educator, vascular surgeon and orthopaedist) was created to follow diabetic patients with foot problems. Between January 1991 and December 1995 we studied 192 patients: 120 men and 72 women with a mean age of 62±8 and 66±10 years, respectively. The type of diabetes, clinical presentation, metabolic control (HbA1c, blood pressure, total cholesterol, HDL cholesterol and triglycerides), prevalence of other diabetic complications (retinopathy and nefropathy) and amputation rates were analysed. Results: A total of 192 diabetics were studied and 172 (89.6 %) had NIDDM and 20 (10,4%) IDDM. The foot was predominantly neuropathic in 133 (69.2%) cases and predominantly vascular in 59 (30.7%). The mean HbA1c was 8.4±1.5%. Hypertension was present in 107 (55.7%) patients and dyslipidemia in 36 (18.7%). 123 patients had other diabetic complications: retinopathy in 123 (64%) and nefropathy in 56 (29%). The prevalence of amputed patients was 18.2% (n=35) and the prevalence of amputations was 24.4% (n=47): major 31.9% (n=15) and minor 68% (n=32).The evolution of annual results was also analysed and there was not any significant difference between years, except for the lower major amputation rate found in the last year (9%). Conclusions: According to St. Vincent declaration a multidisciplinary team approach was created for diabetic patients with foot problems. In this cohort the high amputation rate, was probably justified by the fact that all this diabetic patients had important foot problems. The lower major amputation rate of the last year suggests an improvement of diabetes care in this area.

1853

EVALUATION SOME OF POTENTIONAL RISK FACTORS IN THE SCRINING PROGRAM FOR DIABETIC FOOT .

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In addition to well known risk factors for diabetic foot ulcers (i.g. peripheral sensomotor and autonomic neuropathy, large vessel disease) such factors as limited joint mobility (cheiroarthropathy and limited hallux dorsiflexion) may lead to foot leasions. The poor selfcare plays the essential role due to the lack of patient education . The aim of this study was to investigate the trigger mechanism leading to breakdown of the foot and the influence of limited hallux dorsiflexion and cheiroarthropathy at the foot damage.145 IDDM and NIDDM patients aged 18-69 years , 2-35 years of diabetes duration took part in this study.There was the high correlation between the limited hallux dorsiflexion and peak plantar pressures in the two points (measured by EMED pedography system): hallux and 1 metatarsal head (r_1=0,9 p<0,001, r_2 = 0,8 p< 0,001); between the LJM and peak plantar pressures points (r_1= 0,8 p<0,01, r_2= 0,9 p< 0,001).

The patients were divided into 3 groups: 85 without ulceration (U-), 29 with plantar (intrinsic) ulcers (UI) and 31 with dorsal (extrinsic) ulcers (UE). In UI group there was nobody with the signes of large vessel desease, but in UE group 31,4 % of patients had ankle/brachial ratio< 0,6.The patients of UI and UE groups were intrviewed about the provoking factors for the foot damage. In UI group 33% of ulcers were the results of poorly fitting shoes, 23% related to failed self care, 19% to minor trauma, 14% could not mentioned any reason. In UE group 32% of ulcers was due to mycosis , 17% related to poor shoes, 23% to mechanical trauma.

CONCLUSION. The data suggest that cheiroarthropathy and limited hallux dorsiflexion are simple methods and could be included in scrining program for the diabetic foot. All the patients should be screened for adequate selfcare by interviewing or special questionnarie.Whatever once identified patients with the signes of interdigital mycosis and large vessel disease should be considered as the risk for a foot ulceration.

1854

PREVENTIVE CARE OF THE DIABETIC FOOT, PROPOSAL OF A COSTA RICA ATENTION MODEL

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Introduction: Most physicians and nurses in Costa Rica don't educate and don't examine the feet of the diabetic patient. It is important to teach the Health Care Personal to pay more attention to the diabetic foot and to educate the patients in its preventive care.

Objective: Propose an attention model from the EBAIS (Basic Equipment of Integrated Health Care) to the tertiary hospital in order to reduce in 50% foot amputations in the next 5 years.

Material and Methods: **1)** To create a National Committee for the Diabetic Foot Care. That will be in charge of continuous educational programs for health care teams. **2)** The PATON-A classification scale will be used by the EBAIS: the green feet (low risk) will be taken care by the EBAIS, the yellow feet (medium risk) will be sent to the secon level of attention hospitals and the red feet will be sent to the tertiary level attention hospitals. **3)** There will be an active communication system among the different attention levels. There will be yearly Seminars about the diabetic foot, to analyze policies and accomplishments.

Conclusions: With this Model we pretend to increase the knowledge of the health professional, the patient and the general population about the preventive care of the diabetic foot in order to decrease at least in 20% the severe complications of the diabetic foot in the next five years.

1855

ANALYSIS OF RISK FACTORS FOR THE DIABETIC FOOT AND EDUCATION STRESS THE IMPORTANCE OF NON-INVASIVE EXAMINATION OF NEUROPATHY AND ANGIOPATHY.

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The aim of the study was to compare various risk factors for the diabetic foot and education level of patients with active or previous foot ulcers (Group 1) and without it (Group 2) examined consecutively by specially trained nurses in 6 diabetes clinics and 1 surgical department in a model area during 1 year. There was no significant difference between Group 1 (44 persons) and Group 2 (278 persons) in mean age (67±10 and 65±10 years, respectively), type of diabetes (Type 2 in 95% and 93%) and its duration (15±14 and 13±7 years). Significant differences between Groups 1 and 2 were found in non-invasive examination of neuropathy by monofilaments (2.7±2.6 vs 5.4±2.8 sensitive points, $p<0.001$) and by bio-thesiometer (vibration pressure threshold - VPT 36±16, CI 30-41 vs 25±12 , CI 23-27 V , $p<0.001$). The number of patients with VPT over 30 V was significantly different (74 and 47%, $p<0.001$), that of patients with VPT of 25-29 V was similar (7 and 8.6%). No significant differences were found in subjective symptoms of neuropathy. The Doppler ankle/arm index was significantly different between Groups 1 and 2 (0.82±0.43, CI 0.68-0.97 and 0.92±0.26, CI 0.89-0.95, $p<0.05$). Only the number of patients with an ankle/arm index under 0.8, not under 1, differed significantly between both groups. In Group 1, a low Doppler index was more common (57%) than AF murmurs (32%), non-palpable peripheral pulsations (29%), and claudications (41%). Diabetes compensation in this group was significantly worse (HbAlc 8.6±2 vs 8.0±1.5%, $p<0.05$) and creatinine levels higher (113±57 vs 94±25 µmol/l, $p < 0.01$). The number of some orthopedic deformities and skin abnormalities was the same in both groups. The level of foot-related knowledge was low in either group, 52% of correct answers. We conclude that simple non-invasive examination of neuropathy and angiopathy with age-related risk values and HbAlc determination are good risk factor characteristics also of elderly patients with the diabetic foot.

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STUDIES OF DIABETIC AMPUTATIONS IN LITHUANIA: SCOPE FOR PREVENTION.

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A comprehensive diabetic foot care service at the Lithuanian Amputee Rehabilitation Hospital has been established with the specific aim of identifying risk factors for ulceration providing preventative education, diabetic footwear and chiropody. Among all 922 amputees referred during 1991 - 1995 for artificial limb provision 188 (20%) of amputations were related to traumatic, 397 (43%) to vascular, 264 (29%) to diabetic and 73 (8%) to other causes. Of 264 patients, 89% had NIDDM, and 49% had diabetes duration < 10 years. From 1994 - 1996, all amputees were examined using quantitative sensory testing and Doppler techniques: 40% were purely neuropathic, 54% neuroischaemic and 6% purely ischaemic. This centre should be able to achieve significant reductions in amputations as many are potentially preventable being of neuropathic aetiology. The screening to identify "high-risk" patients who might benefit from foot care education, chiropody and diabetic footwear is extremely important in Lithuania to reduce diabetic amputations.

1857

PERIPHERAL ARTERIAL OCCLUSIVE DISEASE AND ITS RISK FACTORS IN NON-INSULIN DEPENDENT DIABETIC PATIENTS

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We aimed to assess the prevalence of objectively verified peripheral arterial occlusive disease (PAOD) in NIDDM patients. Another purpose was to detect risk factors for PAOD during a prospective 11-year (range 7-14 yrs) follow-up. 130 (64F, 66M) NIDDM patients were randomly selected from the register of the local Diabetic Association. At baseline, 21 patients (16%) were diagnosed with PAOD, based upon an ankle-brachial index (ABI) <0.90. In an age and sex-matched non-diabetic control group, only 1 out of 47 subjects (2%) had PAOD. Medial sclerosis was excluded by measurement of great toe blood pressure. During the study 29 patients (22%) died and two were lost. Of the 88 non-PAOD patients at baseline 20 patients (23%) developed PAOD during follow-up. At baseline PAOD was associated with microalbuminuria ($p=0.0007$), hypertension ($p=0.03$), duration of smoking ($p=0.01$), and diabetes duration ($p=0.04$), as well as age ($p=0.003$). In a logistic regression analysis independent risk factors for PAOD were microalbuminuria ($p=0.02$), and age ($p=0.009$). At follow-up risk factors for PAOD were current or previous smoking ($p=0.05$), duration of smoking ($p=0.02$), triglycerides ($p=0.0002$), total cholesterol ($p=0.0003$), and LDL-cholesterol ($p=0.009$), at baseline as well as age ($p=0.02$) at follow-up. In the logistic regression analysis HDL-cholesterol ($p=0.009$), cholesterol ($p=0.004$) at baseline and age at follow-up ($p=0.004$) were significant predictors of PAOD Current or previous smoking was borderline significant ($p=0.05$). As to glucose, HbA1c, C-peptide values before and 6 min after a glucagone dose, body mass index, sex or mode of treatment did not predict PAOD. In conclusion, PAOD is much more common in NIDDM patients than in the non-diabetic population. Microalbuminuria, dyslipidemia, hypertension and smoking are the strongest predictors of peripheral arterial occlusive disease in diabetic patients

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FOOT RE-ULCERATIONS IN DIABETES: WHICH IMPORTANT MARKERS FOR EFFECTIVE PREVENTION ?

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Objective: Repetitive foot ulcerations aggravate manyfold (2-6x) the risk of amputation. Factors determining foot reulceration have been analysed at the time of a 2nd foot ulcer episode. **Methods:** 268 patients were followed in a prospective, observational multicentric study from Oct 1994 to May 1996 among 3 VA hospitals and 1 private clinic [US] + 1 Swiss hospital [Geneva]. Trigger events having led to foot ulceration were analysed in patients presenting for multiple episodes. Causal factors of the 2nd ulcer were compared to those of the 1st ulcer. **Results:** Out of the 268 enrolled patients 152 presented a history of a prior ulcer (56.8%), in contrast only 26/268 patients presented during the twenty months study period for a 2nd foot ulcer (10%). During the healing time of the first ulcer NO other ulcer developed. In 2/3 of the 26 patients the same trigger mechanism caused the 2nd ulceration as to compared to the first one. The three major causal factors were 1) shoes 2) blisters 3) errors in self-care. Repetition of the first causal factor at the time of the 2nd ulcer underlines the importance of educational strategies focused on each patients personal pivotal event. **Conclusion:** The low incidence of reulcerations while the patient is under medical supervision for an existing foot ulcer highlights the impact of regular foot clinic visits in prevention of relapses. The repetition of the same causal factor in the same patient having led to foot reulceration gives excellent information for tailoring specific educational and follow-up strategies.

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RISK FACTORS FOR MAJOR LOWER EXTREMITY AMPUTATIONS IN DIABETIC PATIENTS

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The objective of the study was to determine the risk factors for major lower limb amputations. We studied 43 diabetic amputees (29 men and 14 women, with 26 thigh and 17 leg amputations), admitted to a centre for prosthetics 5 to 12 months after the amputation. They were compared to 43 age and sex-matched controls without foot lesions or amputation, admitted to an inpatient diabetic clinic. We found that rural population and those with lower educational level were at higher risk for amputation. The presence of neuropathy, ischemic heart disease and a history of intermittent claudication, but not retinopathy, nephropathy and history of stroke, were predictive for a major amputation. Systolic (158.8±25.6) vs 150.2±21.1) and diastolic (95.3±10.9 vs 90.4±11.9) blood pressure tended to be higher in amputees without reaching statistical significance. Vibration perception threshold measured at the great toe with the tuning fork of Rydel-Seiffer, was significantly lower in amputees (3.47±1.9 vs 4.73±2.0, $p<0.01$). Fasting and postprandial blood glucose measured at the time of admission to the clinics did not show any difference between the groups, neither did BMI, type and duration of diabetes. There were more amputees who reported high alcohol consumption ($p<0.05$), but smokers were similar in both groups. Amputees had less diabetes related knowledge than the control group. Risk factors for major lower limb amputation should be taken into account in the development of a preventive diabetic foot programme.

1860

PREVENTION OF DIABETIC FOOT ULCERS IN PATIENTS WITH AND WITHOUT PREVIOUS FOOT ULCERS.

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Background: Foot ulceration(FU) in relation to diabetic neuropathy and/or peripheral ischaemic vessel disease (PIVD) is triggered by external trauma,e.g.from improper footcare and footwear. **Aim:** to prospectively assess ulcer development in 2 cohorts of patients (81% NIDDM, 20% PIVD, 100% neuropathy, duration of diabetes 18 yrs) over a period of 2 years. **Patients and methods:** 21 patients with neither previous FU nor gross foot deformity comprised group A. Another 31 patients with acute FU, a history of FU(n=31) and minor amputations (n=8), and significant foot deformity were recruited after healing of the FU (group B). Both groups received regular footcare and medical footwear (ready made shoes in 16, bespoke shoes in 36 cases). **Results:** in group A,1 patient developed 1 FU, versus 14 patients developing 22 FUs in group B($p<0.05$). FU was caused by footwear (77%),and self-made chiropody(25%). Footwear-related FU was due to temporarily wearing ordinary shoes (27%), to mismatch between foot and bespoke shoe(27%),and to toe-caps (23%).Compliance with medical footwear was reported to be high(85%)in both groups. **Conclusion:** Failure to prevent FU was related to a history of FU and foot deformity, as well as to bespoke footwear (which may be too difficult to design for deformed neuropathic feet).

1861

RISK FACTORS FOR HIGH LEVEL AMPUTATION IN DIABETIC FOOT

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To realize the risk factors for high level amputation (ie:below-knee & above knee amputation), 107 admission patients who were diagnosed as diabetic foot during the period of January 1995 to June 1995 in Chang Gung Memorial Hospital were studied retrospectively. There were 54 male and 53 female, 70 patients (65%) of them admitted to Metabolic ward with a multidisciplinary foot-care team, the others (35%) admitted to orthopedic, plastic surgery, general medicine or trauma team ward. Two groups were subclassified. The cases who received high level amputation were Group A (35 patients). The cases who received no or minor operation (such as toe amputation, debridement, skin graft,...) were Group B (72 patients). Statistical comparison were performed between these two groups by Student's t test, Chi Square and stepwise logistic regression test. Group A was significantly older, lower ischemic index, higher levels of WBC, BUN, Cr, CRP and lower levels of Hb, albumin, HbA1C. However, the levels of cholesterol, triglyceride, the duration of DM, the existence of proteinuria, hypertension, old CVA, heart disease or smoking history did not reveal significantly different. Stepwise logistic regression show older age and higher white count level were risk factors and for those whose Diabetes duration longer than 5 years, older age and no DM team care had significant increase risk. We concluded that patients with diabetic foot in this Medical Center, older age and higher level WBC were increase risk for high level amputation and DM team care do decrease high level amputation to those DM duration > 5 years.

1862

APPROACHING ST VINCENT; REDUCING THE INCIDENCE OF DIABETIC LOWER LIMB AMPUTATION IN LEEDS, UK.

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Lower limb amputation (LLA) is one of the most important endpoints in diabetes, it is a major cause of morbidity and mortality and is known to be avoidable in the majority of cases through the provision of appropriate education and health care. As such, it is a target of the St. Vincent Declaration which aimed to halve diabetic LLAs within five years. At the end of 1993 a new diabetes service was introduced into a metropolitan health district in the UK. In order to evaluate its effectiveness in addressing LLA outcome we compared data currently being gathered (July 1995-June 1996) as part of an international study, with data collected for a 12 month period preceding the establishment of the service (July 1992-June 1993). Identical case ascertainment strategies were utilized for both periods. Using information recorded in operating theatre registers, hospital discharge data and by the regional prosthetic centre we uniquely identified a total of 273 in '92/'93 and 222 LLAs in '95/'96. Of these procedures, 81 and 60 were carried out in people with diabetes respectively. This represents a decrease in LLA incidence of 15.6% in the non-diabetic population and 25.9% in the diabetic population over the intervening 3 year period. Accompanying this decrease there was a concomitant reduction in the level of procedure with only 22% of the amputations being carried out above knee in the more recent time period compared to 35% in '92/'93. In conclusion, set against a background of falling incidence of LLA in the general population, amputations in people with diabetes in Leeds are approaching the target set by St. Vincent.

1863

RISK FACTORS PREDICTING SEVERE FOOT ULCER AND AMPUTATIONS IN PATIENTS WITH NIDDM

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Diabetic foot lesion is a one of the major cause of morbidity in patients with diabetes. Therefore, a case contol study was utilized to quantify the contribution of various risk factors to the risk of severe diabetic foot lesion required surgical intervention including lower extremity amputation. At baseline, risk factors for diabetic foot lesion were determined in 262 NIDDM patients aged 40 to 70 years in 1990. These patients were followed up annually for 5 years with respect to foot lesions. Case patients had had an incident surgical intervention between 1990 and 1995; control subjects had no diabetic foot lesion by 1995. Foot conditions and health status prior to the pivotal event led to the surgical treatment were obtained by medical record review. We identified 25 subjects including 11 amputees and randomly selected 237 contols for comparison. After adjustment for sex and age, risk for severe diabetic foot lesion required surgical intervention was associated with peripheral neuropathy (OR 12.5, 95% CI 5.6-52), peripheral vascular disease (OR 11.3, 95% CI 5.0-34), autonomic neuropathy (OR 10.0, 95% CI 3.6-43), retinopathy (OR 7.7, 95% CI 3.6-27), glycated hemoglobin (OR 2.9, 95% CI, 1.7-6.2), smoking (OR 2.6, 95% CI, 1.0-3.0) and microalbuminuria (OR 1.8, 95% CI, 1.1-2.1). After controlling for these differences, the ORs for severe diabetic foot lesion with peripheral vascular disease was 4.1 (95% CI 2.1-10.4), with peripheral neuropathy, 4.0 (95% CI 1.8-12.4), with glycated hemoglobin, 2.5 (95% CI 1.5-5.3), with retinopathy, 2.5 (95% CI 1.0-4.6), with autonomic neuropathy, 2.3 (95% CI 1.0-7.5), with smoking, 1.6 (1.0-3.0) and with microalbuminuria, 1.0 (95% CI 0.7-1.6).

1864

THE FATES OF PATIENTS WITH DIABETES MELLITUS AFTER AMPUTATION OF A LOWER LIMB

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The aim of the study was to analyze factors that affected the survival time of 125 patients with diabetes mellitus (DM) and 121 patients with normal glucose tolerance (NGT), to whom amputations of the lower limb in consequence of gangrene were performed. In a 5-year follow-up study 80 patients with DM (64 percent) and 39 patients with NGT (32 percent) died.

Among the examined patients with DM were 72 men and 53 women [mean age 69.2 ± 10.1 (± SD) yrs], and among these with NGT 92 men and 29 women (mean age 66.2 ± 12.2 yrs).

In statistical analysis (Cox proportional model) in patients with DM the following predictors of death were identified:
age (p=0.0001), duration of DM (p=0.03), systolic arterial hypertension (p=0.006), peripheral arterial disease (p=0.0007), diabetic nephropathy (p=0.0065). In the patients with NGT only age was significant predictor of death (p=0.0001).

Conclusion: The necessity of performance of amputation of a lower limb in a patients with DM provides information on unsuccessful course of the disease.

1865

PREDICTION OF DIABETIC FOOT ULCER OCCURRENCE BY A TREE STRUCTURED ANALYSIS OF PROSPECTIVE SURVIVAL DATA.

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There has been little prospective research on risk factors for diabetic foot ulcer that simultaneously considers the structural, neurologic, and vascular factors obtained from a clinical evaluation. We prospectively followed 729 diabetic general medicine clinic enrollees without foot ulcer for the development of this lesion. Baseline assessment included a medical history and physical examination that focused on diabetes and the lower limbs; testing for sensory neuropathy using the 5.07 monofilament (SN); autonomic function (orthostatic blood pressure drop (BPD); foot deformity; and glycosylated hemoglobin (GHB) and serum creatinine (CR). Foot ulcer was defined as a full thickness defect that required > 2 weeks to heal. Mailed, telephone, and in-person contacts at regular intervals were used to assure capture of all incident foot ulcers. A recursive partitioning and amalgamation algorithm (RECPAM) that considers time to event (foot ulcer) was used to separate this population into groups based on magnitude of risk. Subjects were elderly (mean age 63.2 yrs) males (98%) with NIDDM (93.4%) and a mean diabetes duration of 11.4 yrs. Subjects were followed for 2.5 years on average for foot ulcer occurrence, during which time 118 ulcers occurred. Seven of twenty variables considered were found to significantly alter risk (SN, history of peripheral vascular disease (PVD), history of amputation (AMP), BPD, GHB, hallux limitus (HL), and CR. Four different risk groups were identified: 1) SN-, PVD- (n=273); 2) [SN+, AMP+, GHB < 13.7%] or [SN+, AMP-, and BPD > 31 mm Hg or HL+ or CR > 186 μM] (n=123); 3) SN+, AMP+, GHB > 13.7% (n=15); and 4) remaining patients (n=318). Proportion developing foot ulcer and relative risk (RR) with 95% confidence limits in groups 2-4 compared to group 1 are: 1) 2.6%, RR=1 (referent); 2) 39.8%, RR=21.5 (9.8 - 47.2); 3) 80%, RR=79.0 (30.9 - 202.5); 4) 15.7%, RR=5.9, (2.7 - 12.9). We conclude that multiple factors contribute to the risk of diabetic foot ulcer. Information on these easily obtained risk factors may be used to classify diabetic patients as to their risk of developing foot ulcer.

1866

POTENTIAL ECONOMIC BENEFITS OF STRATEGIES TO PREVENT LOWER-EXTREMITY AMPUTATION IN PERSONS WITH DIABETES

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Objectives. To estimate the potential economic benefits of selected strategies from published literature—educational interventions, multi-disciplinary clinics, and insurance coverage for therapeutic shoes—to reduce the incidence of lower-extremity amputation among persons with diabetes. *Methods.* We developed a model to estimate the expected incidence and associated costs of lower extremity amputation in a hypothetical cohort of 10,000 persons with diabetes. Prevention strategies were assumed to be targeted at persons with a history of foot ulcer, and benefits were estimated over a period of three years. *Results.* The total potential economic benefits (discounted) of strategies to reduce amputation risk ranged from $1.9 to $2.9 million ($2,798 to $4,286 per person with a history of foot ulcer) over three years. Benefits were highest for educational interventions. Most benefits were found to accrue among persons aged 70 years and older. *Conclusions.* Strategies to reduce the risk of lower-extremity amputation may generate substantial economic benefits, and should be a standard component of routine diabetes care. These strategies may best be achieved through government payers, the health care team, patients and their advocates, in partnership with the health care industry.

1867

RISK FACTORS FOR FOOT ULCERATION IN A DIABETIC OUTPATIENT CLINIC: A FIVE YEARS FOLLOW-UP

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1007 diabetic patients (age 64±11 yrs, M/F 49.6/50.4% duration 13.5±9 yrs, 71 IDDM and 929 NIDDM) were observed for the first time in the outpatient clinic in the period 7/11/91-30/9/95.They were fully characterized for metabolic control and long term diabetic complications, expecially of the lower limbs, with an extensive protocol. No patients showed foot ulceration at the inclusion in the study. In the follow-up period (42.3±11.2 (12.3-59.2) months) the ulcer incidence was 4.8% with a mean annual rate of 1.2%. Between them 4 subjects (~8%) had lower limb amputation after 1 yr ulceration. Age, weight, BMI, type and duration of diabetes, metabolic control at the time of inclusion, severity of retinopathy and nephropathy, previous cerebral or cardiac vascular diseases, the presence of claudicatio, as well as the absolute value of the ankle brachial index were unable to differentiate patients who would or would not develop foot ulceration. In the univariate analysis the factors able to differentiate those patients were represented by: absence of pedal pulses ($p<0.01$), absence of ankle jerks ($p<0.005$), VPT >25 mV ($p>0.001$), presence of foot deformities ($p<0.01$) and presence of a previous foot ulceration ($p<0.001$). In the multivariate analysis adjusted for all parameters the factors still able to discriminate patients at risk of ulceration were: previous ulceration (OR 6.4 (CI 95% 2.95-14.3)), absence of ankle jerks (OR 4.1(CI 95% 1.59-10.61)) and VPT >25 mV(OR 3.81(CI 95% 1.69-8.61)). In addition for each unitary increment of VPT in the absolute value there was a 3% increment in the risk of ulceration. In conclusion this prospective study demonstrates that an accurate clinical examination with the combination of few simple parameters discriminates patients with an increased risk of foot ulceration.

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MANAGEMENT OF THE DIABETIC FOOT IN HOSPITAL : COSTS AND BENEFITS

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The aim of this work is to assess the costs of the diabetic foot lesions seen at a university hospital from january 1989 through december 1993. A total of 163 cases of diabetic foot were reviewed in 132 patients (88 women and 44 men). Lesions were allocated into three groups according to the Wagner classification. We evaluated direct costs (hospital stay, medical treatment, surgical treatment, investigation) and indirect costs (amputation and in-hospital mortality). X^2 test and analysis of variance were used to compare the different groups. More than nine percent (9.16%) of all hospitalized diabetic patients were reported to have foot problems. Almost all the patients (95.50%) were older than 40 years. The first foot lesion appeared at 59.64±11.74 years. The M/F ratio was significant (M/F = 1.64; $p<0.01$). The mean costs and mean durations varied according to the Wagner classification. The mean costs of hospitalization were calculated to be 79.31, 136.18 and 176.41 KDA (1KDA = 1000 algerian dinars), for the groups 1, 2 and 3 respectively ($p<0.001$). The mean cost of a foot lesion was evaluated to be 5611$ US (1$ US was about 23.84 dinars in 1993). The mean durations of hospitalization were calculated to be 26.87, 48.25 and 57.12 days for the groups 1, 2 and 3 respectively ($p<0.001$). More than seventeen percent (17.43%) of patients underwent major amputation. Foot function was preserved in 74% of cases. In-hospital mortality (9%) was present only in patients staged Wagner 4+5 (group 3). More than seventy nine percent (79.11%) of the total financial costs were attributed to hospital stay. Forty two out of 57 patients (73.68%) that had an occupation obtained a rest. The total duration of rest was 3780 days. Direct and indirect costs due to diabetic foot are high. It is necessary to invest more money in Prevention in order to minimize this complication and lessen those costs.

1869

WHO'S AT RISK FOR FOOT ULCERATION?

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Previous studies of risk factors for ulcerations have identified peripheral neuropathy, vascular disease, limited joint mobility, body mass, foot deformities, abnormal foot pressures, a history of ulceration or amputation, nephropathy, and impaired visual acuity as important factors. However, we have been unable to identify any existing work that considers all of these factors in the same analysis. Therefore, the purpose of this study was to evaluate risk factors for foot ulcerations among persons with diabetes.This project was conducted as a case-control study, 225 age-matched patients, 46.7% male, were enrolled with a ratio of approximately 1:2 case:controls (76 cases:149 controls). All patients met the following criteria: 1)The presence of diabetes mellitus based on World Health Organization criteria (6.2% type I), 2) evaluation by medicine and ophthalmology services within the past 6 months, 3) glycosylated hemoglobin, complete blood count, urinalysis, creatinine and blood urea nitrogen laboratory studies performed in the past 3 months. Cases were defined as subjects that met the above criteria with an existing foot ulceration or a history of a foot ulceration. Controls were defined as subjects with no history of foot ulceration. Using a stepwise logistic regression model (alpha < 0.05), elevated plantar pressure (> 65 N/cm^2), previous history of amputation, duration of diabetes (>10 years), foot deformities (hallux rigidus or hammertoes), male gender, poor diabetes control (HbA1c >9%), one or more subjective symptoms of neuropathy, and elevated vibration perception threshold (> 25 V) were significantly associated with neuropathic ulceration. 77.6% of ulcerated patients had a rigid deformity directly associated with the site of ulceration. No significant associations were noted between vascular disease (ankle brachial systolic pressure index <80, nephropathy, transcutaneous oxygen tension <30mmHg, or palpable pedal pulses), level of formal education, nephropathy, retinopathy, impaired vision, or obesity and foot ulceration. The results suggest that neuropathy, deformity, commensurate high plantar pressures and history of amputation are significantly associated with presence of foot ulceration.

1870

INCREASED FOLLOWED BY DECREASED NUMBER OF MAJOR AMPUTATIONS IN DIABETICS DURING ECONOMICAL SANCTIONS

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The National programme in order to reduce amputations for diabetic gangrene have included the following diagnostic procedures: bidirectional CW Doppler flowmetry of the arteries with 8 and 4 MHz sound, the segmental blood pressure measurements, digital photopletismography of the big toe, evaluation and calculation of the neuropathy disability score. 1993. year was characterized by serious deficiency of all drugs especially antibiotics. The number of the lower limb amputations was observed and estimated for the period 1991.-1995. year. There are about 300,000 inhabitants on the territory covered by the Regional Health Centre, and out of this number 8,000 patients were diabetics treated with antidiabetics. 6,400 patients were treated with drugs and 1,600 patients with insulin.

	No of amputations (A)		*A.Crural*		*A.Femoral*	
	Total	**DM** (%)	Total	**DM**(%)	Total	**DM**(%)
1991.	158	79 (50)	43	24 (55.8)	72	25(34.7)
1992.	165	***84*** (50.9)	41	***25***(61)	69	***28***(40.6)
1993.	184	**102 (55.4)**	51	***34*****(66.7)**	89	***39*****(43.8)**
1994.	142	***56*** (39.4)	28	***9*** (32)	80	***30***(37.5)
1995.	130	63 (48.5)	25	18(72)	78	30(38.5)

Statistical evaluation showed increase in the major limb amputations in 1993. year (X^2 p 0.089), followed with a significant decrease (X^2 p <0.01) in 1994. year. The number of transmetatarsal and toes amputations in this period was unchanged. The possibilities to perform diagnostic procedures and surgical interventions were not diminished in this period, but it was concluded that the above results show the importance of the drug therapy, especially of the antibiotics which were deficient to pre the performed amputations. The significant decrease of the amputations from 1994. could be the matter for further speculations.

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MORE THAN HALF OF THE DIABETES POPULATION HAS INCREASED RISK TO DEVELOP CHRONIC FOOT ULCERS.

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Preventive care has been effective in diabetes to lower the rate of foot ulcers (FU) and amputation. The aims of the study were to characterise diabetes patients with FU and to find an easy screening method to identify patients at risk. 50 consecutive diabetics referred to the foot clinic at Karolinska Hospital with chronic FU were examined and answered a questionnaire. A model protocol for foot examination was designed. 323 diabetics, randomly selected, in Primary Health Care (PHC) were examined in accordance to the protocol. The patients were diagnosed to have no signs of complications (group 1), signs of neuropathy (np) (group 2), or both neuropathy, angiopathy (ap) and/or earlier chronic FU (group 3). The examination was performed by trained chiropodists, practical or registered nurses. The results showed that patients with chronic FU (mean age 66.4 years) had a mean diabetes duration of 20.5 years. All had signs of np and 67 % had ap. Mean HbA1c was 11.8%. The healing time for a recurrent chronic FU was longer than for the previous one. The patients without FU (mean age 60 years), had diabetes duration of 9.4 years. Six % had been treated for chronic ulcers. Neuropathy was found in 54%. Autonomous np was most common (70%). 19% had deformities and 38% calluses. Signs of microangiopathy were found in 17.5% and severe macroangiopathy in 5.2%. 26% had impaired vision, which made their self care difficult. The mean value for HbA1C was 7.8% (reference<5.2%). 36% had regular chiropody. In conclusion patients with chronic FU had a longer diabetes duration and worse metabolic control. The healing time for a recurrent FU was significantly prolonged. More than half of patients with diabetes in PHC had risk factors for chronic FU, especially np, local changes and unacceptable metabolic control. Only 40% had no or low risk, 10% a high risk to develop chronic FU. Only a minority had access to medical chiropody. The model protocol to identify patients at risk was easy to use in regular foot examinations and patient education.

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THE ROLE OF NEUROPATHY IN THE DEVELOPMENT OF DIABETIC FOOT ULCERATION.

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There are many factors playing a role in the development of diabetic foot ulceration, mostly are combination of neuropathy, compromise vascular condition and superimposed infection. The aim of this study was to determine the contribution of neuropathy in the development of foot ulceration. A case control study was conducted, recruiting patients admitted to Ciptomangunkusumo hospital for diabetic foot ulcer from.April to September 1996. As a control group, diabetic patients without foot ulceration attending our diabetic clinic were asked to join this study, matched for sex, age, and nutritional status. Neuropathic parameters performed in this study were Semmesweinstein monofilament (5.07), vibratory sensation as measured by vibrameter, heart beat variation (RR interval) during deep breathing measured by SRR5 heart rate recorder, orthostatic hypotension and knee tendon reflex test. Patients with an ankle brachial index of < 0.9 (using simple doppler apparatus) were excluded. Univariate analysis was used to calculate odds ratio for each independent factor. There were 67 subjects with diabetic foot ulceration and 84 diabetics without foot ulceration eligible for statistical evaluation. No differences in age, gender, BMI, years of known diabetes and means HBA1c were found between both groups. Monofilament was the best predictor for the development of foot ulceration (OR = 10.48, 95 % c.i. 4.02-28.22). Abnormal heart beat variation had the OR of 7.71 (95 % c.i. 2.77-22.27), while absent of knee reflex gave rise to OR of 4.32, 95 % c.i. 1.86-10.19). Abnormal vibratory sensation (>43 vhz) contributed OR of 2.63 (95 % c.i.1.23-5.65), while orthostatic hypotension showed the lowest OR (0.89, 95 % c.i. 0.33-2.35). In conclusion, diabetic neuropathy plays an important role in the development of foot ulceration. Semmesweinstein monofilament 5.07 was the best predictor for the development of foot ulceration. Other neurologic parameter abnormalities had less important contributions as a predictor of the development of diabetic foot ulceration

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CONSERVATIVE SURGERY VERSUS CONVENTIONAL THERAPY FOR DIABETIC FOOT: A RANDOMIZED TRIAL

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We compared conservative surgical treatment of non infected neuropathic foot ulcers to conventional therapy in a group of diabetic outpatients. 41 patients who came to our foot clinic for the first time from January to December 1995 with a non complicated neuropathic ulcer were randomized into two groups: Group A [N. 20 patients (17 NIDDM/3 IDDM), age 63.24±13.46 yrs, duration of diabetes (DD) 18.2±8.41 yrs, HbA1c 9.5±3.8%] received a conventional treatment, consisting in medications and relieving of weight; Group B [N. 21 patients (19 NIDDM/2 IDDM), age 65.53±9.87 yrs, DD 16.84±10.61 yrs; HbA1c 8.9±2.2%] underwent surgical ulcerectomy, eventual remodelling or removal of bone segments underlying the lesion and surgical suture. Healing rate was lower (79.2%) in group A than in group B (95.5%; p<0.05), and healing time was longer in group A (128.91±86.60 days), than in group B (46.73±38.94 days; p<0.001). Infective complications occurred significantly more often in group A patients (12.5%) than in group B (4.5%, p<0.05), as well as relapses of ulcerations (8 in group A versus 3 in group B patients; p<0.05). Patients' satisfaction was higher in group B patients (p<0.05) than in group A ones. Surgical treatment of neuropathic foot ulcers is an effective approach compared to conventional treatment in terms of healing time, complications and relapses.

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THE METHODS OF DIAGNOSTICS AND TREATMENT OF THE DIABETIC FOOT.

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The results of investigation and treatment of 320 severe cases of diabetes mellitus with diabetic foot have been summarized and analized The age of the patients ranged from 21 to 69. The average dose of 63,5±12,1 units of insulin was injected daily.

The following investigations were performed: polarography, capillaroscopy, ultrasonic dopplerography of the vessels of the lower extremity, roentgenoscopy of pedal bones. The immune condition of the body, the level of hormones (hydrocortisone, aldosterone, corticotrophin, somatotrophin, calcitonin) in the blood serum and the process of lipid peroxidation were studied.

All patients were observed to develop the thinning of the osseous cortical layer; 87% had 2 to 3-fold increase of calcitonin level and the decreased level of calcium in the blood. 92% of patients had disturbances of pallesthesia, thermosthesia and algesthesia. 78% were noted to have the increased concentration of fibrin and fibrinogen in the blood plasma and the increase of thrombocytes' aggregation qualities. Those changes caused the disturbances of flow-rate characteristics, i.e., slowed-down blood flow venous stasis, thrombogenesis. Magistral flow and pulse index in crural and pedal arteries were diminished in 96% of patients.

Conservative complex treatment proved beneficial for every stage of metabolism and for microcirculation.

196 patients with phlegmone were operated. In cases of marginal dry gangrene on the foot, necrectomy was performed In cases of destructed bones and purulent foci on the foot, excision within healthy tissues was made. The wound was sutured and drained. When gangrenous hallux was aggreavated by progressing infection the tendon of the musculus flexor longus hallucis was excised within healthy tissue.

Complex treatment was aimed at the adequate correction of carbohydrate, lipid, protein, water-electrolyte metabolism and flow-rate characteristics and at the possibility to perform "smaller" surgery. As a result, the number of amputations of lower extremities decreased considerably.

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VASCULAR DISEASE OF THE LOWER EXTREMITIES IN RENAL DYSFUNCTION PATIENTS

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Background: Renal Failure is one of risk factors of diabetic foot. Amputation rate in patients under hemodialysis is high. Hemodialysis patients have arteriosclerosis obliterans so often. Objective: To determine the incidence of vascular disease in renal dysfunciton diabetic and nondiabetic patients with creatinine level above 1.4mg/dl. Research design and method: Subjects: 110 diabetic and nondiabetic patients who took the color doppler ultrasonography. Age: average 64yr(range 34-88yr). Of the 82 diabetics 35 were receiving hemodialysis and 47 were not. Of the 25 nondiabetics 12 subjects were receiving hemodialysis and 13 were without hemodialysis. Results: Results of the doppler were divided into two groups: normal and significant findings (plaque, calcification, stenosis or obstruction). 1. Diabetic prehemodialysis patients group: the percentage of patients who had the significant findings was 62%. Especially the percentage of patients who had the findings of stenosis or obstruction was as high as 47%. 2. Diabetic hemodialysis patients group: the percentage of the significant findings was 74%. The percentage of stenosis or obstruction was 54%. 3. Nondiabetic prehemodialysis patients group: the percentage of significant findings was 30%. Only two(15%) patients had the findings of stenosis. 4. Nondiabetic hemodialysis patients group: the percentage of significant findings was 33%. The number of patients who had stenosis was four(8%). Conclusion: The probability of development of vascular diseases of diabetic renal dysfunction patients even in prehemodialysis stage is high.

1876

FROM THE EXPERIENCE OF «DIABETIC FOOT» CENTRE IN MINSK

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In April 1996 Specialized Center «Diabetic Foot» was stared in Minsk as outcome and consequence of St'Vincent Declaration and introduction of National Program «Diabetes Mellitus in Republic of Belarus». The Center has two parts: an out-patient consulting room and an in-patient department. A qualified doctor and a nurse with a special training work at the out-patient department. 615 patients had their examinations in this out-patient consulting room. Specialized examination included: taking the patients case history, follow-up of the disease, determining risk factors, foot examination, Doppler ultrasound measurements of vessels, vibration perception threshold and temperature discrimination of threshold measurements. It was found out that 13,1% of the patients examined had a diabetic foot syndrome. 86,3% them had neuropathic, and 13,6% had severe neuro-ischemic lesions. 54,5% of patients with ulcers received complex out-patient therapy. 18,1% of patients had severe infections lesions or gangrene. They were hospitalized to the city hospital № 10 where a 30-bed department for diabetic foot patients is located. The in-patient department staff includes experienced surgeons and a diabetologist who have a special training in this field. The joint efforts of the medical personnel allowed to decrease by 18,8% the number of high (above the knee) amputations and to determine risk groups and introduce prophylactic treatment. In the future the Center is planning to carry out their screening program for early diagnosis of the syndrome of the diabetic foot to prevent amputations.

1877

GRANULOCYTE COLONY STIMULATING FACTOR (G-CSF) IMPROVES NEUTROPHIL FUNCTION AND CLINICAL OUTCOME IN LIMB THREATENING INFECTION.

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Lower extremity infection continues to be a cause of significant morbidity and mortality. Neutrophil superoxide generation, a crucial part of neutrophil bactericidal activity, is impaired in diabetes. In a randomised, double-blind placebo controlled trial we assessed the effects of systemic treatment with the haematopoietic growth factor G-CSF on neutrophil superoxide generation and clinical outcome in patients with severe foot sepsis, necessitating hospital admission. Individuals were matched for age, sex, duration of diabetes, vascular disease and were randomised to either **G-CSF** therapy (**G**; n=20), 5 $\mu g\ kg^{-1}\ day^{-1}$, subcutaneously for 7 days or **Placebo** (**P**; n=20). Both groups received similar antibiotic and insulin treatment. Baseline metabolic control was similar, HbA1c [median; (range)], **G**9.2%; (5.5-12.3) vs. **P**8.7%; (5.5-12.9), *p=0.49.* Initial opsonised zymosan stimulated superoxide levels (nmol/10^6 neutrophils/30 min.) were also similar [mean ± SE]. **G**6.8 ± 1.9, **P**7.2 ± 1.4, *p>0.05.* After 7 days treatment the G-CSF group showed neutrophilia, absolute neutrophil counts, [mean ± SE, $x10^9$ /L], **G**20.9 ± 1.7, **P**4.1 ± 0.4, *p<0.0001* with significantly improved superoxide levels, **G**15.2 ± 1.5 vs. **P**6.6 ± 0.8, *p<0.001.* No G-CSF patients required surgery/amputation vs. 4 placebo, χ^2=4.44, *p<0.05.* Deep tissue swabs became sterile quicker in G-CSF patients, [median; (range)], **G** 4 (2-10) days vs. **P** 8 (2-79), *p=0.007.* Improved neutrophil function in the G-CSF group was also associated with more rapid cellulitis resolution, **G** 7 (5-20) days vs. **P** 12 (5-93), *p=0.01,* shorter hospital stay **G** 10 (7-31) days vs. **P** 16.5 (9-100), *p=0.007,* and reduced intra-venous antibiotic requirements. **G** 8.5 (5-30) days vs. **P** 14.5 (8-63), *p=0.02.* G-CSF increases neutrophil superoxide generation in patients with foot infections and this may have clinical benefits.

1878

DIABETIC FOOT ULCER MANAGEMENT - EXPERIENCE OF IBRAHIM MEMORIAL DIABETES HOSPITAL.

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Foot ulcer is a dreadful complication of diabetes mellitus. About 27% of our surgical admissions are due to foot ulcer. In the surgical out-patient dept. 40% of the patients are treated for foot ulcer. One recent study on 167 patients showed that 65% of these patients are known diabetic, 57% on admission had blood sugar >10 mmol. Elderly patients with a mean age of 52 years are more commonly affected. Male female ratio was 4 : 1. Only 21% patients gave any historyof trauma and 22% of these patients and macroangiopathy. Various reasons, like microangiopathy, poor nutrition, uncontrolled diabetes, delay in treatment, wrong or inadequate treatment by different types of healers, all account in many ways for the devastating results of these cases and at the same time makes management difficult. Patience, time, enthusiasm and above all a challenge to fight the problem is all that is required to treat this condition. Patients has taught us many important lessons like (a) Diabetic foot ulcers are deceptive - what is seen from outside more is hidden inside. (b) Once bone and joints are involved it is better to sacrifice that part early. (c) Serial amputations can reduce number of major amputations. (d) Diabetic patients can tolerate very large incision far better then any deep infection. (e) Initial antibiotic therapy should be aggressive, etc. At present our policy is aggressive debridement, 2-3 times daily dressing, not to use any corrosive in the wound, frequent follow up, and amputations when necessary. We are not yet happy with the results. We are using hydrocolloid dressing which seems to be helping granulation tissue to grow in clean cases. Local musle rotation was found helpful to save heelpad. When patients are gravely ill due to septicaemia with renal or cardiac failure an early guillotine amputation even under local anesthesia can save many life.

1879

TOPICAL APPLICATION OF GROWTH HORMONE CAN ACCELERATE WOUND HEALING IN DIABETIC FOOT ULCERS

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Diabetic foot ulcers are a serious complication of Diabetes Mellitus, carrying enormous economic and social costs for the Institutions and patients. Previous studies have demonstrated that there are GH receptors in the different structures of the skin and the promotion of local growth could be done directly by GH and throughout mRNA IGF-1 production. In order to evaluate if the topical application of r-hGH could accelerate the rate of closure of diabetic foot ulcers, the authors designed a randomised, single blind and crossed study. Fourteen patients (7 male, 7 female) with neurophatic foot ulcers matched with a control group (6 male, 4 female) for age, duration of diabetes, HbA1c, IGF-1, IGFBP3 and baseline wound's area were assigned to be treated with a solution containing r-hGH (0,2 IU/cm^2 of wound area), thrice a week. For ethical reasons foot ulcers should be crossed to the opposite group every 2 weeks if a reduction greater than 25% of the baseline area was not seen. The wounds were photographed weekly and images were submitted to computerised planimetry. The data obtained were plotted statistically. Mean rate of closure at the second week of treatment was significantly greater in the r-hGH treated group compared with the control group (34,6 ±21,1% Vs 7,8 ±24,9 %, p<0.01). Since the patients in the control group significantly crossed to the r-hGH treated group, the placebo group was reduced to 4 patients at the second week, which became subsequent statistical analysis quite difficult at the 30th day and totally impossible at the 45th day of the survey. At the 30th day the results still showed an higher mean rate of wound closure in the hGH treated group compared with the control group, although statistically not significant (37,2 ±27,5% Vs 15,4±13,6%, p=0,08). However the rate of cure of the 20 r-hGH treated ulcers was halved in relation to the ulcers treated classically in our Department (43,2 ±15,6 days Vs 90,2 ± 25,3 days). The authors concluded that topical application of r-hGH might be a valuable complement to the classical treatment of neurophatic foot ulcers, promoting the acceleration of wound closure. Larger studies will be needed to confirm these data and to evaluate if this kind of treatment could be extensible to the neuroischaemic foot.

1880

THE DIABETIC FOOT: SOME ASPECTS OF EARLY DIAGNOSIS

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Macrovascular disorders appear more frequently and proceed more difficult in patients with diabetes mellitus but the specific mechanisms of their development are studied insufficiently.We have carried out the analysis of pecualiarities of enzymes activity, levels of phospholipids and fatty acids, level of the ^{125}I-6-ketoprostaglandin F_{1a} (6-ketoPGF$_{1a}$), ^{125}I-thromboxane B_2 (TXB$_2$) in the membranes of erythrocytes, blood plasma and the lower extremities arteries' wall in patients with diabetic macrovessel complications (n=17).Simultaneously, 11 patients with atherosclerotic macrovessel complications and invariable indices of oral glucose tolerance test were examined. More considerable 'anaerobization' of metabolism has been found in vessel's tissue diabetic patients which is accompanied by increase of total lactatedehydrogenase (LDH) of activity and deplacement of isoenzymes spectrum to catode fraction.The considerable depression the activity of protein-kinase C (PK-C), Na^+,K^+-ATPase, Ca^{2+},Mg^{2+}-ATPase in RBC's membrane and arteries' wall at progressing diabetic vascular disorders indicates their role in diabetic angiopathy pathogenesis.Between decreasing of PK-C and Na^+,K^+-ATPase and Ca^{2+},Mg^{2+}-ATPase activity there exists certain correlation which in probably conditioned by Ca^{2+}-phospholipid-dependent phosphorylation of those ATPases. That may lead to disorders of the metabolism of phosphatidilnositole,the synthesis of eicosanoids and to promotion the accumulation of free calcium in the cell's cytoplasm.The progressing of the diabetic macroangiopathies cause leads to considerable increase of TXB$_2$and decrease of 6-ketoPGF$_{1a}$ levels, which may promote to vasoconstriction effect,proaggregability, blood rheologic disorders and to intensification the adhesion of blood cells to endothelium. The increase of TXB$_2$/6-ketoPGF$_{1a}$ ratio is a sensitive criterium of introduction and progress of diabetic angiopathies. The appearance and progressing of diabetic angiopathies is accompanied by the decrease of phospolipides, polyunsaturated fatty acids and the increase of free cholesterol and saturated fatty acids in the membrane erythrocyte's and in the tissues of arteries' wall. Our results support the hypothesis that disorders of the metabolism of phosphatidilinositole, the synthesis of eicosanoids may be beneficial in development of diabetes macrovascular complications.

1881

FOOT AMPUTATION IN DIABETICS: PREVENTABLE AND NONPREVENTABLE FACTORS. I. Vereanu, C. Ionescu-Tirgoviste, S. Pruna, R. Strachinariu, D. Avram and L.C.Nwabudike. Clinic of Diabetes, "Institute N.C.Paulescu" Bucharest, Romania.

This is an analysis of 473 diabetic patients admitted into the surgical department of the Cantacuzino Hospital for diabetic gangrene in two different years: 1991 (238 cases) and 1994 (235 cases) of which: 371M (78.4%) /102F(21.5%); NIDDM patients: 298 (63.0%); 49 cases (10.4%) were primary insulin-dependent and 126 (26.6%) were secondary insulin treated patients. Duration of diabetes was 9.3± 6.9 yrs. The surgical treatment was conservative in 50 cases (11.8%). Minor amputations: toes-124 cases (26.2%); transmetatarsian-82 cases (17.3%); major amputations: below knee-61 cases (12.9%); above knee-51 cases (31.9%). **Medical risk factors** encountered in order of frequency were: high perception thresholds for pain, temperature and vibration in 100% of cases; bilateral absence of Achilles tendon reflexes in 100% of cases; low nutritional blood flow (due to arteropathy or microangiopathy or sympathetic denervation of arterioles) in 100% of cases; type of diabetes (NIDDM in 63% of cases); long duration of diabetes (61%>10yrs.). **Medical risk indicators**: high blood glucose and glycated hemoglobin (87%); high (>30mg./24h) albumin excretion rate in 78% of cases. **Non-medical (socio-economic and behavioural) indicators:** low socio-economic status (84% low income earners); male gender (78%); poor educational level (73%); old age (71.7%>60yrs.); poor clinic attendance (92% without regular check-ups in the past year and 54% in the past 5yrs.); poor self-conservative behaviour leading to late presentation at the physician (lesions>10 days old at presentation in 72% of cases). **Trigger factors for leg ulceration** were divided into 3 categories: *preventable*: ill-fitting shoes, various traumas and burns in 76.7% of cases; *partially preventable*: increased pressure points, hyperkeratoses with fissuring, and fungal infections in 10.9% of cases; *non-preventable*: arteriopathic and microangiopathic ischaemia; diabetic bullae in 12.3%. Our cases were considered predominantly neuropathic in 83% of cases and predominantly vascular in 17% of cases. **Conclusion:** two-thirds of amputations are preventable if the patients are well informed and motivated and if the foot care team (diabetologist, chiropodist, nurse and surgeon) is committed.

1882

Impact of specialised care on time course and outcome of recurrent diabetic foot lesions

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Background: Avoidance of amputation in diabetics with foot ulceration by specialised care has been demonstrated recently. In the healed high risk for reulceration persists. Little is known on the influence of a diabetic foot clinic on the course of a second lesion. **Aim:** To analyse the impact of a dedicated diabetic foot clinic on time course and outcome of recurrent ulceration in diabetic patients. **Patients and methods:** 30 consecutive patients (10m/20f; average age 71,2 years; average diabetes duration 17,9 years) who relapsed after a diabetic foot lesion were analysed. Data on healing time, severity of the lesion at presentation (wagner-classification), hospitalization rate and need of amputation were sampled for both events.
Results: Average healing time was shortened to 24 days (SD 24,5) after initial presentation refering the second lesion compared to 69 days (SD 49,5) on the first (p=0,00001, Wilcoxon). Treatment duration was reduced by 65% for the whole population, 70% for pure neuropathics and 60% in critical limb ischemia respectively. Need for initial hospitalization declined from 73% to 7% (p=0,0001). Amputation had been performed in 23% of the patients before first contacting our clinic. On the first lesion 10% had to be treated by minor-amputation, on the relapses no amputation was required.
Conclusion: Specialised care by a diabetic foot clinic suceeds in improving outcome of high risk patients with recurrent foot ulceration. Treatment duration, need of hospital admission and amputation are proven to be reduced significantly.

1883

FREQUENCY OF LOWER LIMB AMPUTATIONS IN 147 TURKISH PATIENTS WITH DIABETIC FOOT SYNDROME

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There is large discrepancy regarding the incidence of diabetes-related lower limb amputations among various populations and ethnic groups. To our knowledge, there are no data regarding the frequency of lower limb amputations in Turkish diabetic population. In the present study, in a reference hospital setting, we evaluated the medical records (1986-96) of 147 patients (11 IDDM and 136 NIDDM) in respect of 195 hospitalizations due to diabetic foot ulcers. As to the predisposing factors, peripheral macrovascular disease and distal symmetric polyneuropathy were noted in 55.1% and 73.5% of the patients, respectively. History of cigarette smoking (more than 1 pack-year) was positive in 30.6%. of the patients. Medical therapy alone (i.e.,debridement and antibiotics) and lower limb amputations (below toe; minor and above toe; major) were performed in 59.2% and 40.8% of the patients, respectively. Implantation of a skin graft or island flap was performed in 6.8% of the patients in order to accelerate the healing of large defects. The most frequent isolate from deep wound cultures were found to be Staphylococcus Aureus (21.8%) and, as to the whole study period, overall mortality rate was 8.8%. Our data suggest that nearly half of the Turkish patients who admit due to diabetic foot ulcers undergo some kind of a lower limb amputation in our group. High frequency of associated risk factors such as peripheral vascular insufficiency may, at least in part, explain the high frequency of these amputations.

1884

HOW DOES DELAY IN SEEKING CARE FOR A DIABETIC FOOT LESION INFLUENCE OUTCOME?

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Objective: To identify patient characteristics that were associated with delay in seeking care for diabetic foot lesions, and to examine the independent association between time to presentation and foot lesion outcome. Design/Methods: Patients in this study were enrolled in the Diabetes Ulcer Outcome Study (DUOS), an observational study of foot ulcer treatment and outcome conducted at three VA medical centers and 3 non-VA facilities. Patients were enrolled from 1 Oct 1994 - 30 June 1996, and followed prospectively throughout the course of their treatment. A trained research assistant (RA) collected standardized information from each patient regarding demographic factors, diabetes and ulcer history, and general health. Lesion characteristics and treatment were recorded by the RA at each outpatient and inpatient visit. Patients were followed until outcome: healing, amputation, or death. Results: Three hundred two patients were enrolled in DUOS, of whom 272 (90%) provided a lesion onset date. Half of these patients reported seeking care within the first week, while 20% waited over four weeks. A forward stepwise linear regression model yielded two predictors of shorter time to presentation: education regarding proper footwear (26% shorter delay in seeking care, 95% CI 10%-41% shorter), and persons to help with foot care (compared to no helpers, each additional person resulted in 16% shorter delay, 95% CI 4%-27% shorter). Delay in seeking care was positively correlated with ulcer severity at presentation (Pearson's product moment r^2=.16, p=.004). However, after adjusting for confounding factors, a delay in seeking care was not associated with healing, or lesion duration. Conclusions: Half of all patients obtain care within 7 days of finding a foot lesion. Outpatient education regarding footwear, and help with foot care were independently associated with shorter delay in presentation for care. A longer time to obtaining care was associated with increasing ulcer severity, but there is no independent association with ulcer healing or duration.

1885

DEATH AND INSTITUTIONALIZATION FOLLOWING DIABETES-RELATED LOWER EXTREMITY AMPUTATION

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We are unaware of any report in the medical literature that has discussed risk factors for both mortality and discharge disposition following lower extremity amputation (LEA). Our aim was to report risk factors associated with in-hospital mortality and the need for institutional care in diabetics with LEAs. We abstracted data for every hospitalization for a LEA from January 1 to December 31, 1993 in 6 metropolitan statistical areas in South Texas. Amputation level was categorized as foot, leg or thigh. Discharge status categories were: home, nursing home, rehabilitation facility and death. We used the Kaplan scale of cogent comorbidities to determine the relationship of 12 disease categories and their association with discharge status. There were 1,043 LEAs in South Texas in 1993. While only 2.3% of the population was admitted from an institutional care facility, over 25% were discharged to one. Of the total population, 18.5% were discharged to a nursing home, 7.0% to a rehabilitation facility and 5.1% died within the period of hospitalization. We performed a univariate analysis. Factors with a $p<0.25$ were included in a stepwise logistic regression analysis with an α of 0.05. High level (leg or thigh) amputation, peripheral vascular disease, male gender, and absense of advanced locomotor impairment were associated with discharge to a rehabilitation facility. For discharge to a nursing home significant associations were found with: female gender, advanced age (>65), single marital status, high level amputation, and advanced cerebrovascular disease and locomotor impairment. Death following a LEA was strongly associated with female gender, high level amputation, advanced renal disease, anemia, and congestive heart failure. We conclude that a significant number of patients either die or require long-term care following a diabetes-related LEA, thus further adding to the burden of this sequela. Several clinical parameters are significantly associated with discharge status after this procedure. More prospective clinical research is needed to verify the associations and to clarify their application in practice.

1886

GAIT ANALYSIS IN DIABETIC UNILATERAL LOWER LIMB AMPUTEES.

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Unilateral amputation changes the walking pattern and could affect the foot of the remaining limb. Gait was analysed in 23 diabetic amputees (Stanmore mobility grades 4 or 5), at 8 ± 2 months (mean ± sd) and in 7 non-diabetic amputees at 10 ± 3 months after amputation. Diabetic amputees were matched with diabetic non-amputees, with and without neuropathy. Subjects walked at their own selected pace over a Kistler force plate. Maximum horizontal (posterior (P), anterior (A), medial (M) and lateral (L)) and vertical (V) ground reaction force, ground contact time (GCT) and force-time integral (FTI) were measured. For the diabetic amputees, all gait parameters, (except M) were greater in the remaining compared to artificial limb ($p<0.01$), whereas there were no differences between legs in the non-amputees. Maximum A, P, M, L and V forces (% body weight) for the artificial and remaining limb were respectively 8.6 vs 10, 7.3 vs 11.5, 7.1 vs 7.1, 1.2 vs 2.5 and 101.4 vs 105.4, GCT (msec) and FTI (Nsec) were respectively 1042.6 vs 1109.6 and 586.7 vs 701.6. FTI and GCT were greater and maximum V force were smaller in the remaining limb of amputees compared to the non-amputees ($p<0.001$). The non-diabetic amputees had significant lower FTI's than the diabetic amputees (409 and 510 vs 586 and 701 for artificial and remaining limb), although walking speed, GCT and maximum forces were not different between the two groups. Only the FTI and maximum V force were different between legs in the non-diabetic amputees, with a significant difference for FTI. The results of the non-diabetic amputees suggest greater differences between legs for the diabetic amputees. Thus, the asymmetric walking pattern and the greater force-time integral in the remaining limb of the diabetic unilateral amputees may further increase the already high risk for foot ulceration, thus exacerbating the risk for amputation of the remaining limb.

1887

DIABETES MELLITUS SURVIVAL AFTER LOWER EXTREMITY AMPUTATIONS.

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Lower Extremity Amputations (LEAs) in diabetics are an important medical social problem in Brazil. Lack of proper medical care and metabolic control, result in disability, earlier retirement of work, and preventable deaths. The aim of this paper is to determine the survival of amputee diabetic patients. This is a prognostic coorte study, involving all diabetics LEAs that occurred between January 1990 trough December 1994, living in Rio de Janeiro municipality, metropolitan region, followed up by three years after the surgical procedure. Amputation data came from all sources available to identify Diabetic cases, also including amputtee register (AR).Amputtee death certificates were reviewed in the same period for all deaths registered in the State of Rio de Janeiro (336,182). The end point for analysis was death of diabetic amputtee or the closing date of the study. Data were analyzed trough a survival table. Sex differences were compared by x^2 Mantel test. There were 419 diabetic LEAs, being 221 in men (52,7%) and 198 in women (47,9%). In this period occurred 111 deaths in diabetic patients (26,5%), the rate was 24,0% for men and 29,3% for women. Survival probability after 36 months from diabetic amputtees, was 57,9% for men,and 49,5% for women (x^2 1.39 $p>0.005$). During the first 15 days the survival probability was 90% for men, and 87% for women;after 60 days, 84% for men and 79,8% for women. However no statistically significant difference was observed in the survival probability between genders at 36 months; it was always lower for women, since the first days. These results suggest poor medical care or late demand, a finding not expected.The speed survival rate decreased in the first month of follow-up,suggesting death by infection (sepsis),even when amputtee are more prone to this kinds of risk. All deaths were reviewed except if any patient migrated to other state and died there. These results pointed out to the necessity of appropriate health care and monitoring metabolic changes proper to Diabetes Mellitus.

1888

DOES DIABETIC NEUROPATHY AFFECT THE FORCES USED DURING WALKING ?

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Neuropathy might affect the forces controlling walking, and abnormal forces may be an important cause of elevated foot pressures. The aim of the study was to investigate the effect of neuropathy on ground reaction forces. We measured vertical, medial and lateral impulses (force-time integrals) and ground contact times using a Kistler force plate mounted in an 8 meter walkway, in 15 non-diabetic controls (C), 35 diabetic controls (D), 35 diabetic patients with neuropathy (DN) 17 with previous neuropathic ulceration (DNU), and 14 patients with Charcot neuro-arthropathy (CH). The mean (sd) vertical impulses (expressed as % body weight) were C-55.4 (5.5), D-59.0 (6.1), DN-58.3 (4.9), DNU-61.5 (6.0) and CH-62.5 (7.2). D, DNU and CH were significantly greater than C ($p<0.001$). The lateral impulses were C-0.12 (0.07), D-0.09 (0.08), DN-0.10 (0.09), DNU-0.12 (0.10), CH-0.06 (0.05). CH was different from all others, but only significant for DNU and C ($p<0.05$). No differences were observed in medial impulses. The ground contact times (msec) were C-664 (71), D-712 (73), DN-702 (64), DNU-746 (69), CH-767 (88). CH and DNU were significantly longer than C ($p<0.01$). Increased impulses were mainly due to increased ground contact times and not directly attributable to neuropathy. Thus, neuropathy does not seem to alter the impulses except when combined with structural changes as in Charcot neuro-arthropathy.

1889

AMPUTATION AND REAMPUTATION OF THE DIABETIC FOOT

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We have observed that while numerous studies have focused on lower extremity amputations in general, amputations at the level of the foot are often treated as a single level or not stratified by specific site within the foot. In the majority of cases, this is due to the fact that data is gathered from secondary databases which do not provide specific documentation about amputation location. It is perhaps for this reason that we have not encountered any works in the medical literature that have reported on a large number of foot amputations, categorizing them by specific level and etiology. The purpose of this manuscript is to compare level of foot amputation by age, prevalence of arterial disease as a precipitating factor, gender, and ethnicity in persons with diabetes mellitus. We abstracted the medical records for each hospitalization for a lower extremity amputation from January 1 to December 31, 1993 in six metropolitan statistical areas (MSA's) in South Texas. Amputation level was defined by ICD-9-CM codes and were categorized as foot, leg, and thigh amputations. Foot level amputations were further subcategorized as hallux/first ray, middle, fifth, multiple digit/ray, and midfoot amputations. Only the highest amputation level for each individual was used in the analysis. Of 1043 subjects receiving a lower extremity amputation in South Texas in the year 1993, 477 received their amputation at the level of the foot. African Americans requiring a foot level amputation were at significantly higher risk to receive a midfoot-level amputation than were the rest of the population. Nearly 40% of all subjects receiving a foot-level amputation had a previous history of amputation. However, nearly 40% of subjects receiving foot amputations were without any pre or peri-admission diagnosis of peripheral arterial occlusive disease, suggesting a causal pathway dependent primarily on neuropathy. This implies that better screening of diabetic patients with appropriate risk-directed treatment at the primary care level may significantly impact the large number of preventable diabetes-related lower extremity amputations.

1890

DIABETIC NEURO-ISCHAEMIC FOOT: ELEVATED PLANTAR PRESSURES AND CONTACT TIME, BUT NO PLANTAR ULCERATION

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Diabetic neuropathic feet tend to develop ulceration on the plantar surface, whereas neuro-ischaemic feet tend to ulcerate on the margins of the foot. The reason for this difference in pattern of ulceration is not fully elucidated. High foot pressures have been implicated in the aetiology of foot ulceration, but time of pressure loading (contact time) on the soft tissues of the foot may also be important. The latter has not been studied previously in the neuro-ischaemic foot. The aim of our study was to measure dynamic barefoot pressures and contact time using the Musgrave Footprint System (Musgrave Systems Ltd.) in 15 neuropathic patients (vibration perception threshold (VPT)=40±11.5V (Mean±SD), pressure index (PI)>1), 20 neuro-ischaemic patients (VPT=39.3±10.5V, PI<0.7) and 8 non-diabetic controls (VPT=6.8±2.4V, PI>1). Peak pressures (at the first metatarsal head) were significantly higher in both the neuropathic (4.0±0.48 kg/cm^2, $p<0.05$) and neuro-ischaemic patients (3.36±0.34 kg/cm^2, $p<0.05$) as compared with non-diabetic controls (2.43±0.15 kg/cm^2). Contact time was 602.9±30.3 msec in the control group, whereas both the neuropathic (854.0±54.1 msec, $p<0.005$) and the neuro-ischaemic group (962.4±53.4 msec, $p<0.001$) showed significantly elevated contact time. There was no statistically significant difference in plantar pressures and contact times between the neuropathic and the neuro-ischaemic groups despite the trend to different sites for ulcer formation in the two groups. Further factors, in addition to high plantar pressures and prolonged contact time need to be considered in the aetiology of diabetic foot ulceration.

1891

DIABETIC FOOT ULCER - RISK FACTORS OF AMPUTATIONS

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The incidence of diabetes related amputations is an declared index of diabetological-care system in the country. Related to this a substantial reduction of amputations was declared a primary objective by WHO and IDF (St. Vincent Declaration). The purpose of this study was to estimate risk factors of amputations with advanced symptoms of diabetic foot - a neurop[athic ulcer. Analysis was made on 58 patients with symptoms of neuropathic ulcer - 42 men and 16 women aged 61 ± 14 and 67 ± 12 respectively. A mean period of diabetes lasting 12 years. All patients were hospitalised after an attempt of treatment as out-patients including paramedic procedures. In most of them (85%) the predominant symptoms was general infection, in some cases accompanied by a high fever. Diabetic retinopathy was present in 54 (94%), diabetic nephropathy in 50 cases (86%). Arterial blood flow was diminished to a different degree in all patients. In 20 cases (34%) pharmacological treatment and minor surgical procedures were not sufficient and required total amputation.. Patients who had undergone amputations were mostly males and subjects who did not attend courses on diabetic education. The group of patients with amputations in comparison with paliatively treated had more advanced signs of nephropathy (proteinuria and diminished creatinin clearance - 49.3 ml/min vs 71.2 ml/min, $p<0.001$)), neuropathy (Valsalva manoeuver) and retinopathy. It is concluded that the diabetic foot ulcer problem refers mainly to patients with microangiopathic complications. Our results also indicate that proper education of diabetics results in reduction of incidents of complication of diabetic foot and in consequence a dramatic reduction in amputations.

1892

A NOVEL METHODOLOGY TO EVALUATE THE DURABILITY OF INSOLE MATERIALS FOR HIGH RISK DIABETICS

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Viscoelastic inserts are commonly used as artificial shock absorbers to prevent neuropathic foot ulcerations by decreasing pressure on the sole of the foot. The aim of this study was to develop a rational platform for biomechanical characterizations of insole material durability. This novel methodology consists of *in vivo* gait analysis and *in vitro* bioengineering measurements, applied on 2 insole materials (PPT and Pelite) worn by 8 diabetic volunteers for 12 weeks. Gait analysis, using the Emed pedar system, was performed every 3 weeks, to obtain temporal changes in the plantar foot pressure profile. Mechanical testing (compressive modulus) was also performede at 3 week intervals. The hypothesis was that a correlation exists between *in vivo* gait analysis and *in vitro* mechanical testing. Results demonstrate significant differences in the compressive stiffness of the 2 insoles as well as the rate of change over time. PPT insoles had a compressive stiffness 125% greater than Pelite insoles at time 0. After 12 weeks, insoles were essentially the same. Peak pressures decreased by 20% for both materials over 12 weeks. Neither material showed an elevation in pressure. Comparisons of peak pressures and compressive moduli show a relationship between the two measurements, such that a drop in pressure corresponds to a drop in modulus. Good correlations were found between pressure-time integral and modulus (r^2=0.93), and energy and modulus (r^2=0.87). In conclusion, we have established a new bioengineering protocol, based on gait analysis, viscoelastic characterizations, and biomaterial analyses, to study temporal variations in the structure-function relationships of insole materials. Based on these results, it is conceivable that a quick, inexpensive biomechanical test, which is based on creep or stress relaxation responses, can be established to indirectly evaluate pressure distributions on patients' feet as well as material "wear".

1893

TOE AMPUTATION AND TISSUE DEBRIDEMENT USING LARVAE OF THE GREEN BOTTLE FLY (LUCILIA SERICATA).

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The management of gangrenous lesions in the ischaemic foot where vascular intervention is not possible or has been unsuccessful is limited. Local surgical and enzymatic debridement (varidase) may be hazardous and auto-amputation of necrotic toes may be protracted with the risk of intercurrent infection. We describe our experience with the use of the larvae (maggots) of the green bottle fly (Lucilia sericata) to excise necrotic tissue. Three diabetic patients with black gangrenous tissue including toes which had not separated for 12, 8, 6 months respectively were treated. Between 100-150 immature (1-2 mm length) larvae were applied at each treatment under a fine nylon mesh. Larvae were removed after 48-72 hours. Approximately 50% of the larvae survived, growing to between 6 and 10 mm in length. The number of treatments varied with the extent of the lesions and the survival of the larvae, but between 2 and 5 treatments, applied at weekly intervals were used. In all patients necrotic tissue and mummified toes were completely separated to reveal healthy granulation tissue. One patient found the treatment moderately uncomfortable and psychologically disturbing but despite this was willing to continue because of the obvious benefit. Thus, our initial experience suggests that larval treatment is a successful, safe and relatively quick way of amputating gangrenous toes and debriding necrotic tissue in the ischaemic foot.

1894

QUANTITATIVE ASSESSMENT OF THE PLANTAR PRESSURE DISTRIBUTION IN DIABETIC PATIENTS WITH NEUROPATHIC FOOT DISORDERS

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Pressure distribution on the sole during walking has been considered a useful indicator in the assessment and treatment of neuropathic foot disorders. **Methods:** Using the fastscan dynamic insole pedobarograph (Megascan Hannover) pedobarograms of 30 normal adult subjects with no foot disorders and 30 diabetic patients (9 IDDM and 21 NIDDM) with neuropathic foot without ulcers were recorded and the pressure distribution patterns of the metatarsal head I-III region, the metatarsal head IV-V region and the heel for each feet in both groups were analysed, also the area of peak plantar pressure on the whole sole. **Results:** Plantar pressure [g/cm^2] of the metatarsal head IV-V region in the diabetic group was significantly higher compared with the control group in both feet (Mean +/- SD: 1971+/-614 vs.1422+/-605 for the left feet, 2441+/-1832 vs.1469+/-667 for the right feet respectively). P<0,03 and 0,005. For statistical analysis we used the U-test. A non-significant higher plantar pressure was found in the metatarsal head I-III of the right foot and in the heel of both feet in both groups. The area of the maximum peak pressure [cm^2] of the whole sole of both feet was significantly smaller (90+/-21 vs. 106+/-10, p<0,004 for the left feet respective 84+/-20 vs. 106+/-20, p<0,005, for the right feet) in the diabetic group compared to the control group. **Conclusion:** In contrast to the current literature we recorded high plantar pressures especially under the metatarsal head IV-V region of diabetic patients with neuropathic foot disorders. 28 % of recorded cases of the metatarsal head IV-V region showed a plantar pressure above 2500 g/cm^2.

1895

TREATMENT OF INFECTED DIABETIC FOOT ULCERS:
TOPICAL MSI -78 VS. ORAL OFLOXACIN.

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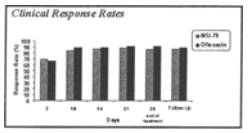
MSI-78, a novel synthetic antibiotic peptide, was evaluated for treating patients with infected diabetic foot ulcers. In a prospective, double-blind trial, 490 diabetic adults with an infected foot ulcer suitable for outpatient management were randomized to either MSI-78 1% topical cream and placebo ofloxacin, or active ofloxacin and topical placebo cream (vehicle). Infection was diagnosed by clinical criteria, i.e., purulence or ≥2 signs of inflammation. Patients with severe infections, osteomyelitis or gangrene were excluded. All ulcers were cultured at baseline and day 3 of treatment, and subsequently if clinical signs of infection persisted. Treatment lasted from 14 to 28 days, and patients were followed-up 14 days after the end of treatment. Treatment efficacy was assessed primarily by clinical response, i.e., improvement or resolution of infection, and secondarily by overall microbiological response, i.e., resolution, improvement, colonization, superinfection, failure. The intent-to-treat analysis of clinical response rates showed statistical equivalence of the two treatment regimens at all time points (95% incidence interval of the difference method), with rates ranging from 84-93% (see figure). Overall microbiological responses were similar for the two groups. The frequency of amputation or osteomyelitis of the affected site were similar for the two groups. Treatment was well tolerated in both groups, but insomnia was significantly more frequent with ofloxacin. Thus, topical MSI-78 was highly effective and offered a similar therapeutic benefit as systemic ofloxacin, with a somewhat better adverse experience profile.



1896

BIOMECHANICAL COMPARISON OF TREATMENT STRATEGIES TO REDUCE PRESSURES AT THE SITE OF NEUROPATHIC ULCERS

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High pressure on the sole of the foot has been identified as an important causative factor in the development of neuropathic ulcers in persons with diabetes mellitus. There is little scientific data to compare the effectiveness of commonly used modalities to reduce these areas of high pressure. Our aim was to compare the effectiveness of total contact casts (TCC), "half-shoes" (HS), rigid soled post-operative shoes (RSS), accommodative felt & polyethylene foam padding (AD), and removable walking casts (RWC) to reduce peak plantar foot pressures at the site of neuropathic ulcerations in diabetics. We compared the reduction in peak pressures at ulcer sites under the great toe (n=7) and ball of the foot (n=19) using the 5 treatments described above. A rubber soled canvas oxford shoe was used to establish baseline pressure values. With the Novel Pedar in-shoe pressure measurement system, data from 32 mid-gait steps was collected for each treatment. Data was analyzed by evaluation of mean pressure and percent change from the baseline. We used Tukey's Studentized Range Test for simultaneous multiple comparisons between treatments with an α =0.05. RWC's reduced plantar pressures significantly better than other treatments for ulcers under the ball of the foot. For great toe ulcers, TCC's and RWC's reduced pressure better than the other treatments. There was a consistent pattern in the ability of the devices evaluated to effectively reduce foot pressures at ulcer sites. Removable cast walkers were as effective or more effective than TCC's. Half-shoes were consistently the third most effective modality followed by accommodative dressings and post-operative shoes. Clinical studies are needed to fully evaluate healing times, complications and patient satisfaction.

AVERAGE PEAK PRESSURE &PERCENT CHANGE FROM BASELINE*

* N/cm^2 (%Δ)	RWC	TCC	HS	AD	RSS
Forefoot	7.5(85) >	12.4(76) >	17.8(66) >	27.1(48) >	33.7(36)
Hallux	4.9(79) =	3.5(85) >	8.7(64) >	15.8(34) >	22.2 (7)

1897

ANTIBIOTIC TREATMENT OF DIABETIC FOOT ULCERS, DOES IT MATTER? AN ANALYSIS OF 95 CASES

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Background: The role of antibiotics (AB) in the treatment of diabetic foot infections is unclear. **Aim**: to relate the treatment response of diabetic foot infection to microbiological findings and to AB treatment. **Materials and methods**: 95 consecutive cases of foot lesions, Wagner stage < 4, were analysed, of which routine microbiological assessment was available (swabs, isolation of up to 4 germs per case, sensitivity testing against to 30 different AB). Swabs were taken because of suspected nosocomial infection (n=51), because of high risk feet (n=31) and at random (n=13). Standard treatment, i.e. absolute pressure relief, daily wound care (necrosectomy) and sterile dressing, was applied in every case. **Setting**: outpatient diabetic foot clinic. **Results**: Of the 95 cases, 5 were sterile; 191 isolates were cultured (22 different germs, 43% staph.). 22 cases, stage <4, were treated without AB (all improved). 73/95 cases started oral AB [at random cephalexin (CE) or clindamycin (CL)] prior to knowing culture results; 64 of these 73 improved, although 20% of isolated were resistant to CE, and 16% to CL. 9/73 cases improved only after changing AB according to culture. **Conclusion**: In diabetic outpatients, low-grade foot-lesions may improve despite unsuitable (or even without any) AB in approx. 50% of cases and in 40% with CE or CL as first-line AB. Cases requiring differentiated AB treatment need to be defined.

1898

TRANSCUTANEOUS OXYGEN TENSION AND TOE PRESSURE AS PREDICTORS FOR OUTCOME OF DIABETIC FOOT ULCERS

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The possibility to predict outcome of chronic foot ulcers in diabetic patients is limited. A common method is measurement of toe blood pressure (TBP). Transcutaneous oxygen tension ($tcPO_2$) is another noninvasive method, which reflects local blood flow and oxygenation. The present study was undertaken to compare the predictive value of $tcPO_2$ and TBP for ulcer healing in patients with diabetes and chronic foot ulcers. Fortysix (34 male) patients were investigated. The age was 61±12, and diabetes duration 26±14 years. $TcPO_2$ (mmHg) was measured at the dorsum of the foot, and TBP (mmHg) with a mini cuff using laser Doppler fluxmetry as detector. The ulcer healing was evaluated by measuring the ulcer area. After a follow up time of 1 year the patients were divided into 4 groups according to outcome, i. e. increase or decrease of ulcer area, healed with intact skin, or amputated. The results are presented as x±SD.

	Amputation n=4	Increase n=8	Decrease n=15	Healed n=19
$TcPO_2$ (mmHg)	0.5±0.6*	18±14*	49±11	48±19
TBP (mmHg)	58±33	50±24	76±28	47±31

* $p<0.01$ as compared to patients with healed or decreased ulcer area.

Ten out of 12 patients, who deteriorated had $tcPO_2 < 25$ mmHg, while 31 out of 34 patients who improved had $tcPO_2 > 25$ mmHg. The sensitivity and specificity for $tcPO_2$ at 25 mmHg were 83 and 91%, respectively, while the corresponding values for TBP at 50 mmHg were 58 and 79%. $TcPO_2$ measurement provided a higher positive predictive value (77%), as compared with TBP (50%). In conclusion, the results show that $tcPO_2$ is a better predicator for ulcer healing than measurement of TBP in diabetic patients with chronic foot ulcers, and that the probability of ulcer healing is low when $tcPO_2 < 25$ mmHg.

1899

BACTERIAL FLORA OF DIABETIC FOOT INFECTIONS

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Foot infections are common and most serious complications of diabetes mellitus. The management of the infection requires deep.tissue cultures. The aim of this study was to determine to what degree each particularmicroorganism is present in the different tissues and the different grade of lesion. In 399 isolates taken from deep tissue in 217 patients we confirmed the polymicrobial nature at the diabetic foot infection. The most frequently isolated species were gram-positive (55.9%) and gram-negative (39.1%) aerobes, but only 1% anaerobes (because of technical difficulties - great distance bewteen hospital and laboratory). The most commonly isolated microorganisms were Staphylococci and Streptococci, followed by Proteus, Pseudomonas, Enterobacter, Escherichia colli and Candida species. The average number of isolates per patient was 1.8. Additionally, in 39 patients the biopsy of deep tissues and bones was performed, and similar flora was obtained.

1900

TIP-THERM: A SIMPLE SCREENING METHOD FOR TEMPERATURE SENSATION

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Loss of temperature and pain sensation is responsible for most neuropathic diabetic foot lesions. The determination of vibration sensation is often used as screening method for diabetic neuropathy. However, the relationship between the loss of vibration and the increased risk for foot lesions is for patients not directly obvious, whereas, temperature sensation is related with the contents of diabetes education programmes. Therefore, we tested the validity of Tip-Therm® as a screening method for the loss of temperature sensation in 50 patients with diabetes (age 48 ± 15, diabetes duration 16 ± 11; mean±SD). Tip-Therm® looks like a thick pencil and is made out of 2 materials with different conductivity for heat (steel and plastic) and has a defined geometry. When touching the skin a subjective temperature difference is felt. For comparision, temperature sensation was measured with a Thermocross® and vibration sensation with a graduated tuning fork. All temperature sensation were determined on the dorsum of the foot. In an earlier study, normal temperature sensation measured with a Thermocross® was <10°C temperature difference. All 26 patients with normal temperature sensation (<10°C) recognised the different subjective temperature sensation with Tip-Therm®, and 20 of the 24 patients with disturbed temperature sensation (≥10°C) were unable to recognise the difference. In conclusion, Tip-Therm® (Axon, Düsseldorf, FRG) is a simple, reliable and inexpensive screening method for the determination of a disturbed temperature sensation in diabetic neuropathy.

1901

DIABETIC FOOT: CLINICAL AND BACTERIOLOGIC ANALYSIS.

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The **aim** of the study was to analyze the clinical and microbiological characteristics of foot ulcers treated at the Out-Patient Diabetic Clinic in the last five years. **Methods:** We have reviewed 160 NIDDM patients (86 female, 74 male) with foot ulcers; mean age: 68'13 ± 11 years and diabetes duration of 26'17 ± 11 years. 67% of these patients were treated with insulin (mostly with two doses NPH). Swabs from the base of the ulcer were taken in 131 patients and cultivated in aerobic and anaerobic conditions. **Results:** Foot ulcers begin in diabetic women about 60-70 years old and later in diabetic men. There was no significant difference between men and women regarding the kind of lesion. An average of 2'26 ± 1'4 species per patient were isolated. Sixty-four percent of diabetics had two or less organisms per lesion. The most frequent isolates were: Gram-positive cocci, 50%; Gram-negative bacilli, 42%: (Enterobacteriaceae, Pseudomonas) and Gram-positive bacilli, 3'19%: (Corynebacterium). When we compared this with the species isolated in the reinfection we can state that the infection caused by gram-positive cocci (50 vs. 43'40%, p< 0'05) and gram-negative bacilli (42 vs. 41%, p< 0'05) decreases whereas the infection caused by gram-positive bacilli increases (3'19% vs. 15'09%, p<0'05). 60% of our patients were permanently discharged. **Conclusions**: The diabetic foot infection has a polimicrobian nature being the combination of gram-positive cocci and gram-negative bacilli the most common association assessed. The bacteriological features change in the reinfection. We conclude that early diagnose and treatment allow us to avoid more aggressive therapies and at the same time we are able to increase the discharge rate so that an improvement in the life quality of the patients is achieved.

1902

THE LIPSTICK TEST, AN EASY WAY TO STUDY LOCAL PRESSURE ON THE DIABETIC FOOT.

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Treatment of chronic foot ulcers (FU) in diabetes patients includes the relief of pressure at wounds and yet make it possible for the patient to walk. Due to neuropathy the patient does not feel the pressure of ill-fitting shoes or insoles. The aim of the study was to find and use an easy method to test local pressure on wounds from shoes, insoles or orthosis and the effect of non-weight-bearing corrections. About 100 patients with diabetes and FU are regularly treated at the foot clinic, Karolinska Hospital (Stockholm) by a diabetes team of physician, chiropodist, nurse and orthopaedic technician. All patients are treated with orthotic shoes with supportive insoles. At each visit the pressure from shoes and insoles at the FU is examined using the lipstick test; a piece of surgical tape is put on the bandage covering the wound. The site of the wound is marked with lipstick and a similar piece of tape is put on the sole where the wound is to be relieved, thereafter the patient puts on his shoes and walks about 10 m. If the site for pressure relief is coloured with lipstick the orthopaedic technician adjusts the sole and/or the orthotic shoe. We have found that the supportive insoles need, when they are new, several adjustments followed by repeated adjustments at least every third month. The fitness of the soles are crucial for wound healing. In conclusion the lipstick test has proved to correctly demonstrate local pressure at the wound site from ill-fitting shoes or insoles. The lipstick test is easy to perform and the cost is practically none.

1903

MICROBIOLOGY OF DIABETIC FOOT INFECTIONS

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In this study we recorded the microbiological flora of diabetic foot infections and the susceptibility of isolates to antibiotics. We studied 22 diabetic patients with lower extremity ulcers (12 neuropathic, 8 neuroischemic and 2 unclassified). Infections were followed by amputation in 5 cases (22.7%) . Swab cultures from the bottom of ulcer were obtained from all patients. *Results :* 2 cultures were sterile; most of the remaining 20 were polymicrobial (mean : 2.25 organisms). The commonest microorganism was *Staphylococcus* sp (21.7% of isolates), followed by *Pseudomonas* sp (19.5%), *E. coli* (17.3%) and anaerobes (13% of isolates). Gram (+) aerobes were isolated in 65% of positive cultures, whereas Gram (-) aerobes in 80% and anaerobes in 20%. 50% of Staphylococci were methicillin-resistant. *Staphylococcus* isolates were 100% susceptible to netilmicin and 90% to amikacin, followed by quinolones (80%), ticarcillin (70%), amoxycillin/clavulanate, co-trimoxazole (60%) and 3rd gen. cephalosporins (40%). *Pseudomonas* isolates was 88% susceptible to quinolones, followed by imipenem, aztreonam, netilmicin, amikacin (77%), ticarcillin (66%), 3rd gen. cephalosporins (44%); isolates were 100% resistant to amoxycillin/clavulanate, co-trimoxazole, 2nd gen. cephalosporins. *E. coli* isolates were 100% susceptible to ticarcillin, 3rd gen. cephalosporins, imipenem, aztreonam, quinolones, 85% to netilmicin, amikacin, 2nd gen. cephalosporins, and 57% to amoxycillin/clavulanate & co-trimoxazole. *In conclusion :* Initial empirical therapy of diabetic foot infections must be active against *staphylococcus, pseudomonas, E. coli,* and also against anaerobic bacteria.-

1904

FACTORS ASSOCIATED WITH AN ABNORMAL RESULT OF THE SEMMES-WEINSTEIN TEST FOR PERIPHERAL NEUROPATHY

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Peripheral neuropathy is a major factor in the pathogenesis of the diabetic foot which is a major source of morbidity. A predictive test is of great benefit by identifying patients at higher risk for the development of a diabetic foot. The Semmes-Weinstein monofilament test (SWM) assesses the pressure threshold and has been shown to predict development of diabetic foot. We assessed clincal and demographic factors associated with abnormal SWM. One hundred consecutive patients with insulin-dependent diabetes mellitus were investigated. Mean age: 38.2 years (range: 18-65), median duration of disease: 14 years (range: 0.1-61); 38 were male. Medical history: smoking, use of alcohol, hypaesthesia and/or hyperaesthesia. Physical examination: weight, length, blood pressure, Achilles tendon reflex (ATR), pedal vibration threshold. Laboratory variables: mean HbA1c during the previous year, last HbA1c, plasma lipids, creatinine and urinary albumine/creatinine ratio (UAR). Presence of nephropathy, proliferative retinopathy, and hypertension were defined using preset criteria. Variables were either dichotomous or dichotomised using median or established cut-off points. The results from two legs were pooled (200 feet examined). The filament 5.07 was used and applied to 7 plantar sites. SWM was abnormal if the filament was not felt on ≥1 site. The associations were tested with the Chi square test; variables with a $p<0.05$ were entered in a logistic regression model. Abnormal SWM was associated with younger age (≤36 years; $p<0.001$), longer duration of disease (>14 years; $p<0.001$), hypertension ($p=0.02$), nephropathy ($p<0.001$), retinopathy ($p<0.001$), presence of both hyperaesthesia and hypaesthesia ($p<0.001$), absence of ATR ($p<0.001$), higher plasma creatinine (≥75 μmol/l; $p=0.014$) and elevated UAR (UAR >3.0; $p=0.046$). Older age ($p=0.047$), longer duration of disease ($p=0.048$), presence of hyperaesthesia and hypaesthesia ($p=0.047$) and absence of ATR ($p=0.025$) were independently associated with abnormal SWM. Thus, duration of disease, simultaneous presence of negative and positive neuropathic symptoms and the absence of the Achilles tendon reflex are associated with abnormal SWM, indicating decreased protective sensitivity and increased chance of ulceration.

1905

BACTERIAL INFECTIONS IN DIABETIC FOOT : INDIAN EXPERIENCE.

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Bacterial infections in foot lesions in diabetes greatly increase morbidity. We studied infections in diabetic foot in 270 (M = 212; F = 58), age (61.78 ± 10.85 yr). Samples for culture were collected during surgery from incised tissue or drainage material. Results showed presence of a mixed bacterial flora. A total of 551 aerobic and 224 anaerobic microorganisms were isolated with an average of > 3 per sample. Aerobic organisms included Staphylococcus (aureus, intermedius, epidermidis, haemolyticans, homonis) 42.06%, Streptococcus (pneumoniae, pyogenes, viridans) 34.13%, Proteus (mirabilis, vulgaris) 36.51%, Klebsiella (pneumoniae, pneumoniae-ozaene, oxytoca) 30.56%, Enterobactor (aerogenes, agglomerans, cloacae) 22.22%, E-coli 19.44%, Pseudomonas (aeruginosa, maltophilia, putrefaciens) 11.90%. Anaerobic organisms included Anaerobic Streptococci 34.13%, Peptostreptococcus (anaerobius, magnus, micros) 29.76%, Bacteroides (fragilis, melaninogenicus) 18.25% and MRSA 4.71%. Gram-positive aerobes were sensitive to Ampicillin-Sulbactum 91.33%, Quinolones (Ciprofloxacin, Ofloxacin) 93.16 - 94.53% and Cephalosporins (Cefotaxime, Ceftazidime, Ceftriazone and Cefaperazone) 90.42 - 91.79%. Gram-positive anaerobes were sensitive to Vancomycin 92%, Piepracillin 86.3%, Ampicillin-Sulbactum 75.4% and Cephalosporins 84 - 91.4%. Gram-negative aerobes were comparatively more resistant, they were sensitive to Amikacin 95.15%, Quinolones 74.78 - 82.68% and Cephalosporins 59.58 - 79.56%. Gram-negative anaerobes were sensitive to Metronidazole 91.9%, Cephalosporins 79.6 - 89.8% and Ampicillin-Sulbactum 63.3%. Proper isolation and identification of microorganisms and use of appropriate therapeutic agent can be of great value in the management of diabetic foot lesions.

1906

DECREASE OF PAIN SENSATION AS MEASURED BY NOCICEPTIVE FLEXION REFLEXE IN DIABETIC POLYNEUROPATHY.

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Foot ulcers are the main complication of diabetic polyneuropathy (PNP) and could lead to amputations. They are associated with loss of pain perception, wihch is now objectivable with the use of R III reflex threshold (R III). This was measured in 24 normal subjects 32 ± 9 years old, and in 50 diabetics, 52 ± 12 years old, suffering of NDDM (39) or IDDM (11) of 15 ± 9 years duration. They were distributed in 5 groups following clinical PNP status (G1 = no PNP, G5 : history of plantar ulcer and/or toe amputation). R III Threshold is lower in normal subject (10,0 ± 1,21 mA), than in all diabetic groups ($p < 0,04$). Among diabetics, only group 5 is higher (39,3 ± 8,2 mA) than the others ($p < 0,001$). There is no significant difference between the others. R III is correlated to Thompson's sensitivo-reflex score ($p < 0,001$), the amplitude of sural nerve action potential ($p < 0,01$), tuning fork graduated index ($p < 0,05$) but not to sympathetic cutaneous response ($p = 0,10$). Increased R III threshold is in good relation with severe PNP with attendant foot lesions, but cannot be used alone, as a fully reliable predictor of « at risk foot », before occurrence of foot lesions. The PNP pronostic seems better related to clinical sensitivo-motor score ($F = 19,9$, $p < 0,001$) and to tuning fork vibratory perception ($F = 10,3$, $P < 0,001$) than to R III ($F = 4,4$, $p < 0,005$), amplitude of sural nerve action pentotial ($F = 3,85$, $p = 0,01$) or sympathetic cutaneous response ($F = 3,25$, $p = 0,02$). R III threshold measure is interesting to study the Aδ fibers in diabetic PNP, but its clinical usefulness is to be assessed

1907

MICROBIOLOGICAL FINDINGS OF DIABETIC FOOT INFECTIONS IN HUNGARY

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Diabetic foot infections have well known microbiological characteristics. As infections can vary depending upon geographical location and as such data have not been collected from the Hungarian diabetic population microbiological investigations were carried out in 60 Hungarian diabetic patients (21 women, 39 men; age 43 - 89 years). Specimens from infected foot lesions were taken for aerobic and anaerobic culturing immediately after the patient's hospitalisation. Two lesions proved to be sterile. *Candida* alone was identified in one patient. In the remaining 57 patients a total of 134 bacterial strains was isolated and, in addition, *Candida* species were identified in 3 cultures. The average number of species per lesion was established at 2.38. Of the 57 patients 45 were positive for only aerobic isolates whereas the cultures of 12 patients were positive for both aerobic and anaerobic isolates. The most common gram-positive aerobic organisms were *Staphylococcus aureus* (24), *Streptococcus* species (16) and *Enterococcus* (13) while *E. coli*, *Enterobacter cloacae* and *Proteus mirabilis* predominated within the aerobic Gram-negative enterics. In anti-microbial susceptibility testing amoxicillin-clavulanate proved to be the most effective. The first Hungarian microbiological survey corroborated that diabetic foot infections have a polymicrobial nature regardless of geographic occurrence.

1908

.INSENSIBILITY TO THE 5.07 MONOFILAMENT IN NON-INSULIN-DEPENDENT DIABETICS Llussà J., Tomàs P., Méndez A., Gimbert RM., Bundó M., CanoJF. and GedapS Group. Catalan Family and Community Medicine Society. Barcelona (Spain)

Background: Diabetic foot is a prioritary problem in Non-Insulin-Dependent Diabetic Patients (NIDDM) and its precocious detection and prevention should be pioritary in Primary Health Care (PHC).**Aims:** Estimation of the prevalence of 5.07 monofilament insensibility in a non selected NIDDM population. Measurement of the interexplorator concordance , in order to prove its reproducibility.**Methods:** Sixty-two NIDDM from five urban PHC Centres were included, in a transversal multicentric design study. Twenty-four men (38.7%), and 38 female (61.3%), aged between 50 and 70 years (Mean age 67 years), with more than five years from the diagnosis , and without previous feet ulcers/amputations, were tested with the 5.07 monofilament in three points of each foot. The test was repeated at the insensible points, and the patient was considered "insensible to the monofilament" when one or more points were insensible twice. A trained explorator instructed four doctors and a nurse in order to repeat the test, and the concordance between both explorations was measured by the Kappa index.**Results:** Sixteen patients were insensible to the monofilament in the second exploration (25.8% CI95%:14.4-36.7). The Kappa index between the first exploration (performed by different professionals in PHC Centres) and the second (single trained explorator) was 64.5% (CI: 43.12-85.43).**Conclusions:** The estimated insensibility prevalence to the 5.07 monofilament is slightly higher than the found in the medical literature. The test is useful and easily applicable to PHC. The obtained concordance has been acceptable. but it needs a minimal training referred to the exploration itself as well as to the way of interrogating the patient.Further studies are needed to validate the 5.07 monofilament as a screening test in the detection of NIDDP at risk for diabetic foot.

1909

MICROBIOLOGY OF INFECTED DIABETIC FOOT. L. Mancini, G. Marra, C. Saponara, F. Toscanella, P. Magnani, D. Pitocco, Ghirlanda G. Department of Internal Medicine and Geriatry. Rome, Italy.

Infections of the lower extremity resulting from skin ulceration in the patients with diabetes mellitus cause significant disability and morbidity, and are expensive in term of medical care.The microbiology of an infected foot ulcer is complex because of the presence of both aerobic and anaerobic Gram-negative bacilli and Gram-positive cocci. Aim of our study is to describe the bacterial population in infected foot lesions of 51 consecutive diabetic outpatients referred to our Foot Clinic between January '95 and June '96. Eighty-five cultures were performed on the specimens obtained from foot ulcers, abscesses, bullae, osteomyelitis.We isolated 115 bacteriological species; Gram-positive cocci were the patogens most common isolated (76.5%;n=88). *Staphylococci* were the microbiological agents most frequent(46%;n=53), particularly *Staph Aureus* was isolated in 19.1%(n=22), *Staph Epidermidis* in 12.1%(n=14), *Staph Simulans* in 10.4% (n=14).*Streptococci* were isolated in 20% (n=23), particularly *Enterococci* in 12,1%. Gram-negative bacilli were isolated in 23.5% (n=27), particularly *Pseudomonas Aeruginosa* (6%; n=7). The 45.8% (n=39) of the cultures perfomed were polimicrobial. *Staphylococci* were observed in 69.5% (n=32) of the monomicrobial infections, particularly *S. Aureus* in 38.4% (n=15). *Streptococci* were observed in 48% (n=19) , Gram neg in 38.4% (n=15) of the polimicrobial infections. The association between *Streptococcus* and *Staphylococcus* was the microbiological association most frequently observed (38.4%;n=15).There was only two cases of anaerobical species observed(1.7%). Our study shows that *Staphylococci* are the bacteriological species more present in infected diabetic foot and are the only pathogen in two-third of monomicrobial infections. Streptococcical species were the pathogen more present in polimicrobial infections, particularly with *Staphylococci*, supposing a sinergistic action of these species. We observed few anaerobic species, probably because outpatients clinic shows a specific infection pattern, different from that seen in hospitalized patients.

1910

MANAGEMENT OF DIABETIC FOOT:COMPARATIVE ANALYSIS IN DIFFERENT STAGES.

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To study bacteriological spectrum & sensitivity in advanced foot lesion, Comparison of new therapy "Collagenase(Salutyl) & Tetrachlorodecaoxide (Oxo-ferrin) Solution" with traditional methods. 38 cases with diabetic foot ulcers(6-14cm) in various stages slough, suppuration, pregangrene were vigorously treated by division into controls n-12 & study group n-26 & result is encouraging in cure & lowering morbidity. Good glycaemic control was kept. Both groups were investigated alike & pus/exudate culture was done. Controls underwent mechanical debridement Saline wash & antiseptic dressing with appropriate antibiotic. n-26 had Saline wash, Salutyl ointment for slough removal followed by combined Oxoferrin soaked twice daily dressing from day 4-7 till end. Treatment documented with serial photographs, measurements & ful thickness biopsy showing increased granulation infiltrated cells, capillary nos, & tissue strength. 19 pts. had complete healing with epithelialisation by 28 days,3 pts. by 37 days & 2 needed graft 2 pts. failed due to concomitant disease 5 of n-12 had partial healing in 45 days followed by graft 2 had gangrene & underwent amputation. Study was cost effective, statistically ($p<0.05$), clinically & epidemiologically successful & significant for a third world country.

1911

THE PREVALENCE OF FUNGAL INFECTION IN DIABETICS

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The Diabetic Foot Disease is one major complication associated with diabetes. Regular examination of the feet, identifying diabetics at risk for foot ulceration and the treatment of the predisposing factors such as peripheral atherosclerosis, neuropathy and abnormal pressure loading and increased susceptibility to infection make up the preventative and educational strategy in the management of the diabetic foot

We examined the feet of 105 consecutive diabetics aged 21 to 58 years, who took part in an education course at the Finnish Diabetes Center in Tampere in autumn 1996. If any clinical lesions were noticed in the nails,in the toe webs or in the skin of the soles specimens for mycological examination. were taken.

In all 96 samples from 68 patients were cultured. The prevalence in 105 diabetics of any lesions in the nails was 40%, in toe webs 40 % and in the skin of the soles 12 %. The real prevalence of dermatophyte infection was 12.4 % for nails, 8.6 % for toe webs and 11% for skin. The prevalence of yeast infection was 7.6 % for nails, 1% for toe webs and 1 % for skin. 3 patients had both dermatophyte and yeast infection in nail specimen and 1 in skin specimen. The fungal infection rate was therefore 17.1 % for the nails and 19 % for the skin and overall 26.6 % for both .

1912

RECURRENT VS.NON-RECURRENT DIABETIC FOOT ULCER DISEASE: IMPACT OF PHYSICAL ACTIVITY

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Background: Increased plantar pressure, inappropriate footwear, and poor foot care are established risk factors for neuropathic diabetic foot ulcer disease(NDFUD). Is physical activity (e.g. walking) also a risk factor? Aim: To assess the average physical activity in patients with recurrent vs. non-recurrent NDFUD (ulcer vs. no ulcer within 12 months prior to the study). Patients and methods: 60 diabetic patients were studied(48% females,28% IDDM,72% NIDDM,mean(SD) age 61(10) yrs,duration of diabetes 23(11) yrs), with non-recurrent NDFUD (n=20),and with recurrent NDFUD(n=20). 20 diabetic control patients without any NDFUD, and 20 non-diabetic control subjects were also studied, all matched for sex and age. Physical activity was recorded in arbitrary units(AU) by an accelerometer(Suzuken Ltd. Japan) worn at the hip for 7 consecutive days. Results: patients with recurrent NDFUD displayed similar activity (24(8)AU) as control subjects, either diabetic (23(12)AU),or healthy(27(9)AU)). However, patients with non-recurrent NDFUD exhibited significantly less physical activity (18 (11)AU,$p<0.05$ vs.healthy controls). Conclusions: patients with non-recurrent NDFUD were the least active of all study groups. Thus, low (habitual) physical activity may protect against recurrent NDFUD.

1913

DIAGNOSIS OF OSTEOMYELITIS IN THE DIABETIC FOOT WITH A COMBINED BONE AND HMPAO LEUCOCYTES SCINTIGRAPHIES.

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The specificity of routine radiography and bone scintigraphy are too low to reliably detect foot osteomyelitis in diabetic patients because of osteoarthropathy, previous skin ulcers or postoperative healing. On the contrary, the accumulation of leucocytes is more specific of an infectious process. 27 diabetic patients with possible foot osteomyelitis (37 foot ulcers) were included in a prospective study with standart radiography, 99mTC MDP three-phase bone scintigraphy and 99mTc-HMPAO leucocytes scintigraphy performed within four days. The congruence of abnormal focal uptake of HMPAO and MDP was considered as positive. The final diagnostic was based on the following criteria : a) osteomyelitis if standard radiography and/or surgical biopsy (bacteriology and/or histology) were positive ; b) absence of osteomyelitis if two successive radiographies within 3 weeks were negative and a good evolution was observed without antiobiotherapy. Results a) 20 ulcerations were associated with osteomyelitis : the combined scintigraphy was positive in 18 cases (2 false-negative) including 7 lesions one to two weeks before the radiological abnormalities. At the end of a eight-week antibiotherapy a second combined scintigraphy was performed in 9 patients (10 ulcerations) : all sites were negative for HMPAO leucocytes. b) Of the 17 cases without osteomyelitis, the combined scintigraphy was negative in 16 (one false positive) including 13 cases with focal uptake of HMPAO leucocytes without focal uptake of MDP (soft tissue infection). Conclusion : The combined bone scintigraphy and HMPAO leucocytes scintigraphy with a very good spatial resolution is a very sensitive (90%) and very specific (94%) method to detect osteomyelitis in the diabetic foot ulcerations.

1914

EFFECT OF INDUSTRIALLY PRODUCED TOVEY-LIKE FOOTWEAR ON DIABETIC FOOT ULCER RECURRENCE.

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Background: Ordinary fashion footwear is an important trigger of diabetic foot ulcer disease. Bespoke medical footwear, hand-made with shock-absorbing insole (at least 30% reduction of peak plantar pressures),and soft upper (no toe cap!), as suggested 1984 by F.Tovey,has proven to prevent foot ulcer recurrence (FUR).Aim: To assess whether ready-made medical footwear can have similar effects. Patients and methods: 46 patients (28% females,83%NIDDM,17%IDDM; median (95%CI) age 63(60-66) years, without relevant foot deformities were supplied with <3 pairs of ready-made medical shoes (Diabeticus/Thanner, FRG) after healing of a foot ulcer. They were followed-up for 12(10-14) months. Results: There were 20 patients with FUR after 8(6-10) months, in 16 of whom FURs were related to footwear. Patients without footwear related FUR (n=30) had worn the medical shoes for 50 (20-75)% of the day; patients with footwear-related FUR (n=16) had worn the medical shoes for 10(0-30)%, and ordinary footwear for the rest of the day ($p<0.05$). Conclusion: Ready-made Tovey-like shoes, when worn most of the day, satisfactorily protect undeformed diabetic feet against footwear related FUR.

1915

DIABETIC FOOT OSTEOMYELITIS : PROSPECTIVE EVALUATION WITH ^{99m}Tc - LEUKOCYTE / BONE SCINTIGRAPHY AND MR IMAGING.

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Diagnostic of foot osteomyelitis among diabetic patients may be extremely difficult because of coexistent disease processes such as chronic oedema, soft-tissue infection and neuroosteoarthropathy. In this prospective study, we compare the bone scan with ^{99m}Tc leukocyte scintigraphy and MR imaging to diagnostic osteomyelitis in twenty diabetic foot neuropathic ulcerations with forteen osteoarthropathy. The diagnostic of osteomyelitis is made clinically or with plain film if it was evident (bone involved) or with the follow up without antibiotherapy if the imaging process tested are discordant. Osteomyelitis is present in thirteen diabetic foot ulcerations, only seven are suspected clinically. High signal intensity in the bone marrow is demonstrated on T1 and T2 fat-saturation MR imagings with Gadolinium enhancement, in twelve cases who developed osteomyelitis (sensitivity 92,6%). Bone scan with ^{99m}Tc leucocyte scintigraphy is effective in 9 cases (sensitivity 69.2%). Among the seven diabetic foot ulcerations without osteomyelitis, six have no element for this diagnostic in MR imaging and in bone scan with ^{99m}Tc leucocyte scintigraphy. One false positive result reduce the specificity to 85,7% for both methods. Osteoarthropathy did not influence these results. In conclusion, our study suggests that MR imaging appears to be the most sensitive method for diagnosing osteomyelitis in diabetic foot neuropathic ulcerations with or without osteoarthropathy. The specificity is identical with bone scan with ^{99m}Tc leukocyte scintigraphy and MR imaging. Furthers studies are necessary to confirm these preliminary results especially in diabetic foot ulcerations with osteoarthropathy.

1916

THE FOOT-EYE-KIDNEY DIABETIC SYNDROME

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During the last 4 years we have been using the nemotecnic clinical classification of the diabetic foot PATON (Big Foot in English). This approach allow our health care system to have a clear idea of the foot's rish of the diabetic and handle is by a stratify system that cover all the country. This classification is very simple and can be done by any health care profesional.

Frecuently we found that the high risk (red) foot has also a severe diabetic retinopathy and nephropathy. We call this the PIORI Syndrome by its spanish initial for foot, eye and kidney.

In this study we wanted to show the prevalence of diabetic retinopathy and nephropathy in the patients with high (red), moderate (yellow) and low (green) risk feet.

We take at randon from our Out-Patient Diabetic Clinic, 10 patients with low rish diabetic foot accordind with the PATON classification, 10 moderate risk and 10 high risk feet. All with more tan 15 year of been knowing as diabetics. The patients were seen by an oftalmologist and fluorescein angiogram were practiced. A 24 hrs urine was collected and the nephropathy was defined as the excretion of more than 300 mg of albumin.

Table I. Prevalence of nephropathy and retinopathy

	Green	Yellow	Red
Nephropathy	3	4	10
Proliferative Retinopathy	0	0	9

No difference was found in term of year of evolution, metabolic control, age, sex, BMI and type of diabetes.

We conclude that clearly a Syndrome that involved the foot the eye and the kidney is identified. The PATON classification allow a selection of the severity of all the microangiopathic lessions in the diabetic.

1917

DIABETIC NEUROPATHY AND OSTEOPOROSIS IN THE DIABETIC FOOT

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Diabetic foot is more vulnerable to the calcaneus avulsion fracture than non-diabetic foot The complications of diabetes mellitus, neuropathy, vascular insuficiency and Charcot arthropathy may accelerate the osteoporosis of the calcaneus of diabetic patients. The aim of this study was to evaluate whether the severity of the diabetic complications correlate with the bone mineral density of calcaneus bone. We studied the correlation of bone mineral density of diabetic patient and the severity of their diabetic complications. We recruited 24 diabetic and 20 non-diabetic control women with age matching. We measured bone mineral density of femur and bone mineral density of calcaneus, nerve conduction velocity and other biochemical parameters. We evaluated the severity of their retinopathy and nephropathy. also. We classified study population into mild, moderate and severe group according to their neuropathic severity. Bone mineral density of calcaneus were 0 612 ± 0.19, 0 565 ± 0.13 and 0.432 ± 0.14 (g/cm^2) in mild, moderate and severe group($p < 0\ 05$). Severely complicated group had more retinopathy and nephropathy. No statistically significant differences were found in the age, osteocalcin, deoxypyridinoline, alkaline phosphatase, BMI and HbA_1C between groups. In conclusion, the severity of the diabetic complications is correlated with degree of the osteoporosis of the diabetic foot bone and may be the risk factor of foot fracture in the diabetic foot.

1918

TRANSCUTANEOUS OXYGEN TENSION - REPRODUCIBILITY IN DIABETIC PATIENTS WITH PERIPHERAL ARTERIAL OCCLUSIVE DISEASE

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Transcutaneous oxygen tension ($tcPO_2$) is a non invasive method, which reflects local blood flow and oxygenation. The aim of the present study was to evaluate the reproducibility of $tcPO_2$ measurements in diabetic patients with peripheral arterial occlusive disease (PAOD). Ten male diabetic patients with PAOD were investigated (toe/arm blood pressure index ≤ 0.7). Age was 65±13 (mean±SD) years, and diabetes duration 33±6 years. Toe blood pressure (mmHg) and $tcPO_2$ (mmHg) were measured daily for 3 days. Toe blood pressure was measured with a mini cuff and laser Doppler fluxmetry as detector. $TcPO_2$ was measured at a reference point (I_2 dx), the dorsum of the foot, and at the base of dig I-II. The results showed that $tcPO_2$ at I_2 dx (59±8 mmHg) was higher ($p<0.001$), as compared to the dorsum of the foot (21±20 mm Hg) and base of dig I-II (24±20 mmHg). Significant correlations were seen between $tcPO_2$ day I-II-III at each measuring site: (I_2 dx, $r=0.83$; $p<0.01$); dorsum of the foot, $r=0.95$; $p=0.0001$; base of dig I-II, $r=0.95$; $p=0.0001$). No systemic differences were observed between the three $tcPO_2$ measurements with paired t-test ($p=0.35$-0.96). Neither were there any differences between the three toe blood pressure determinations (toe blood pressure 49±39 mmHg). In conclusion, the present study showed that the reproducibility of $tcPO_2$ and toe blood pressure are good in diabetic patients with PAOD.

1919

TREATMENT OF CHARCOT'S ARTHROPATHY : MEASUREMENT OF RESPONSE BY QUANTITATIVE BONE SCANNING

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Charcot's arthropathy is characterised by gross disorganisation of the foot resulting in intractable neuropathic ulceration. Immobilisation of the affected joint by casting gives the impression of reduced disease activity, but scientific evaluation of treatment options is rarely made due to a lack of suitable objective assessment methods. In this study we investigated quantitative bone scanning (QBS) as a method for monitoring response to casting. Nine patients (mean age 55.5±12.3yr, duration 9.9± 5.5yr and duration of arthropathy before presentation 5.0±5.6mth) had their affected foot cast. Foot skin temperature measured by a Thermistor (Takara Instruments, Japan) showed that the affected foot was significantly hotter than the control foot at baseline (3.4±2.0°C,z=2.3;p=0.02) and 3 months after application (2.5±2.0° C,z=2.2;p=0.03) but not after 6 months (1.3±1.0°C). Patients underwent QBS, at baseline, 3 and 6 months after casting, with 99mTc-labelled diphosphonates to quantitate isotope uptake in an accurately measured area. Repeated measures (ANOVA) of the absolute Tc uptake, and the ratio of the affected vs the unaffected foot, for dynamic (blood flow) and delayed (bone uptake) phases of QBS are shown in the Table. Strong correlation exists between changes in dynamic and delayed phases isotope uptake at both 3 and 6 months ($r=0.8$;$p<0.02$).

	Tc Uptake (%) of affected foot	Dynamic phase (affected/unaffected)	Delayed phase (affected/unaffected)
Baseline	2.0±0.8	2.4 ± 1.5	2.2 ± 1.0
3 months	1.5±0.8 *	1.7 ± 0.8*	1.6 ± 0.4*
6 months	1.4±0.8 *	1.4 ± 0.3*	1.5 ± 0.5

**different to baseline* $p< 0.05$

These results indicate casting produces a decrease in skin temperature and bone isotope uptake of the affected foot in Charcot's Arthropathy. Whether the changes represent an intrinsic improvement in bone disease or reflect a decrease in blood flow to the limb through immobilisation by casting requires further investigation. QBS provides an objective measurement of activity in Charcot's Arthropathy.

1920

DEMONSTRATION OF INSULIN RESISTANCE IN ARTERIOSCLEROSIS OBLITERANS AND PARTIAL IMPROVEMENT BY PROSTAGLANDIN.

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To determine whether insulin resistance is present in arteriosclerosis obliterans (ASO) subjects, we performed both 75g-oral glucose tolerance test (OGTT) and insulin sensitivity test in non obese ,non diabetic and non hypertensive ASO subjects. Furthermore in order to investigate whether administration of prostaglandin E1 derivative(limaprost,Ono, Japan) affects insulin sensitivity in ASO subjects, insulin sensitivity test was performed before and after limaprost (30 μ g/day) administration. Insulin sensitivity was determined by the Steady-State Plasma Glucose (SSPG) method using sandostatin. SSPG significantly elevated in ASO subjects over control(8.1±0.7 vs 5.7±0.4mmol/L,mean±SE,$P<0.01$,t-test) and insulin resistance in ASO was noted. Area under the curve of immunoreactive insulin during 75g-OGTT tended to be higher,but,not significant and the degree of compensatory hyperinsulinemia was not so strong as coronary artery disease. SSPG significantly reduced after limaprost administration (from 10.6±0.7 to 8.5±0.6mmol/L, $P<0.05$), indicating the improvement of insulin sensitivity. These results indicate that insulin resistance is demonstrated in ASO, however, compensated hyperinsulinemia has not been observed. Limaprost administration has improved insulin sensitivity for glucose metabolism.

1921

IS CHARCOT OSTEOARTHROPATHY OF THE DIABETIC FOOT RELATED TO OSTEOPENIA?

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Background: Charcot osteoarthropathy (COA) of the diabetic foot is believed to result from (neurotrophic ?) osteopenia; however, prompt restitution of destroyed bone structures following non-weight-bearing contradicts this view. **Aim:** to assess bone density in the vincinity (distal tibia) of chronic "healed" diabetic COA. **Patients and methods:** 10 women and 12 men were studied, 6 with bilateral,and 16 with unilateral diabetic COA of the feet (n=28 feet with COA).For control purposes, 26 legs of 13 healthy persons matched for age, sex, and body weight were studied. Bone mineral density(BMD,g/cm²) was assessed by photon absorptiometry. **Results:** BMD was 0.98 (SD 0.15) in 16 legs with COA, versus 1.03 (SD 0.18)in 16 unaffected legs($p<0.05$.).In all 28 legs with COA,BMD was 1.09(SD 0.25). In the controls,BMD was 1.05(SD 0.31)in the right,and 1.14(SD 0.14) in the left leg, respectively (n.s.).**Conclusion:** No difference was found by overall comparison of BMD in COA and control legs. In legs with COA, BMD was diminished by $\leq$ 5% compared to legs without COA within the same diabetic patients; this is supposed to be the consequence of therapeutic pressure relief rather than the cause of COA.

1922

ENDOVASCULAR REVASCULARIZATION BY MECHANICAL ATHERECTOMY IN DIABETIC FOOT.

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Diabetes is the leading cause of amputations in developed country. Owing to the infrapopliteal site of arterial occlusion classical percutaneous balloon angioplasty cannot be performed. Revascularization is achieved mainly by distal by-passes with autologous saphenous vein, that cannot always be performed for general or local contraindications. Mechanical atherectomy with rotational Auth's device appears to be a particularly suitable technique in the treatment of distal arterial occlusions. We studied 66 patients with type 2 diabetes that were referred to our department for revascularization or amputation because of ischaemic trophic lesions of the foot. Digital subtraction angiography showed an occlusion of the femoral superficial artery in 18 patients, while popliteal and tibial arteries were occluded in 29 patients and significantly stenotic in 19 patients. Four patients were not treated due to the length of the obstruction and/or the lack of a suitable pedal circulation. Mechanical atherectomy was performed in 62 patients without any significant side effect and resulted in immediate patency of the treated arteries. In 57 patients vessels have been patent for a mean follow-up period of 29 months (range 4-49 months) with a limb salvage rate of 95%. One patient had occlusion of the treated segment after 3 months without recurrence of necrosis. Two patients were amputated, one did not improve because of a poor run-off of the pedal circulation and one had a severe infection. We conclude that mechanical atherectomy is a safe and effective endoluminal recanalization procedure in diabetic patients with limb threatening ischaemia due to infrainguinal arterial disease, mainly in those with general or local contraindications to by-pass surgery.

1923

EFFICACY OF DIFFERENT SHOES AND INSOLES IN REDUCING PLANTAR PRESSURES IN DIABETIC NEUROPHATIC PATIENTS

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Diabetic patients with peripheral neuropathy develope foot ulcerations in areas of high plantar pressure.The recent development of small thin sensors has allowed the reliable measurement of in-shoe foot pressures. We have utilized this device (Dinatto by BT Italy) to evaluate the effect of various manufactured shoes and different insoles (Podiabetes by Buratto, Italy) in reducing plantar pressures in diabetic neuropathic patients. 9 diabetic patients (7M/2F) age 61±5 yrs, duration of disease 13±8yrs, VPT 35±7mV were included in this study. Mean plantar pressures (N/cm²) under metatarsal heads in static (SMP) and dinamic (DMP) condition and peak plantar pressures in dinamic condition (DPP) were evaluated in the following experimental conditions: Barefoot (B), with the own old shoes (OS), with two types of manufactured shoes designed with super depth in soft thermoformable leather and flexible soles (Type 1) or rigid with a rocker bottom (Type 2) completed with three different insoles : a single layer of Alcapy (3mm) (Type A); a layer of alcapy (3mm) + a layer of alcaform 167 (5mm) (Type B); a customized insole made by alcapi+ alcaform 167 and 200 (Type C).

	B	OS	1A	1B	1C	2A	2B	2C
SMP	4.6±3	4.6±2.6	4.6±2.3	4.1±2.2	4.3±2.9	3.4±2*°	3±1.5*°	2.8±1*°
DMP	17±7	20±11*	17±8°	16±7°	14±7°	14±7°	12±4*°	12±5*°
DPP	269±63	328±84*	291±92°	269±55°	265±59°	264±77°	249±49°	250±42°

* $p<0.01$ vs B; ° $p<0.01$ vs OS

Our data show the ability of manufactured shoes and insoles in reducing plantar pressures. The different results obtained with the different solutions suggest that the perscription of shoes and insoles may be tailored to the specific needs of diabetic patients with peripheral neuropathy and high plantar pressures.

1924

EVALUATION OF ANGIOGRAHPIC FINDINGS OF THE LOWER LIMB IN DIABETICS TREATED AT A FOOT CLINIC - A 3-YEAR RETROSPECTIVE STUDY

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The aim of the study was to evaluate retrospectively angiographic findings of the lower limbs of diabetics, treated at a diabetic foot clinic, in terms of the indications, outcome, future therapeutic strategy, and the incidence of complications over a 3-year period. A retrospective study was conducted to evaluate 71 diabetics (17 and 54 with Type-1 and 2 diabetes, respectively, 47 males, 24 females, with a mean age of 60 ± 12 years and diabetes duration of 16 ± 9 years) undergoing angiography (AG) of the lower limb End-stage diabetic nephropathy was present in 16 pts (234%), with 10 treated by hemodialysis. The criteria assessed included indications for AG, angiographic findings, future therapeutic strategy, and the incidence of immediate complications post-AG in a total of 89 limbs. Angiographic findings were evaluated as positive in the presence of arterial occlusion or a stenosis > 50%. The examination was performed using intra-arterial digital subtraction angiography with a non-ionic contrast medium during a short hospitalization in a diabetes center. In 49 pts (69%) the indications for examination included defects involving the leg (gangrene 39%, non-healing ulcers 30%), resting pain in 9 pts (13%), claudications in 8 pts (11%), and elective amputation in 5 pts (7%). An analysis of 89 AG demonstrated most often crural bed involvement (96%), with involvement of the femoral region in 53%, the popliteal artery in 22% and the pelvic artery in 15%. Based on AG, vascular intervention was indicated in 68 limbs (76%), it was technically impossible in 15 limbs (17%) because of an advanced finding; AG was negative in 6 limbs (7%). Out of the 89 limbs, percutaneous transluminal angioplasty was indicated in 46 (51%), bypass in 17 limbs (19%), combined revascularization in 5 (6%). AG-related complications occurred in 3 pts (4.2%); all involved bleeding from the groin which required surgical revision in 1 case and was associated with acute renal insufficiency with full renal function normalization in 1 case. In diabetics with the diabetic foot of suspected vascular etiology, it is appropriate to indicate early AG since, if adequate care is provided to the patient, it is relatively safely and often results in a vascular intervention, primarily PTA.

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1925

CYTOKINES AND SOLUBLE CELL ADHESION MOLECULES IN VITREOUS HUMOR

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To explore mechanisms in the pathogenesis of diabetic retinopathy, we examined the vitreous of sixty six patients with proliferative diabetic retinopathy(PDR) and fifty patients without diabetes mellitus for the presence of tumor necrosis factor-α(TNF-α), interleukin-6(IL-6),soluble intracellular adhesion molecule-1 (ICAM-1), vasculer cell adhesion molecule-1(VCAM-1), P-selectin and E-selectin. Vitreous samples were obtained, undiluted from the patients with PDR and patients with miscellaneous retinal diseases prior to vitrectomy. Cytokines and cell adhesion molecules were measured by enzyme-linked immunoadsorbent assays. The levels of TNF-α, VCAM-1, P-selectin and E-selectine were not different for patients with PDR and those without diabetes mellitus. However, levels of IL-6 and ICAM-1 were significantly(P<0.001) elevated in patients with PDR compared to levels in patients without diabetes mellitus(IL-6: 175±46pg/ml vs 10±2pg/ml; ICAM-1:12.8±9.4ng/ml vs 2.1 ±2.8ng/ml). Elevated levels of IL-6 and ICAM-1 in the vitreous of patients with PDR, implicating a role for these cytokine and cell adhesion molecule in the pathogenesis of PDR by enhancing immune action and inflammation.

1926

BIMOCLOMOL (BRLP-42) PREVENTS AND CORRECTS EARLY RETINAL NEUROSENSORY DYSFUNCTION IN DIABETIC RATS

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Oscillatory potentials (OPs), the neuronal components of flash-evoked electroretinogram (ERG) are of clinical importance in prognosis and diagnosis of diabetic retinopathy (DR). Diabetes reduces the amplitude and increases the latency of OPs. The effect of Bimoclomol (former code BRLP-42; Biorex, Hungary) on streptozotocin (STZ 45 mg/kg)-induced ERG abnormalities was assessed using scotopic ERGs under anaesthesia. Daily oral treatment with 20 mg/kg BRLP-42 was either preventive or curative, given 1 day or 1 month following the onset of diabetes. 50 mg/kg aminoguanidine served as reference. Groups of 10 male Crl Wistar rats were investigated: nondiabetic, diabetic, and BRLP-42- or aminoguanidine-treated diabetic groups. All the ERG waves were impaired with 2-month diabetes. The reduction ($p<0.001$) in amplitudes of glycin sensitive OP3 (70.2%) and of GABA mediated OP2 (90.0%) was protected by both BRLP-42 (by 48.0±4.2%, and 37.4±7.4%, resp.; $p<0.01$) and the reference compound to a similar extent. Curative treatment improved the deficits by 86.3±30.5%, and 36.7±10.0%, resp.; $p < 0.05$). The significant prolongation of peak latencies due to diabetes was normalised for OP3 and inhibited by 68% for OP2 after pretreatment with BRLP-42. Diabetic alterations in the main waves of ERG were also improved. Our results demonstrate the reduction in severity of DR possibly by protecting basement membrane thickness of retinal vessels which is known to correlate with OP latencies. This effect can be beneficial in the treatment of retinopathy. The mechanism of action of BRLP-42 on retinal dysfunctions is unknown The relationship between changes in OP latencies and the thickness of basement membrane suggests a direction for further studies.

1927

VITREOUS LEVELS OF VASCULAR ENDOTHELIAL GROWTH FACTOR ARE NOT INFLUENCED BY ITS SERUM CONCENTRATIONS

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Vascular endothelial growth factor (VEGF) plays a major role in the development of intraocular neovascularization in proliferative diabetic retinopathy (PDR). The source of intravitreous VEGF is presumably ischemic retina, but increased levels derived from serum could not be excluded. The aim of the study is to determine the intravitreous concentrations of VEGF in diabetic patients with PDR and to investigate whether serum VEGF could contribute to intravitreous concentration. For this purpose, we included 20 diabetic patients (5 type 1 and 15 type 2) with PDR in whom a vitrectomy was performed (group A). Nondiabetic patients ($n=13$) with other conditions requiring vitrectomy served as a control group (group B). In all cases, a recent vitreous hemorrhage was excluded. In both groups, VEGF was determined in serum and undiluted vitreous samples obtained simultaneously. Furthermore, serum VEGF was determined in 69 healthy controls (group C) and 39 diabetic patients without late complications (group D) . Vitreous and serum VEGF was determined by ELISA (R&D Systems, Abingdon); intra-assay CV 3.8 %, interassay CV 5.1 %. Statistics: Student's t-test and ANOVA. Results: Intravitreous concentrations of VEGF were strikingly higher in group A (2101.6 ± SD 1628.9 pg/ml, median 1756 pg/ml, range 334.5-6662) in comparison with group B (12 ± SD 8.1 pg/ml, median 9 pg/ml, range 9-38.2); $p<0.0001$. We did not observed differences in serum VEGF among the groups included in the study (group A 183.2 ± 123 pg/ml, group B 221.5 ± 147.1 pg/ml, group C 231.5 ± 188.1 pg/ml, and group D 214.7 ± 171.8 pg/ml); p=ns. We conclude that the high vitreous levels of VEGF obtained in diabetic patients with PDR could not be attributed to serum diffusion across blood-retinal barrier. Furthermore, our results suggest that intraocular synthesis is the main contributing factor for the high VEGF concentrations observed in these patients.

1928

NUMBER OF (A-C)n REPEAT IN THE UPSTREAM OF ALDOSE REDUCTASE GENE CORRELATES WITH DIABETIC RETINOPATHY

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The polyol pathway has been implicated in the pathogenesis of diabetic microangiopathies. We studied the correlation between the number of (A-C)n repeat region in 2.1 kb upstream of the transcription start site of aldose reductase (AR) gene and retinopathy in Japanese patients with non-insulin-dependent diabetes mellitus. We also measured erythrocyte AR activity. We classified the 45 patients into two groups. The first group consisted of 20 patients with proliferative retinopathy within 8 years' duration and defined as "with retinopathy". Second group consisted of 25 patients with no evidence for retinopathy, nephropathy or neuropathy even after 10 years' duration and defined as "without retinopathy". Japanese subjects showed 10 allelic types in this region. The most prevelent allele was 24 times in (A-C) repeat and designated as Z allele. Those with Z+2 allele (25 times in (A-C) repeat) have significantly less chances to develop retinopathy, whereas those with Z-4 allele (22 times in (A-C) repeat) have significantly more chances to develop proliferative retinopathy. Most of the patients with proliferative retinopathy had nephropathy and neuropathy as well. Erythrocyte AR activity was significantly elevated in those with Z-2 allele compared to those with Z+4 allele. In addition, erythrocyte AR activity was significantly lower in those with Z+2 allele compared to those with Z allele. Our study also revealed two nucleotide changes from "TC" to "CT" in this region compared to Chinese. The present study suggests that the number of (A-C)n repeat in 2.1 kb upstream of AR gene may determine AR activity and may be one of the factors influencing predisposition to diabetic microangiopathies.

1929

SERUM LEVELS OF VASCULAR ENDOTHELIAL GROWTH FACTOR ARE NOT INCREASED IN DIABETIC RETINOPATHY

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Vascular endothelial growth factor (VEGF) has been involved in the etiopathogenic events of diabetic retinopathy (DR) and increased levels have been observed in the vitreous of diabetic patients with proliferative retinopathy. However, there are no studies about the possible relationship between serum VEGF concentrations and DR. For this purpose, we included 28 consecutive diabetic patients (6 type 1 and 22 type 2) with DR attended by the Diabetic Retinopathy Unit (8 non-proliferative DR and 20 proliferative DR). Diabetic patients (n=39, 18 type 1 and 21 type 2) without DR and 69 healthy controls matched by age were used as control groups. In all cases we excluded systemic diseases other than diabetes mellitus. VEGF was determined in duplicated serum samples by ELISA (R&D Systems, Abingdon, UK); intra-assay CV 3.8 %, interassay CV 5.1 %. Statistics: Student's t-test and ANOVA. Results: we did not observed differences in serum VEGF concentrations in diabetic patients compared with healthy control group (210.9 ± SD 157.5 pg/ml vs 231.5 ± SD 188.1 pg/ml; p=ns). In diabetic patients with DR we obtained similar levels of VEGF than in diabetic patients without DR (205.6 ± SD 138 pg/ml vs 214.7 ± SD 171.8 pg/ml; p=n.s). Furthermore, we did not observed any relationship between serum VEGF concentrations and the severity of diabetic retinopathy (nonproliferative DR patients: 261.7 ± SD 163.6 pg/ml vs proliferative DR patients: 183.1 ± SD 123 pg/ml; p=n.s). We conclude that serum levels of VEGF are not related with the presence and the degree of diabetic retinopathy.

1930

DIABETIC-LIKE RETINOPATHY: EARLY AND LATE INTERVENTION THERAPIES IN THE GALACTOSE-FED RAT MODEL.

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The aim was to determine whether diabetic-like retinal capillary basement membrane thickening (RCBMT) in the galactose-fed rat could be halted or ameliorated with early or late interventions by withdrawal of the galactose diet or treatment with the aldose reductase inhibitor ARI-509. Weanling S-D rats were divided randomly into 8 groups and fed Purina laboratory chow (#5001) plus: 50% starch—*CONTROL (**CON**)*; 50% D-galactose—*GALACTOSE (**GAL**)*; 50% D-galactose with ARI-509 at 10 or 25 mg/kg/day—*LOW and HIGH DOSE PREVENTION (**LDP; HDP**)*; 50% D-galactose for 4 or 8 months, then intervention by adding ARI-509 (25 mg/kg/day)—*4-MONTH and 8-MONTH INTERVENTION (**4IN; 8IN**)*; or galactose withdrawal from the diet at either 4 or 8 months—*4-MONTH and 8-MONTH GALACTOSE WITHDRAWAL (**4GW; 8GW**)*. At 4, 8, 16, and 24-month time points, tissue carbohydrate levels and RCBMT in the outer plexiform layer were determined for all groups (n = 5 to 8). Retinal polyol was reduced by 95% and 100% in all ARI-treated and galactose withdrawal groups, respectively. Mean RCBMT in ***GAL*** rats, became almost 2-fold greater (189 ± 9.4 nm) than in ***CON*** rats (103 ± 3.4 nm) by 24 months. Prevention of RCBMT was statistically significant ($p < 0.05$) in both the ***LDP*** and ***HDP*** groups at all time points. In contrast, a significant decrease in mean RCBMT compared to ***GAL*** rats was not found in either the intervention or withdrawal groups until 24 months (***4IN***, 166 ± 10.3 nm; ***8IN***, 161 ± 8.2 nm; ***4GW***, 136 ± 5.1 nm; ***8GW***, 163 ± 9.6 nm). The lack of a reversal and the long delay before the effects of either early or late intervention treatment were observed are consistent with findings from the Diabetes Control and Complications Trial.

1931

SUPPRESSION OF ENDOGENOUS OSMOREGULATORY GENES IN HUMAN RETINAL PIGMENT EPITHELIAL CELLS TRANSFECTED TO OVEREXPRESS ALDOSE REDUCTASE.

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Human retinal pigment epithelial (RPE) cells, a pathophysiologically relevant model for diabetic complications, demonstrate heterogeneous basal aldose reductase (AR) gene expression and activity. In 20 mM glucose, high AR-expressing RPE 91 cells exhibit exaggerated sorbitol accumulation and depletion of taurine and myo-inositol rendering them rate-limiting for their normal metabolic functions. The mechanism linking high AR gene expression to intracellular osmolyte depletion is unknown. The relationship of basal AR gene expression to steady state mRNA levels of the Na-taurine co-transporter (TT) was explored in low AR expressing RPE 47 cells and RPE 47 cells stably transfected to overexpress AR (to reproduce the phenotype of the RPE 91). Total cellular RNA was extracted from hypertonically-stressed RPE cells, size selected (>1 kb) and used to prepare a cDNA library in pcDNAI. Screening with a partial human AR cDNA yielded recombinant plasmids which were screened for function by transiently transfecting COS cells and measuring AR enzymatic activity. The clone with highest AR activity was selected for stable transformation in RPE 47 cells. RPE 47 cells were co-transfected with pRSV-neo-pcDNA1-AR27 and a clone identified (RPE 75) which expressed 4 x elevated AR enzymatic activity relative to untransfected RPE 47 cells and reproduced basal RPE 91 AR activity. To assess the effect of AR overexpression on other osmoregulated genes, RPE 47 cells, stably transfected RPE 75 cells and RPE 91 cells were exposed to 5 mM glucose for 24 h, and Northern blots prepared and sequentially hybridized with a 900 bp TT cDNA and a partial human AR cDNA and normalized to 28S. In RPE 75 cells, basal expression of the transfected AR gene was increased 3.5-fold vs. RPE 47 cells, reproducing the RPE 91 level. In RPE 75 cells, basal levels of the endogenous AR and TT were decreased by 38% and 44%, respectively compared to the untransfected RPE 47, the decreased level of TT mRNA being identical to that in RPE 91 cells. These data are consistent with reciprocal suppression of endogenous osmoregulatory genes in response to overexpression of AR, suggesting complex interactions among osmoregulatory genes.

1932

EFFECT OF ANTIOXIDANTS ON DEVELOPMENT OF RETINOPATHY IN DIABETES AND EXPERIMENTAL GALACTOSEMIA.

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Dietary supplementation with 0.1% α-tocopherol acetate and 1% ascorbic acid has been reported by us to inhibit the diabetes- or galactosemia-induced increase in lipid peroxides (measured as TBARS) in retina and the decrease in activities of retinal ATPases and enzymes involved in protection from oxidative stress. Long-term studies of the effects of antioxidants on the development of retinopathy were conducted using this same diet. Alloxan-diabetic rats and nondiabetic rats fed 30% galactose diet randomly were assigned to receive diet supplemented with the antioxidants or standard diet. 18 months of diabetes or galactosemia resulted in pericyte loss, acellular capillaries and basement membrane thickening (i.e., lesions consistent with the early stages of retinopathy), and consumption of antioxidant diet significantly ($P<0.05$) inhibited the development of acellular capillaries in retinas of diabetic rats. The number of pericyte ghosts in antioxidant-treated rats tended to be less than in diabetic controls, but the decrease did not achieve statistical significance. Thickness of capillary basement membrane was not influenced by the antioxidant diet. GHb intentionally was kept comparable between diabetic groups, so the beneficial effect of antioxidants on the frequency of acellular capillaries seems not due to a difference in glycemia between groups. The antioxidants normalized a diabetes-induced increase in osmotic fragility of erythrocytes (an estimate of oxidative stress, demonstrating efficacy of the therapy. Galactose-fed rats showed no defect in osmotic fragility, and antioxidants caused no significant inhibition of galactose-induced metabolic abnormalities or histopathology in the retina. Galactosemic rats were not hyperphagic like diabetic rats (consuming only 48% the amount of antioxidants consumed by diabetics), perhaps accounting for the lesser effect of antioxidant diet on galactose-induced retinopathy. Supplemental antioxidants inhibited the development of retinal acellular capillaries in diabetic rats, but whether oxidative stress is a major factor in the development of diabetic retinopathy requires further investigation.

1933

ANTI-ANGIOGENIC ACTIVITY OF AQUEOUS HUMOR DECREASED IN DIABETIC MAN.

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Neovascularization is an important event in diabetic ocular disease such as proliferative retinopathy. VEGF, bFGF and other cytokines play a role to progress angiogenesis. On the other hand, we have previously shown that anti-angiogenic activity has been present in aqueous humor(AH). Recently we found that TGFß2 was the major factor of anti-angiogenicity in AH. In the present study, we checked this activity and TGFß2 concentration of AH from diabetic patients. AH was obtained from patients undergoing ocular surgery. Angiogenic activity was estimated by the proliferation of HUVECs cultured with 20% AH. TGFß2 concentration was measured by ELISA (Amersham's kit) with or without acidifing AH. The number of HUVECs increased from 0.5(x10^4/well) to 14.7±0.8(m±SE, n=6) in the control culture, 6.1±0.7(n=5) ($p<0.01$ vs control) in nondiabetic AH supplemented, and 9.3±0.8(n=8) ($p<0.01$ vs control, $p<0.05$ vs nondiabetic)in diabetic AH supplemented. Total TGFß2 concentration of diabetic AH (diabetic 6.4±0.7 ng/ml, n=27) wasn't different from of non-diabetics (7.0±0.7, n=27). But the ratio of activated form was lower in diabetics(7.7±0.7 %, n=10) compared to nondiabetic (12.4±2.0, n=10, $p<0.05$). These results suggests that less activation of TGFß2 in diabetic AH caused lowering anti-angiogenic activity. The less anti-angiogenic activity of diabetic AH might be involved in promotion of neovascularization in diabetic ocular disease.

1934

EARLY TREATMENT WITH PHENSUCCINAL ATTENUATES DIABETIC RETINOPATHY DEVELOPMENT IN RATS

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Taking into consideration the susceptibility of diabetic retinas to oxidative stress we evaluated the impact of a new low-toxic succinic derivative phensuccinal (P) with antioxidant properties on diabetic retinopathy (DR) development in rats. The newborn (2-3 days of age) rats were injected with streptozotocin (100 mg/kg i.p.) and randomized into a control diabetic group (CD, n=6) and P-treated one (PD) (P-25 mg/kg/day in the diet for 3 months, n=7). Antioxidant effect of P was assessed in liver homogenate by spectrophotometric determination of malonic dialdehyde (MDA), vitamin A and β-carotenoids. The severity of the alterations in retina was evaluated by scores in light and electron microscopic studies (blind). CD animals showed retinal capillary basement membrane (RCBM) irregularities, a significant (1.7 fold, $p<0.01$) increase in RCBM thickness (RCBMT) compared to normal controls (n=7), substantial edema and vacuolation in neural elements, abnormalities in endothelial cells and pericytes. P-treatment inhibited DR development reflected in absence of retinal neural elements edema, almost normal arrangement of endothelial cells and pericytes, more compact and even RCBM as well as a significant decrease RCBMT in comparison with CD rats (by 27.4%, $p<0.02$). Treatment with P improved glucose tolerance ($p<0.05$), reduced by 2.5 fold ($p<0.01$) MDA content, increased by 2 fold ($p<0.001$) vitamin A and β-carotenoids contents. Thus P possesses angioprotective effect due to improvement of oxidative parameters in diabetic rats and could be a beneficial agent for DR treatment.

1935

AGEs PROMOTE DIABETIC RETINOPATHY BY THE PRODUCTION OF VASCULAR ENDOTHELIAL GROWTH FACTOR

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Recent studies have suggested that advanced glycation endproducts (AGEs) play an important role in the pathogenesis of diabetic complications. The aims of the present study were to prove the increment of AGEs and vascular endothelial growth factor (VEGF) in vitreous fluid of patients with proliferative diabetic retinopathy (PDR) and to clarify the effect of AGEs on VEGF mRNA expression *in vitro*. We evaluated AGEs production by measuring crossline, a novel AGE-product which has both crosslink and fluorescence similar to AGE-proteins *in vivo*. Vitreous samples were obtained during vitrectomy from 14 patients with PDR and 14 patients without diabetes received vitrectomy due to retinal detachment (n=7) or epiretinal membrane (n=7). Crossline and VEGF levels were determined by competitive ELISA. *In vitro* study, the expression of VEGF mRNA was analyzed by quantitative RT-PCR after the treatment of Müller cells, the strong candidate for VEGF production, with 50 μg/ml of AGE-BSA or BSA. The crossline levels in the vitreous of patients with PDR were significantly higher than those without diabetes ($P<0.05$). The VEGF levels in the vitreous were also significantly higher increased in patients with PDR ($P<0.01$). Furthermore, there was the positve correlation between crossline and VEGF levels in the vitreous samples of patients with PDR ($P<0.05$). The expression of VEGF mRNA was enhanced by the presence of AGEs when compared with the absence of AGEs. AGEs in vitreous fluid could promote intraocular neovascularization in diabetes through the production of VEGF from retinal cells. These results demonstrated that AGEs plays an important role in the initiation and progression of intraocular neovascularization in diabetic retinopathy.

1936

EFFECTS OF TAURINE AND VITAMIN E SUPPLEMENTATION ON OXIDATIVE STRESS AND NA/K ATPASE ACTIVITY IN THE RETINA OF STREPTOZOTOCIN-INDUCED DIABETIC RAT.

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To examine the effect of dietary supplementation with two endogenous antioxidant agents, taurine and vitamin E, on diabetic retinas, we evaluated the formation of conjugated dienes (CD) and the changes of membrane-bound enzymes such as (Na,K)-ATPase activity in retinas from male Wistar adult non-diabetic and streptozotocin-induced diabetic (STZ-D) rats. Diabetic animals were assigned to three groups: untreated; taurine supplementation by 5% w/w; vitamin E supplementation by 200 IU/kg of diet. After induction of diabetes, (STZ-D) rats were sacrificed at 2, 4 and 8 weeks and their retinas removed. (Na,K)-ATPase activity and conjugated dienes (CD) were measured spectrophotometrically and expressed as μmol Pi/h/mg protein and μmol/mg protein, respectively. Results are given as means ± SD. The levels of CD were 3.7 ± 0.3 in control retinas. We found a significantly increase ($p<0.0001$) of CD at 4 weeks (8 ± 0.3) in diabetic retinas of untreated rats, but not in retinas of vitamin E-treated and taurine-treated rats. Retinal (Na,K)-ATPase activity was reduced at 4 weeks (55.4 ± 7.7; $p=0.03$) and at 8 weeks (56.3 ± 6.9; $p=0.04$) in untreated animals with respect to that of control rats (104.3 ± 18.1), whereas ATPase activity in taurine-treated diabetic rats was found to increase by 2.1-fold after 2 weeks, and also after 1 and 2 (1.5-fold) months. Supplementation with vitamin E did not significantly reduce ATPase activity in (STZ-D) rats, although a progressive reduction was found at 4 weeks (84.7 ± 26) and at 8 weeks (73.8 ± 25.5). Our results suggest that hyperglycaemia produces an oxidative damage in diabetic retinas causing a dysfunction of membrane-dependent (Na,K)-ATPase. Treatment with taurine and vitamin E can modify diabetes-induced abnormalities in rat retinal membrane activities.

1937

IGF SYSTEM GENE EXPRESSION IN HYPOXIC RETINA.

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Neovascular response in the diabetic eye is related to the development of retinal ischemia. To study the effect of relative ischemia on the IGF system in retina we used a model of neonatal rat retina that produce neovascularization when exposed to alternating levels of oxygen. We examined the influence of 24 hours hypoxia (10±1% oxygen), 48 hours hyperoxia (75±5% oxygen) and relative hypoxia (shifting from 48 hours 75% oxygen to 24 hours in room air) on the expression of the IGF system e.g. IGF-I, IGF-I receptor (IGF-IR)and IGF binding proteins 1,2 and 3 (IGFBP1,2,3) in retina using solution hybridization RNase protection assay. Gene expression was analyzed by densitometry and the numbers are given as percent of control (room air). Hypoxia induced a significant increase in IGF-IR, IGFBP2 and IGFBP3 mRNA and significant decrease in IGF-I mRNA (see table). Relative hypoxia also caused an increase in IGF-IR, IGFBP2 and IGFBP3 mRNA but did not change the expression of retinal IGF-I. We showed that relative hypoxia, similar to hypoxia, caused increase in IGF-IR, IGFBP2 and IGFBP3 gene expression, but unlike in hypoxic state IGF-I mRNA was not decreased. Thus we conclude that during relative retinal hypoxia that induces retinal neovascularization in newborn rat, there is overall up-regulation of the IGF system gene expression that might cause increased local activity of IGF-I in retina.

	Hypoxia 10% O_2	Hyperoxia 75% O_2	Relative Hypoxia
IGF-I	68%	117%	111%
IGF-IR	196%	97%	150%
IGFBP-2	208%	135%	161%
IGFBP-3	411%	109%	216%
IGFBP-1	Not Detected		

1938

PREVENTION OF THE DEPLETION OF NITRIC OXIDE SYNTHASE CONTAINING NEURONS IN THE DIABETIC RAT BY AMINOGUANIDINE.

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A close association of nitric oxide synthase immunoreactive neurons (NOS-ir) to the retinal vasculature has been demonstrated suggesting a mechanism by which retinal blood flow and metabolism are linked. Aminoguanidine (AG), an inhibitor of advanced glycation and NO synthase activity, has been shown to attenuate the progression of retinal disease in experimental diabetes. The present study has examined the effects of diabetes on the population of NADPH diaphorase-positive (NADPHd) and NOS-ir neurons in the diabetic rat retina. In addition, the role of advanced glycation and the NO system in these diabetic retinal changes has been explored by the use of pharmacological inhibitors of these systems. Male Sprague Dawley rats (200-250g) were randomised into Control or rendered Diabetic via streptozotocin injection (50mg/kg). Control rats were randomised to receive no treatment or AG (2g/l). Diabetic rats were further randomised to receive no treatment, AG (1g/l) or a non-pressoric dosage of the NOS inhibitor L-NAME (5mg/L). Rats were followed for 3 or 32 weeks. At the various time points, the right eye from each rat was dissected and fixed in buffered 4% paraformaldehyde for 2h. Retinas were stained for NADPHd. Counting of cells was performed double blind (mean ± SEM).

		NADPHd histochemistry	
Group	n	3 weeks	32 weeks
Control	9	2715±39*	2944±144
Diabetic	9	1946±48	2283±148†
Control+AG	6	2278±129	2587±186
Diabetic+AG	7	2137±131	2939±31‡
Diabetic+L-NAME	7	1689±230	2392±93†

*p<0.05 versus all groups, †p<0.05 versus control, ‡p<0.05 versus Diabetic

A correlation between NOS-ir and NADPHd staining in the treated diabetic rat retina was demonstrated. The number of NADPHd-positive neurons per retina was reduced after 3 weeks of diabetes and persisted at 32 weeks. AG therapy restored the number of NADPHd-positive neurons at week 32 whereas L-NAME did not affect this parameter. This study indicates that the retinoprotective effect of aminoguanidine cannot be reproduced by L-NAME and is likely to be mediated by a decrease of advanced glycation rather than via inhibition of NOS.

1939

TREATMENT BY SOMATOSTATIN ANALOG, OCTREOTIDE, OF ISCHEMIA INDUCED NEOVASCULARIZATION IN NEWBORN RAT.

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Octreotide was shown to prevent angiogenesis in diverse in vitro models of neovascularization. It's effect on proliferative (ischemia related) diabetic retinopathy is controversial. We used 48 hour cycles of alternating 10% and 50% oxygen during the first 14 days of newborn rats, followed by 4 days in room air to induce neovascularization. Half of the rats were injected with octreotide 10 µg x 2 daily during the whole period. At 18 days the rats were sacrificed, blood samples were taken and the eyes were enucleated and placed in formaldehyde. Ten representative slices of each eye were examined. Assessment of pathology was done using four parameters (table 1), each was assigned a value between 0 (non existent) and 3 (severe). Octreotide group included 52 eyes and the control group 59 eyes. In spite of the high octreotide levels in the treated group, growth hormone (GH) levels were not significantly decreased (from 64±33 to 45±13 µg/l) and IGF-I levels were not changed at all (75±12 to 86±19 µg/l). We failed to demonstrate any inhibition of neovascularization by octreotide. Octreotide did not prevent ischemia related neovascularization. However in this model octreotide did not significantly decrease levels of GH and IGF-I. Other drugs that better suppress GH are still worth trying.

Table 1. Pathologic findings in the eyes

Parameters	*Octreo.*	*Control*	*P-value*
Dilated vessels	1.6 ±0.9	1.8±1.0	0.09
Retinal hemorrhage	0.9±1.2	0.8±1.0	0.17
Vitreal hemorrhage	0.9±1.2	0.8±1.1	0.48
Neovascularization	0.7±1.1	0.5±0.9	0.11
Numbers represent average score ± SD.			

1940

OXIDATIVE DAMAGES TO OCULAR STRUCTURES IN DIABETIC PATIENTS ARE RELATED TO RETINAL FREE RADICAL PRODUCTION

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Retinal participation to the genesis of ocular complications during diabetes is supported by the observation of a concorrent higher rate of lipid and protein oxidation and decreased concentration of antioxidants both in the vitreous and in cataractous lenses obtained from diabetic subjects affected by background retinopathy compared to diabetic patients non affected by retinopathy and to non diabetic subjects. These alterations were even more pronounced in the presence of a proliferative retinopathy, thus suggesting that the retina may be a place for free radical production. Therefore, to confirm this hypothesis, the concentration and redox state of the proteins in the subretinal fluid (SF) of patients undergoing retinal detachment surgery have been evaluated.

Diabetic (n=8) and non diabetic (n=9) subjects with comparable age, degree of myopia and evolution time of the retinal detachment were considered for this study. The SF was obtained by puncture after sclerotomy; the concentrations of total proteins, sulfhydryl (P-SH) and carbonyl (DNPH) proteins were determined in the ocular samples with spectrophotometric methods. The levels of malondialdehyde (MDA) was measured by HPLC.

The protein concentration in the SF did not differ between diabetic and non diabetic patients. A higher concentrations of MDA (2.58 ± 0.31 vs 1.12 ± 0.22 nmol/mg protein, $p<0.01$) and DNPH (3.18 ± 0.36 vs 2.02 ± 0.28 nmol/mg protein, $p<0.02$) were observed in the SF of diabetic subjects, compared to non diabetic patients; the highest values were documented in patients affected by proliferative retinopathy. In the same subjects the lowest content of P-SH was noted (5.16 ± 1.32 nmol/mg protein, $p<0.01$ compared to diabetic patients without proliferative retinopathy).

In conclusion, it can be suggested that the retina partecipates to the genesis of diabetic eye complication by free radical production and that lipid and protein oxidation are events associated with retinal detachment and are involved in the onset of diabetes related ocular injury.

1941

IMMUNOHISTOCHEMICAL CHARACTERISATION OF RETINAL GLIAL CELL CHANGES IN DIABETIC RETINOPATHY

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Purpose: Occlusion of the retinal vascular bed is supposed to be a key factor in the development of proliferative diabetic retinopathy. A previous study has shown that the material occluding retinal vessels displays immunoreactivity to retinal glial cells. In the present study the glial cell changes secondary to diabetic retinopathy were further characterised. **Methods:** Immunohistochemistry to glial fibrillary acid protein (GFAP) and vimentin (all glial cell types), S-100 protein (perivascular glial cells), carbonic anhydrase (CAH-II) and CD-57 antigen (Müller cells), and CD-68 antigen (microglia) was performed on five eyes of five normal persons and on ten areas of vascular occlusion from ten eyes of six diabetic patients. **Results:** In the retina from diabetic patients immunoreactivity to S-100 protein in perivascular glial cells was significantly higher than in normals, except for areas of vascular occlusion where S-100 protein immunoreactivity was absent. The material accumulated to occlude the retinal vessels displayed immunoreactivity to vimentin, GFAP, CAH-II and CD-57 antigen, but not to S-100 protein, or CD-68 antigens. **Conclusions:** Diabetic retinopathy is characterised by activation of the retinal perivascular glial cells, but this cell type disappears in areas of vascular occlusion. The material accumulated to occlude retinal vessels represents invaded Müller cells.

1942

OXIDATIVE STATUS AND ANTIOXIDATIVE DEFENSE MECHANISMS OF DIABETIC CATARACTOUS AND CLEAR LENSES

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Oxidative status and antioxidative defense mechanisms were investigated in diabetic cataractous and clear lenses. Lens malondialdehide concentration(MDA) was measured to evaluate oxidative stress and lens total vitamin C (Vit C), non-protein sulphydryl compounds concentration (NPSC), glucose-6-phosphate dehydrogenase (G6PDH), catalase, superoxide dismutase(SOD), glutathione peroxidase (GSHPx)enzymes activities were measured in 15 diabetic cataractous lenses and 6 clear lenses. Lens MDA concentration of diabetic cataractous lenses were higher three times than that of clear lenses($p=0.02$). However, lens total vitamin C concentrations were decreased than clear lenses($p = 0.000$). Difference between diabetic and clear lens NPSC concentrations was not significant. Lens specific G6PDH, catalase and GSHPx enzyme activities were higher than that of clear lenses($p= 0.001$, $p=0.01$, $p=0.001$, respectively). SOD activities were lower than clear lens($p= 0.002$). As a preliminary report an important positive correlation between lens G6PDH and catalase activities($p=0.000$ $r= 0.81$) and negative significant correlation with SOD activities were determined($p=0.000$ $r=0.70$, $p= 0.000$ $r=0.82$). H_2O_2 is occurred in lens by photochemical oxidation and lens metabolism. Antioxidatives, Vit C and NPSC are used, and catalase, GSHPx activities increase to remove H_2O_2. In diabetic lens, insufficiency of these antioxidative mechanisms and inhibition of SOD by increased concentration of H_2O_2 led to oxidative damage of lens and development of cataract. Beside of this, increased activity of diabetic cataractous lens G6PDH that is key enzyme of pentose phosphate pathway (HMP) shows that HMP possesses an important antioxidant role in lens.

1943

DIABETES PREVENTS DARK-INDUCED RETINAL HYPEREMIA.

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These studies were undertake to assess the effects of diabetes of 3 weeks duration on dark-induced retinal hyperemia. Dark adaptation increases retinal glucose metabolism and blood flow and causes borderline to mild hypoxia in the outer retina. Increased glucose metabolism via the sorbitol pathway in diabetic rats also is associated with increased retinal blood flow and with 'hypoxia-like' cytosolic reductive stress (an increased ratio of free cytosolic NADH/NAD^+). Dark-induced hyperemia was assessed in 2 independent studies in control (C) and streptozotocin-diabetic (D) male Sprague-Dawley rats and in diabetics treated with aldose reductase inhibitors, sorbinil (D+S) and zopolrestat (D+Z), at a dose of 0.2 mmol/kg bwt/day. After anesthesia with thiopental (80 mg/kg bwt) the head was wrapped with black cloth except for 1 eye. Vascular conductance (blood flow in µl/g retina/min normalized to 100 mmHg mean arterial pressure, mean±SD, N=8 to 12) was assessed 30 min later in the dark-adapted (DA) and the contralateral room light-exposed (LE) eye with a plasma soluble tracer (^{3}H-desmethylimipramine, mol wt=276). Conductance in C increased 9.6±2.1 % in DA eyes to 434±39 vs 387±41 in LE ($p<0.0001$). Conductance in DA and LE eyes did not differ in D in either study, although conductance in LE of D was increased 12 % vs LE in C to 432±42 in one study ($p=0.026$) and 32 % to 507±46 in the other ($p=0.0027$). Conductance was not increased in DA vs LE in D +S, but was increased 6.5±4.5 % ($p=0.0023$ for DA vs LE in D+Z, and $p=0.15$ vs the 9.6 % increase in C). In conclusion, these observations demonstrate that dark adaptation causes retinal hyperemia in normal rats and that this response is prevented by diabetes. The role of increased sorbitol pathway metabolism in mediating impaired dark-induced hyperemia in diabetic rats is unclear in view of the normalization by zopolrestat, but not by sorbinil, of dark-induced hyperemia.

1944

TRACE ELEMENT CONTEND IN THE DIABETIC CATARACT AND TRANSPARENT LENS

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The contend of trace elements Cu, Zn, Mn, Cr, Fe, Pb, Sb, and Ni in 15 diabetic cataract lenses and 12 transparent lenses were determined by graphit furnace atomic absorption spectrophotometry. It was faund that the contend of the elements in diabetic cortical cataract lenses were markedly increased from those in transparent lenses. Comparing transparent lenses with diabetic cortical cataract lenses, accumulation of Cu, Ni, Cr and Fe about six times, Mn and Pb about tree times, Zn and Sb about two time were higher in diabetic cataractous lenses than transparent lenses. There were significant relationship between Mn and Zn ($r=0.0739$, $p<0.05$), Sb and Zn ($r=0.763$, $p<0.05$), Pb and Zn ($r=0.824$, $p<0.01$), Sb and Mn ($r=0.994$, $p=0.000$), Sb and Ni ($r=0.986$, $p=0.000$), Pb and Cr ($r=0.741$, $p<0.05$), Ni and Mn ($r=0.992$, $p=0.000$). It was therefore inferred that the occurance of diabetic cataract was directly or indirectly related to the deficiency of trace elements.

1945

RELATIONSHIP BETWEEN ERYTHROCYTE ALDOSE REDUCTASE LEVELS AND DIABETIC RETINOPATHY IN NIDDM PATIENTS.

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The most important diabetic complication on the eye is diabetic retinopathy, because its progression leads directly to blindness. Since the acceleration of polyol pathway may initiate diabetic retinopathy, we consider that the enzyme aldose reductase (AR) probably modify the progression of diabetic retinopathy. In this study, we have measured AR levels in erythrocyte of diabetic patients in order to elucidate the relationship between the incidence of diabetic retinopathy and AR levels. The AR levels in erythrocyte of peripheral blood were determined in 531 NIDDM patients and 219 healthy human subjects by the ELISA method. Clinical parameters of diabetic patients (n=531) : gender (male/female), 284/247; age, 60.7±11.3 years; duration of diabetes, 12.1± 8.8 years; fasting blood glucose, 9.5±3.1 mmol/L; HbA1c, 8.2±1.8%, AR, 10.5±3.0 ng/mgHb. The levels of AR did not correlate with age, duration of diabetes, fasting blood glucose and HbA1c. In the all diabetic patients, the incidence of retinopathy (including non-proliferative and proliferative) was 47.1%. The AR levels in the patients with retinopathy was significantly higher than in those without ($p<0.02$), and the levels of the patients without retinopathy was almost same with that of healthy human subjects (10.1±2.0 ng/mgHb). In 531 patients, the levels of AR showed no significant correlation with the incidence of diabetic retinopathy. However, in 194 patients with duration of diabetes from 10 to 20 years, the incidence of diabetic retinopathy was significantly increased in proportion to the amount of AR ($p<0.05$, χ^2 test). The incidences were 72.2% and 33.3% in the patients having not less than 13 ng /mgHb and not more than 7 ng/mgHb of AR, respectively. We concluded that high AR levels are related to the development of diabetic retinopathy.

1946

IRON METABOLISM OF DIABETIC CATARACTOUS AND CLEAR LENSES; PRELIMINARY STUDY

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Lens iron, transferrin and ferritin concentrations were determined in 15 diabetic cataractous lenses and 6 clear lenses. Lens iron concentration was determined by electrothermal atomic absorbtion spectrometer, lens transferrin concentration was investigated by two different techniques, immunological method and dynamic colorimetric iron binding measurement method, lens ferritin concentration was firstly determined by immunochemiluminecance methods. Lens iron concentrations are 13.82 ± 12.4 ng/mg dry weight and 2.57 ± 0.94 ng/mg dry weight, in diabetic cataractous lenses and clear lenses respectively($p=0.001$), no lens transferrin concentration was determined by immunological and colorimetric iron binding method in two groups. Lens ferritin concentrations are 64.96 ± 47.05 U/mg protein, 294.50 ± 56.50 U/mg protein, , in diabetic cataractous lenses and clear lenses respectively($p=0.001$). In diabetic cataractous lenses, accumulation of iron may be result of loss of selective permeability of lens, and the decrease of ferritin concentration may be result of the destruction of natural protein structure of ferritin by oxidants. Both of the increased iron and the decreased ferritin concentrations will be attend to the acceleration of the oxidative mechanisms of the lens and the development of the cataractogenesis.

1947

HIGH LEVELS OF ERYTHROCYTE ALDOSE REDUCTASE IN NIDDM PATIENTS WITH DIABETIC RETINOPATHY

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The key role of aldose reductase (AR) in the development of various diabetic complication has been postulated. There has been, however, little study on the association between susceptibility to diabetic complications and the level of tissue AR in human subjects. To elucidate relationships between AR levels and diabetic complications, AR levels in erythrocytes of peripheral blood were determined in 470 NIDDM patients by the specific two-site ELISA based on purified recombinant human AR. AR levels showed no significant correlation with sex, age, duration of diabetes, fasting blood glucose and the average HbA1c of the patients. No significant difference was demonstrated between the enzyme levels of the 470 patients and 50 healthy controls. In 182 patients with duration of diabetes equal to and more than 10 years, the AR levels of patients with retinopathy (n=96) were higher than those in patients without retinopathy (n=86), but the average HbA1c levels during the recent 2-year period were also higher in the patients with retinopathy. To reduce the influence of the differance of the average HbA1c levels, we compared the AR levels in the patients with HbA1c level less than 7%. In these patients, the AR levels of patients with retinopathy (n=14, non-proliferative) were significantly higher than those in patients without retinopathy (n=45) (12.1 ng/mg Hb vs 9.8 ng/mg Hb, $P<0.01$). Between the two groups, there were no statistical differences in age, duration of diabetes, average HbA1c levels and average maximum and minimum blood pressure recorded during the recent 2-year period. AR levels were not significantly different between the patients with and without nephropathy. These results suggest that high AR levels in erythrocytes are related to the development of diabetic retinopathy.

PS 50

Retinopathy – Epidemiology, Clinical and Treatment

1948

Can digitised colour 35mm transparencies be used to diagnose diabetic retinopathy?

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[a]Diabetes Research Unit, Llandough Hospital and [b]Departments of Media Resources and Medical Statistics, UHW, Cardiff, UK.

Retinal photography is an adjunct to ophthalmoscopy in screening for diabetic retinopathy (DR). Digital retinal cameras allow a retinal image to be immediately displayed on a high resolution video display monitor. We conducted a pilot study to investigate the agreement in retinopathy grading from digitised images in comparison to the original colour transparencies as 35mm slides. 150 macula centred, 45°, non stereoscopic retinal images were digitised onto CD ROM by Kodak at base resolution of 768x512 pixels. The anonymised images were displayed on a 17" monitor running Windows at 800x600 resolution in 64,000 colours and graded in random order. Alternatively the transparencies were graded on a Slidex viewer. A quality control set were also graded with exact agreement in 93% of cases overall (91% (73/80) of PC images and 94% (75/80) of slide images). Compared to colour transparencies, 95% (84/88) of sight threatening diabetic retinopathy (STDR) and 100% (62/62) of non STDR cases were diagnosed using the PC. One case of pre-proliferative DR showing multiple cotton wool spots masked by the light reflections in the image viewed by the PC in a young patient was graded as non STDR. Three cases of non proliferative DR, one of poor photographic quality and two of which had hard exudates discernible despite reflections on the retina when viewing the colour transparencies but masked by the reflections when grading from the PC were graded as non STDR from the PC. There was good agreement between PC displayed digitised retinal images and 35mm colour transparencies.

1949

HBA_{1c} FROM DIABETES ONSET AND PREVALENCE OF RETINOPATHY

CE Kullberg[1], HJ Arnqvist[2], Kerstin Finnström[3] and Johnny Ludvigsson[4]; for the VISS-study group. [1] Dept. of Internal Medicine, Faculty of Health Sciences, and [2] Dept. of Endocrinology, [3] Dept. of Ophthalmology, [4] Dept. Of Child Health, University Hospital, Linköping, Sweden.

The VISS study (Vascular complications In Southeast Sweden) aims to investigate prevalence and incidence of vascular complications in relation to HbA_{1c} from diabetes onset, in a population with of Type 1 diabetes, from well defined geographic area. The study population comprise all patients with Type 1 diabetes onset before the age of 36, onset during 1983-1987, and at the time of onset living within the counties of Östergötland, Kalmar or Jönköping. Retinopathy was examined with fundus photography during 1994-95. All photos were evaluated by two ophthalmologists, and retinopathy was classified according to a modified Airlie House protocol. 353 sets of fundus photographs have been evaluated. For 252 patients (71%) no sign of retinopathy was seen. Patients with mean HbA_{1c} from diabetes onset in the highest quartile (>8.6% HbA_{1c}) had a relative risk of 4.2 (95% CI 2.3<RR <7.8) to have retinopathy compared to patients in the lowest quartile (<6.6% HbA_{1c}). Logistic regression with presence of retinopathy as dependent variable indicated significant impact from mean HbA_{1c} from diabetes onset, duration, and diastolic blood pressure, but no influence from age, sex or systolic blood pressure. Sixty-one of 166 (39%) patients with diabetes onset after 14 years of age had retinopathy, compared to 41 of 188 (22%) patients with younger onset (relative risk 1.7; 95% CI 1.2<RR<2.4). Prevalence of retinopathy was strongly associated with long-term mean HbA_{1c}. Patients with onset after 14 years of age had significantly more retinopathy than patients with younger onset.

1950

SUPERIORITY OF ELECTRONIC IMAGING IN DIABETIC RETINOPATHY SCREENING

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We aimed to compare two systems of instant retinal photographic imaging for diabetic retinopathy screening - the traditional Polaroid system (Canon CR4 45NM) and a new electronic imaging system (Canon CR5 45NM) which can produce an enlarged retinal image on a computer monitor screen. 213 eyes from 107 diabetic patients, including 14 already attending an ophthalmologist, were photographed through dilated pupils by both systems in random order and the images were analysed blind. Diabetic retinopathy was present in 58 eyes of which 55/58 (95%) were detected on the electronic imaging system and only 49/58 (84%) on the Polaroid. Of 34 eyes requiring referral to an ophthalmologist according to standard European criteria, 34/34 (100%) were detected on the electronic imaging system and only 24/34 (71%) on the Polaroid. Side by side comparisons showed the electronic imaging system to be considerably superior to Polaroid at lesion detection. Computer printouts of the images, both colour or black & white, of sufficient quality to show the lesions missed by Polaroid, could also be obtained. Most of the important lesions missed on the Polaroid but detected on the electronic image were hard exudates or haemorrhages within one disc diameter of the macula. Using linear analogue scales to compare the brightness of the flash, the patients assessed the electronic imaging system as significantly less bright than the Polaroid system ($p<0.0001$). Their comments suggested that the discomfort of the flash of the electronic imaging camera was considerably less than the Polaroid equivalent. Other potential advantages of electronic imaging in diabetic retinopathy screening include ready storage/retrieval of digitised images; image enhancement and analysis using image analysis software; and electronic transfer of images to ophthalmologists and primary care doctors. Electronic imaging systems represent a major potential advance for the improvement of diabetic retinopathy screening, with increased detection of sight threatening retinopathy compared to Polaroid.

1951

INCIDENCE AND TIME-TREND OF OCULAR COMPLICATIONS OF IDDM IN JAPAN

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No previous study has reported the prognosis regarding diabetic ocular complications among Japanese with IDDM in a population-based setting. The aim of this study is, therefore, to evaluate the incidence and time-trend of ocular complications by following-up the DERI (Diabetes Epidemiology Research International) cohort consisting of 1428 individuals with IDDM. The subjects were diagnosed as having diabetes during 1965-79 with onset of diabetes <18 years of age. We have traced 75% of the cohort (1064/1428) regarding ocular complications until January 1, 1995. The study revealed that the incidence rates of blindness and photocoagulation therapy were 3.7%, 19.4% for 15-year observation, and 9.7%, 41.0% for 25-year observation, respectively. Of interest is that the incidence rate, for 15-year observation, of blindness for subjects diagnosed during 1965-69 (old cohort) was higher compared to those diagnosed during 1975-79 (new cohort) (353 vs. 106/100,000 person years); in contrast, there was no difference in incidence of photocoagulation therapy between old and new cohort (978 vs. 1023/100,000 person years). The results suggested that the prognosis of ocular complication has been recently improved, the reason of which may be increased prevalence of photocoagulation therapy in Japanese IDDM patients.

1952

SCREENING FOR EARLY DIABETIC RETINOPATHY BY A HIGH RESOLUTION COMPUTER VISION SYSTEM.

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Diabetic retinopathy has been identified as one of the most common causes of blindness. One of the disturbing problems that diabetic patients face is that they may be suffering from a gradual or acute potential loss of vision and not recognize it. Although diabetic retinopathy is a treatable disease, early detection is essential to ensure that the patient receives appropriate preventive treatment. The main aim of this study is to introduce a method for screening for early diabetic retinopathy. The method is based on the use of a high resolution computer vision system. It can provide a resolution of 10,000 pixels per inch and can differentiate over 16 million different shades of colors that no human eyes can match. Computer quantitative algorithms for screening for early diabetic retinopathy have been developed and implemented. A substantial number of early diabetic retinopathy cases have been studied. The results show that the computer system can detect diabetic retinopathy much earlier than an ophthalmologist can. Another major advantage of having such a system is that it provides a mass-screening means for detecting early diabetic retinopathy at low cost.

1953

OPHTHALMOLOGIC COMPLICATIONS IN INSULIN DEPENDENT DIABETES MELLITUS

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We wanted to know the frequency of ophthalmologic complications in 94 insulin dependent diabetes mellitus patients. It was a descriptive observational study. We used an univariate analysis; Interest II and Quattro softwares. We revised results of complete ophthalmologic examination: corrected visual acuity, tonometry, slit lamp inspection and fundoscopic test using midriatic drug. Diabetic retinophaty diagnostic was made by biomicroscopy. We found 19.14% of simple retinopathy, 6.38% of simple retinopathy + maculopathy and 4.23% of proliferative retinopathy. Additionally, we observed 8.51% of cataracts (1.06% on left eye, 2.12% on right eye and 5.31% bilateral). Moreover, we found 23.33% of impairment visual acuity on left eye, 25.84% on right eye and 3.35% of blindness. Finally, 6.66% of patients had photocoagulation. For simple retinopathy, it was present after a mean time of 18.1 years (SD 2.74), for maculopathy, 14.5 years (SD 3.34) and for proliferative retinopathy, 20.8 years (1.75). We concluded that diabetic retinopathy prevalence, loss vision and cataracts were significative.

1954

NEW BLINDNESS IN DIABETES REDUCED BY 11% PER YEAR IN STOCKHOLM COUNTY

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Main aim: To develop a method for assessing all-cause blindness incidence in diabetes, and to evaluate interventions. **Background:** Mobile fundus photography teams for early diagnosis of diabetic retinopathy reached 10 000 persons with diabetes in primary care. **Methods:** Referrals stating diabetes ($n = 2\,272$) to vision rehabilitation centres 1981-1995 were registered (1995 pop. 1 725 756). Blindness was defined (WHO, ICD 10) as best-corrected visual acuity (VA) of the better eye less than 3/60 (0.05); 172 persons were blind from all causes. Trend tests used Poisson regression analysis. **Results:** Referrals with VA 6/15–6/24 increased, perhaps causing differential misclassification bias. The number of referrals with diabetes and blindness per five-year period fell from 93 (1981–85) to 51 (1986–90) to 28 (1991–95); average annual incidence rate for referrals with blindness per five-year period was reduced from 1.2 (95% CI 0.97–1.5) to 0.63 (0.47–0.83) to 0.33 (0.22–0.48) per 100 000 population; mean yearly reduction 11% (7–15%), test for trend $p < 0.001$. **Conclusion:** This is the first geographical region where a proxy measure for new blindness in diabetes was reduced by one third or more in five years, reaching one of the St Vincent Declaration main targets.

1955

MAIN INDEPENDENT RISK FACTORS FOR DIABETIC RETINOPATHY IN MEXICANS.

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Mexicans are a high risk group for non-insulin-dependent diabetes mellitus, and diabetic retinopathy (DR) is one of the main complications and a frequent cause of severe vision loss. Nevertheless few studies have estimated the occurrence of DR in Mexico, and none has studied its risk factors. The authors conducted a cross-sectional study to estimate the strength of the association between DR and its related risk factors. Thirteen percent of the diabetic population in a primary care medical unit were included in the study. A total of 129 men and 261 women received a detailed eye examination by direct ophtalmoscopy trough dilated pupils. Both nonproliferative and proliferative DR were considered for analysis, defined by the more severely affected eye. A logistic regression analysis was used to assess the independent effect of each risk factor while controlling for potential confounders. Odds ratio (OR) and 95% confidence intervals ($CI_{95\%}$) were employed as a measure of effect. The risk of DR increased by 29% for each year of duration of diabetes (OR 1.3; $CI_{95\%}$ 1.2-1.4), and by 46% for each milligram increase in serum creatinine (OR 1.46; $CI_{95\%}$ 0.9-2,4). Those patients with good metabolic control had a 60% diminished risk of DR (OR 0.41; $CI_{95\%}$ 0.23-0.72), even after controlling for age, sex and age of diagnosis of diabetes. Hypertension was related only to proliferative DR (OR 2.3; $CI_{95\%}$ 0.9 - 5.9). DR is closely related to the duration of diabetes and the presence of diabetic nephropathy. Metabolic control is an important predictor of DR in Mexicans, and a potentially modifiable risk factor. An integral diabetes program should be implemented in the Medical System in Mexico.

1956

DIAGNOSING AND TREATMENT OF DIABETIC RETINOPATHY FOR CHILDREN.

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We have examined 38 children aged from three to fourteen with the 1st type of diabetes. In the cases up to five years long diabetes it was revealed initial pathological changes such as: mild venous dilatation, microaneurysmas, single haemorrhages and irregular contours of optic nerve disk. Another group contained the children with duration of diabetes from 6 to 12 years and the 67 % of the patients had changes on eye-bottom, the 38 % of them had irregular contours of optic nerve disk. The 66 % of the children had mild twisted vessels capillary dilatation, microaneurismas, the 28 % of them had more severe current of retinopathy. Development of neovascular abnormalities within optic nerve disk area was found in the 11 % of the cases, the others 17 % of eyes had hard exudates and drusen had been recognized in a part of patients. The reasons for fotocoagulation treatment for children are:

1. presence a lot of microaneurismas, retinal haemorrhages, capillary dilatation;

2. growing pathological changes on the eye-bottom in 3..6 months after revealing diabetic retinopathy in spite of diabetic compensation.

It had been made the fotocoagulation of retinae for 10 children. The 84 % of the patients had the stabilization of process after 6 months.

All children with the 1st type of diabetes mellitus need early diagnoses of retinal changes but it is important to make photocoagulation in the case of disease progress.

1957

RURAL URBAN DIFFERENCES IN THE PREVALENCE OF DIABETIC RETINOPATHY IN MEXICO.

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The prevalence of non-insulin-dependent diabetes mellitus (NIDDM) is increasing in rural areas of Mexico. However, no study has estimated their prevalence of diabetic retinopathy (DR). The authors conducted a cross-sectional study to estimate the prevalence of DR in a small rural town of the southwestern state of Oaxaca. Sixty-two diabetics, which conformed 91% from all diabetics in the town, received a detailed eye examination by direct ophtalmoscopy trough dilated pupils. Both nonproliferative and proliferative DR were considered for analysis, defined by the more severely affected eye. Thirteen percent of the diabetic population in a primary care medical unit from Mexico City, were also included. Theprevalence of DR was lower in the rural population (35.5%), than in the urban (43.1%). However the main difference was in the prevalence of proliferative DR, which was present in 14.8% of the urban diabetics and in only 4.8% of the rural ones. When there was a poor metabolic control the prevalence of DR was equally high in both urban (57.1%) and rural (62%) diabetics, although the prevalence of proliferative DR was higher in those with poor metabolic control living in the city (20.6%) than in those living in the town (10.3%). The estimated prevalence was adjusted by age, sex, duration of the disease and metabolic control. The adjusted prevalence of DR was 42.2% in the urban diabetics and 27.6% in those living in the rural town. Since the occurrence of NIDDM is increasing in rural communities, it is expected that the prevalence of diabetic complications will also increase. DR is an increasing challenge to the Health System in Mexico, even in rural communities.

1958

SPECIFIC FEATERES EYE – LESIONS IN DIABETIC CHILDREN.

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During 1995/96 years 176 children with IDDM aged 3-15 years were examined in children's department of the Endocrinology Center. The average duration of disease was 6,3 ± 0,9. The onset of IDDM in the age up to 5 years was in 65 (36,9%), up to 10 years - 81 (46%) and from 10 to 15 years - 30 (17,1%) patients. Ophtalmoscopy, retinal photography and video recording with laser scan camera (CLSO, Zeiss) were performed. Optical nerve disk hyperaemia and papillit was discovered in 30,7% of patients, blood vessels were dilated in 18,2%, microaneurysms were found in 24,4%, retinal haemorrhages - in 15,9%, early stage of cataract - in 23,3%. Diabetic retinopathy was diagnosed in 21 children (12%). The second observation was performed after 6-12 months. After treatment steady state in fundus was achieved in 89,2% of patients, improvement - in 4%, progression of process in 6,8%. Laser coagulation of retina has been done for 8 children (4,5%). Further progression of diabetic retinopathy was stopped in 6 children. **Conclusion:** Early diagnostics of diabetic retinopathy and laser coagulation are the basis of the favourable prognosis.

1959

DECREASED PREVALENCE OF DIABETIC RETINOPATHY BUT INCREASED PREVALENCE OF PIGMENTARY RETINOPATHY IN DIABETES DUE TO A MITOCHONDRIAL DNA MUTATION

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Pigmentary retinopathy (PRet) is a recognised feature of mitochondrial genome (mtDNA) mutations. While the association of PRet with maternal inheritance diabetes and deafness (MIDD) is well recognised, the relationship between diabetes mellitus (DM), PRet and diabetic retinopathy (DR) has not previously been explored. The aim of this study, therefore, was to determine the prevalence of PRet in subjects with the 3243 mutation of mtDNA, to ascertain whether diabetes alters the phenotypic expression of PRet and to determine if there was any association between the presence of PRet and DR. Twenty maternal line family members (10 DM, 3 IGT, 7 NGT) had ophthalmic examination. Slit lamp was used in 18 of 20, with retinal photography of seven standard fields. Fluorescein angiography was performed in 13 of 20 subjects. Visual acuity was relatively preserved in all subjects (6/6-6/18). Nine of 10 DM subjects had clear cut PRet while 2 of 3 IGT subjects and 2 of 7 NGT subjects had PRet. A strong association between the presence of DM and PRet was found, χ^2_{2df} 6.83, p=0.03, and an even stronger association was found between the presence of abnormal GT and PRet, χ^2_{1df} 6.28, p=0.01. Age was not found to be a significant determinant of PRet. The odds ratio for PRet in subjects with abnormal GT was 13.7 (95% CI 1.5-127, p=0.02, R^2=0.25). The mean age of onset of DM was 44.3 ±15.4 yrs and the mean duration of DM at the time of retinal examination was 15.4 ± 7.4 yrs. Of the 9 DM subjects with PRet, two had mild background DR with microaneurysms only (duration of DM 17 and 21 yrs). No DM subject had proliferative DR. The mean HbA1c in the DM group was 8.9±1.3% (NR<6%) with 8/10 having an HbA1c >8%. Of the 20 subjects examined only one DM subject had evidence of early cortical cataract (80yrs). In conclusion, we propose that changes to normal glucose disposal within the retinal pigment epithelium (RPE) which occur as a consequence of reduced oxidative phosphorylation capacity may explain both the increase in PRet and the absence of DR and cataract. The former may be due to an increase in lactate and decrease in ATP production by the RPE while the latter may be due to a reduction in glucose disposal by the polyol pathway. A greater understanding of the effect of mtDNA mutations has the potential to give insight into the mechanisms of DR and other complications of DM.

1960

DIABETIC RETINOPATHY AND PREGNANCY

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Pregnancy was reported as an important risk factor in the development of diabetic retinopathy in women with Type 1. diabetes. The aim of the study was to estimate the ophtalmological status in Type 1. diabetic women before and during pregnancy as well as postpartum. 116 diabetics divided according to the White scale on 1 A, 63 B, 33 C, 18 D and 1 R were investigated. The control of diabetes was within the criteria of fast normoglycemia. Women included to White's D and R planned their pregnancies. The ophtalmology investigations were performed at each trimester. In 16% women with early diabetic eye complications pharmacological treatment (Calcium dobesilate, Quinax, Pentoxifillin, flavonoides) and/or adequate laser treatment were performed. Diabetic retinopathy was found in D and R classes of White. In A, B and C classes changes in the fundus of the eye before, during and after pregnancy were not confirmed. The exception were two C class patients with I ° retinopathy during pregnancy which persisted in the postpartum period. No progression of retinopathy was observed in 18 patients in White's D with diabetic retinopathy; in only one case regression after delivery was observed. In one patient included in class R the laser therapy during pregnancy caused the involution of diabetic retinopathy. These observations indicate that very good metabolic control of diabetes and ophtalmological investigation connected with adequate therapy of the initial changes in the eye did not progress in diabetic retinopathy in the presented group of diabetic pregnant women.

1961

FREQUENCY OF ADVANCED DIABETIC RETINOPATHY IN HOSPITALIZED PATIENTS.

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In order to ascertain the frequency of diabetic retinopathy (DR) among hospitalized diabetic patients, 354 medical records of such patients seen in consultation by the Endocrinology Service during a 12-year period were reviewed. The presence of chronic complications of diabetes was established according to clinical data and complementary studies obtained from the records. Fundus examination through dilated pupils, performed by trained ophthalmologists, had been informed grading the findings as follows: I and II (background DR), III (preproliferative), and IV (proliferative). Patients' age (mean±SD) was 63.9±11.8 years (range, 16-90). There were 176 women and 178 men; 23 patients had type I and 331 type II diabetes DR was found in 56.3% of women and 50.6% of men. It was present in 52.2% of type I and 53.5% of type II patients. Mean age was similar in subjects with and without DR, but the duration of diabetes was significantly longer in the former (10.4±10.9 vs. 8.4±10.0 years; P<0.05). Subjects with DR had significantly higher frequency of other chronic complications (P<0.001), with the exception of coronary insufficiency, and required insulin treatment more frequently than those without. High blood pressure was present equally in both groups. Of 190 patients presenting DR, 45 had received previous laser photocoagulation. Of the remaining 145 patients, 45 had grade I, 49 grade II, 34 grade III, and 19 grade IV DR. Thus, the relative frequencies of these grades among the 309 diabetic inpatients without previous laser treatment were, respectively, 13.9, 15.8, 11.0, and 6.1%. DR was detected by funduscopy in over 50% of hospitalized diabetic patients. The frequency of advanced grades (III, IV) was 17.1%, exceeding the reported prevalence among ambulatory patients. Funduscopy should be done regularly in diabetic patients admitted to a hospital, especially among those presenting chronic complications of diabetes. This would yield a considerable number of previously undiagnosed cases of DR, including many with preproliferative and proliferative changes, where photocoagulation is urgently needed.

1962

LONG-TERM EFFECTS OF PREGNANCY ON DIABETIC COMPLICATIONS

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The aim of our study was to establish whether pregnancy affects long-term development and progression of retinopathy and nephropathy in diabetics compared to nulliparous diabetic women. Twenty-eight diabetic women who had delivered in 1983-85 at Helsinki University Central Hospital and 17 nulliparous controls matched according to age, duration of diabetes and degree of vascular complications were personally interviewed and the current retinal status and renal function were assessed seven years later, in 1990-92. Serum creatinine, creatinine clearance, nocturnal albuminuria and HbA1c were measured and color fundus photography carried out. The results were compared to the status in 1983-85.
Of those who had been pregnant, five of 26 (19.2%) had experienced worsening of retinopathy. In three of these, proliferative retinopathy had developed out of only minimal background changes. In the control group, progression had occurred more often (8/16, P<0.05). The groups did not differ from each other regarding progression of nephropathy. The frequent controls during pregnancy could have provided the women with more knowledge about their disease and motivated to better self-care.

1963

PREVALENCE OF RETINOPATHY IN DIABETIC PATIENTS ATTENDING THE TIKUR ANBESSA HOSPITAL (TAH) DIABETIC CLINIC. SEYOUM B, MENGISTU Z, BERHANU P, ABDULKADIR J, FELEKE Y, WORKU Y, AYANA G, ADDIS ABABA UNIVERSITY, ADDIS ABABA, ETHIOPIA.

A total of 302 patients was selected by taking alternate patients from regular attendants of the TAH diabetic clinic to determine the prevalence of retinopathy. The mean age was 41.4 years (range 14-85). There were 160 males (53%) and 142 females (47%). One hundred forty (46.45) were type 1 and 162 (53.6%) were type 11. The mean duration of diabetes was 9.4 years; mean haemoglobin A1c (HgA1c) was 10.4%. On the day of the examination the mean fasting blood glucose (FBG) and random blood glucose (RBG) were 195.5mg% and 273.1mg% respectively. The mean serum total cholesterol, triglycerides, LDL, VLDL and HDL were 166.5mg%, 129.9mg%, 94.5mg%, 24.4mg%, and 44.3mg% respectively. The overall prevalence of retinopathy was 37.8% out of which 113 patients (36.1%) had background retinopathy and 5 patients (1.7%) had proliferative retinopathy. The retina could not be visualized in three patients because of dense cataract. Retinopathy correlated positively with age, duration of diabetes and blood pressure r=0.19 (95%CI 0.14 to 0.30), r=0.43 (95%CI 0.33 to 0.52) and r=0.25 (95%CI 0.14 to 0.35) respectively. However retinopathy has no significant correlation with HgAlc and serum lipids. Prevalence of retinopathy was comparable in type I & type II (P>0.05). The prevalence of retinopathy in our patients relative to the duration of diabetes mellitus is high. Therefore, improvement of facilities for the diagnosis and treatment of retinopathy is recommended.

1964

THE EFFECT OF PREGNANCY ON MINIMAL DIABETIC RETINOPATHY

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The aim of our study was to analyze the formation and disappearance rates of individual microaneurysms(MAs) during pregnancy in diabetic patients with mild background retinopathy. Fundus photographs were taken at the 12th, 24th and 32-36th weeks of pregnancy and 3, 6 and 12 months after delivery from 21 type 1 diabetics. MAs were localized twice from fundus photographs using a computerized system. We found that MA count was highest 3 months after delivery and not during pregnancy. Since the hormonal and hemodynamic effects of pregnancy are mostly normalized by six weeks postpartum, there seems to be a delay in the response of the retina to the stimuli aggravating retinopathy. There was a flare up of MAs during pregnancy in patients having HbA1c mean levels below the median value of 6.38. Also patients with a higher than the median (0.76) decrease in HbA1c level compared to prepregnancy HbA1c, developed more MAs during the course of pregnancy. It is known that rapid normalization of glucose levels can aggravate retinopathy. Our results indicate that a similar phenomen occurs also in patients with minimal retinopathy.

1965

Aggravation/progression factors of diabetic retinopathy

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Purpose;To retrospectively assess progression factors for diabetic retinopathy.Methods;555 patients examined in the ophthalmology dept.of our hospital among 710 pts randomly selected.Subjects:361 males,349 females,34 IDDM pts,673NIDDM pts,and 3 other pts.age 61.0 yr;history of illness,11.4±8.2 yr; neglect/interruption,2.5±5.1 yr.period of examination in the ophthalmology dept.:4.6±3.4 yr. Results: Retinopathy at the time of the initial examination was found to be associated with length of both history of illness and neglect in the following order;proliferative , pre-proliferative ,simple,and no retinopathy.271 pts had not yet developed retinopathy ,109 had developed retinopathy,21 with retinopathy that had improved,87 with no change,64 worse,and 3 others ,and also 24 pts with worsening of 2 grades or more.The interval between not having retinopathy and the time when 50% developped it was 11.5yr,and significantly longer than the 7.3-yr progressin interval from simple and the 7.6-yr after proliferative.Conclusion:Mean HbA1c,BP,and history of neglect/interruption of treatment affect the onset and progression of retinopathy after ophthalmologic management,but history of illness has no effect.

1966

THE EFFECT OF LISINOPRIL ON RETINOPATHY IN PEOPLE WITH INSULIN DEPENDENT DIABETES MELLITUS (IDDM).

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Several studies have examined the effects of ACE inhibitors on diabetic nephropathy, but their effects on retinopathy are less well understood. We examined the effect of the ACE inhibitor lisinopril on the progression of retinopathy in the EUCLID study. EUCLID is a 2 year randomised controlled clinical trial of lisinopril versus placebo in 530 IDDM men and women with normo or microalbuminuria, aged 20-59 years recruited from 18 European centres. Resting blood pressure at entry was between ≥ 75 and ≤ 90 mmHg diastolic, and ≤ 155 mmHg systolic. Retinopathy was assessed without knowledge of treatment status from retinal photographs taken at baseline and 24 months. Gradable photographs from both visits were available for 336 patients. Baseline prevalence of any retinopathy was 66% (111/169) in the placebo group, and 60% (100/167) in the lisinopril group (p=0.3). The majority of these had minimal non-proliferative retinopathy (60% in the placebo, and 64% in the treatment groups). Retinopathy was classified into 5 groups (none to proliferative). Progression was defined as a level of retinopathy that was at least one grade higher than that at baseline in the worst eye. The proportion of progressors was 26% and 12% in the placebo and lisinopril groups respectively (p=0.002). Thus the risk of progression was 0.41 (95% CI 0.23-0.74, p=0.003), in the lisinopril versus placebo group. This did not alter when adjustment was made for centre, sex, age, duration of diabetes, glycaemic control, baseline AER and blood pressure (0.45, 95% CI 0.24-0.83, p=0.01). We conclude that lisinopril has beneficial effects on diabetic retinopathy, even in those with little or no renal disease and minimal retinopathy, and that this is not fully accounted for by effects on blood pressure.

1967

THE ROLE OF THE RENIN-ANGIOTENSIN SYSTEM (RAS) IN THE PATHOGENESIS OF RETINOPATHY IN CHINESE NIDDM PATIENTS

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Genetic polymorphisms in the RAS may be involved in the pathogenesis of microangiopathic complications such as nephropathy. Local and systemic haemodynamic factors have been implicated in diabetic retinopathy. A total of 89 Chinese NIDDM patients newly referred to the PWH diabetes clinic were examined by an ophthalmologist for the presence and severity of retinopathy. The relationships between three RAS gene polymorphisms, angiotensinogen M235T, angiotensin-converting enzyme insertion/ deletion (ACE I/D) and angiotensin II type 1 receptor (AT1R) A1166C, were investigated in patients with (n=30) or without (n=59) retinopathy (RET).

Table 1 RAS gene frequencies in patients with or without retinopathy

Chinese NIDDM patients								
ACE	no RET n=59	RET n=30	AGT	no RET n=59	RET n=30	AT1R	no RET n=59	RET n=30
DD	10.2%	6.7%	TT	79.7%	80%	CC	0%	0%
ID	37.3%	40%	MT	16.9%	20%	AC	13.6%	13.5%
II	52.5%	53.3%	MM	3.4%	0%	AA	86.3%	86.5%
D freq	0.29	0.27	T freq	0.88	0.9	C freq	0.07	0.07
I freq	0.71	0.73	M freq	0.12	0.1	A freq	0.93	0.93

Patients with retinopathy had longer duration of diabetes (7.4±6.3y vs 3.8±6.2y, p<0.01), higher systolic BP (153±25 mm Hg vs 131±23 mm Hg, p<0.001) and prevalence of nephropathy (68% vs 28%, p<0.001). There was no significant difference in the RAS genotype frequencies between patients with or without retinopathy, even when analysed according to the use of insulin or prevalence of proliferative retinopathy. Thus in Chinese there is no evidence that the RAS genetic polymorphisms play a role in the development of retinopathy.

1968

INSULIN LISPRO (HUMALOG®) AND THE RENAL AND RETINAL COMPLICATIONS OF TYPE 1 DIABETES

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The renal and retinal changes associated with Type 1 diabetes were compared in subjects randomized to receive either a rapid-acting human insulin analog, insulin-lispro (Humalog®), or Humulin® regular insulin in this pilot study. Twenty-six young subjects (16 females and 10 males) with Type 1 diabetes for a mean duration of diabetes of 12.1 years (range = 2.3 to 24.0 years) participated in this prospective three year clinical trial. The duration of diabetes (p=0.8; Student t-test) and the gender distribution (p=0.4, Fisher's exact test) were similar in the two groups. The glycosylated hemoglobin values were not significantly different at baseline or after one or two years between the two groups. Retinal and renal evaluations included seven standard field color retinal photography and timed overnight urinary measurements of albumin excretion rates (AERs) prior to beginning the study, after one year, and after two years. There were no differences in retinal p=0.2; repeated categorical analysis) or renal (p=0.3; repeated categorical analysis) complications of diabetes between the two treated groups over time. Longitudinal data evaluation did not reveal any differences in worsening or improvement of the grade of diabetic retinopathy or in AER values between the two groups (p>0.05, Fisher's exact test). This pilot data suggests that the new insulin analog (Humalog®) is safe and is not associated with deterioration of diabetic retinopathy or nephropathy.

1969

DIABETIC RETINOPATHY AND HbA1c-ASSESSMENT BY A LOG-LOGISTIC REGRESSION MODEL

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Purpose: The relationship between the onset and progression of diabetic retinopathy and HbA1c was assessed by using a log-logistic model. **Method**: 585 patients examined in the ophthalmology dept. of our hospital among 710 pts randomly selected. Retinopathy progression factors were assessed by using a log-logistic regression model. **Subject**: 299 males, 286 females, 30 IDDM, 554 NIDDM, 1 other. 60.9 ±5.2yr. history of illness 11.8±8.3yr; neglect/interruption of treatment 2.7±5.2yr, period of examination in the ophthalmology dept. 4.6±3.4yr. Insulin 177 pts, SU agents 230, diet 178.

Results: 264pts without retinopathy, 109 with, 21 retinopathy improved, 85 no change, 64 worse, 24 worsening of 2 grades. It would be necessary to control HbA1c to 6.9%(onset alone, 7.0% and progression alone, 6.7%) or less in order to decrease the frequency of onset and progression below 5%. There was no association between HbA1c and the presence or absence of hospitalization for instruction at the initial examination, lipids, dBP, HCV infection, degree of obesity, or past maximum obesity.

Conclusions: To prevent diabetic retinopathy, after start of ophthalmologic examinations, HbA1c must be reduced to below 7.0%, and to prevent progression, to below 6.7%. Even stricter control is required in diabetics with a history of neglect/interruption of treatment.

1970

ACCELERATION OF DIABETIC RETINOPATHY FOLLOWING IMPROVED GLYCEMIC CONTROL: A REPORT ON 13 CASES

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Background: Improving glycaemic control(GC) not always improves the status of diabetic retinopathy(DR); the opposite may occur,i.e. the worsening of background retinopathy (BDR). We collected clinical data of IDDM patients in whom this phenomenon could be ascertained. **Patients and methods:** patients from a diabetes outpatient clinic with a documented decrease in HbA1c by > 0.5% per month and associated impairment of BDR (> 1 step according to EURODIAB complication study) were studied in retrospect (n=10)and prospectively (n=3).**Results:** 5 men and 8 women were identified, mean (SD)age 26(7)yrs, duration of IDDM 13 (3)yrs.Proteinuria was normal in 4/13, <500 mg/l in 5/13,and >500 mg/l in 4/13 patients, blood pressure was elevated in 4/13 patients. The initial HbA1c (HPLC; normal <5.6%)was 11.9(2.4)%,and declined by 1.2 (0.7)% per month upon improving GC.BDR progressed from stage 1-2 by ≥ 1 step, requiring laser coagulation (which came too late for 7 patients to prevent proliferative DR from bleeding).In 3/13 cases(1 with Mauriac's syndrome),serum IGF-1 was monitored prospectively demonstrating an increase by up to 100% prior to deterioration of BDR.**Conclusion:** In certain young IDDM patients improving GC may coincide with deterioration of BDR,which might be mediated by a reduced tolerance of the retina against IGF-1.

1971

VON WILLEBRAND FACTOR AND ITS RELATION TO THE GRADE OF RETINOPATHY IN NON INSULIN DEPENDENT DIABETIC PATIENTS

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Von Willebrand factor (vWF) is secreted by endothelial cells and megakaryocytes, and it promotes the adhesion of platelets to the thrombogenic subendothelial substance. In the present study, the level of vWF has been assayed by the platelets aggregation method; Behring Werke, in 45 NIDDM patients and 10 normal healthy controls to find the relation (if any) between vWF levels and the grade of retinopathy. Diabetic patients were classified into three groups, the first group consisted of 15 patients with no retinopathy, the second group consisted of 15 patients with non proliferative retinopathy, the third group consisted of 15 patients with proliferative retinopathy. All selected diabetic patients were free from medical diseases except diabetes and were not receiving drugs except oral hypoglycemics. The groups were matched for age, body mass index, mean blood pressure and renal function. The diabetic groups were matched for the duration of the disease and the glycemic control. Patients and controls with microalbuminuira were excluded. The results of the present study showed that the mean level of vWF showed no significant difference between diabetics and controls. Using the "F" and Scheffe tests, there was no significant difference between the three studied groups as regards vWF level. Moreover, no significant correlation was found between the level of vWF and any of the following parameters; body mass index, glycosylated hemoglobin, cholesterol or triglycerides in diabetics as well as in controls. From the present study it can be concluded that endothelial damage as evidenced by elevated vWF may not be a major factor in the pathogenesis of diabetic retinopathy to the same degree as it is suggested in diabetic nephropathy.

1972

The effect of glycaemic control and the introduction of insulin therapy on retinopathy in non-insulin dependent diabetes mellitus

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To study the progression of diabetic retinopathy in relation to diabetes treatment and glycaemic control in patients with NIDDM (non-insulin dependent diabetes mellitus), we performed a prospective study in a cohort of 1,378 diabetic patients, aged ≥ 40 years at diagnosis, 333 of whom were treated with insulin, and 1,045 of whom were treated with oral antihyperglycaemic agents or diet alone. In the latter group 174 patients changed to insulin therapy during follow-up. We used the Wisconsin scale to grade retinopathy, recorded blindness (visual acuity ≤ 0.1) and visual impairment (visual acuity 0.2-0.4), and measured the average HbA_{1c} for each patient during a mean 3.1-year study period. Poor glycaemic control and moderate non-proliferative retinopathy at baseline were significantly associated with retinopathy progression. A non-linear relationship was found between mean HbA_{1c} and the 5-year risk of progression of retinopathy ≥ 3 levels with a threshold value of about 8.0 %. In contrast, insulin treatment at baseline was not associated with an increased risk of retinopathy progression. Patients who changed treatment from oral agents or diet alone to insulin therapy had an increased risk of retinopathy progression compared with all other patients in the study. The increase in risk remained even after controlling for mean HbA_{1c}. Progression ≥ 3 levels was significantly associated with a higher incidence of macular oedema and deterioration of visual acuity. The relative risk for blindness/visual impairment due to retinopathy was 2.7 (95 % confidence interval 1.8-4.0) in the group with changed treatment compared with all the other patients in the study. Poor glycaemic control (HbA_{1c} %) before the start of insulin therapy and any retinopathy at baseline were significant risk factors for progression in the group with changed treatment (both $p < 0.01$).

1973

VARIABLES ASSOCIATED WITH RETINOPATHY IN PATIENTS WITH NIDDM FROM A MULTI-ETHNIC URBAN AUSTRALIAN COMMUNITY

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Cross-sectional data from 925 evaluable patients with NIDDM of the first 1,000 recruited to the Fremantle Diabetes Study (a prospective study of ethnic differences in care, control and complications in diabetic patients drawn from a population base of 118,000) were analysed to identify variables associated with the presence of retinopathy. Ethnicity was defined in two ways; using only region of birth and from self-perception of ethnic background in combination with other objective demographic data. Those born in Australia formed 52%, in Southern Europe 19%, in the UK/Ireland 16% and in Asia 4%. Ethnic background was Anglo-Celt in 63%, Southern European in 20%, Asian in 3% and Aboriginal in 1%. Retinopathy, found in 17% overall, was assessed from direct and/or indirect ophthalmoscopy, and/or more detailed ophthalmological data in a proportion of patients assessed for photocoagulation. Multiple logistic regression analysis revealed that the known risk factors duration of diabetes, fasting plasma glucose, HDL-cholesterol, systolic blood pressure and treatment type were significantly and independently associated with the risk of any retinopathy ($P<0.05$). Of simple categorical socioeconomic variables incorporated in the model, educational attainment was inversely associated with retinopathy risk ($P=0.01$). After adjusting for these significant variables, region of birth was not a risk factor for retinopathy although birth in Asia was of borderline significance ($P=0.051$). When ethnic background was included, Asian ethnicity was associated with a signficantly increased risk of retinopathy ($P=0.01$). These data confirm that conventional risk factors for diabetic retinopathy operate in an urban Australian population. Lower educational attainment and Asian ethnicity are also significant risk factors, findings which could be used to ensure better targetting of retinopathy screening.

1974

Complication free duration is not a protective factor for the development of retinopathy in elderly diabetic patients.

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Aim: To assess whether long complication free duration can define elderly patients in lower risk for future development of diabetic retinopathy. **Methods:** 10 year clinic based study of a regional diabetic outpatient clinic serving a 150,000 urban population. Data on 647 type 2 diabetic patients, free of diabetic retinopathy, older than 50 years and followed for more than 4 years is analyzed. **Measurements:** Data included demographic and clinical information on arrival, updated every 3-6 months and yearly direct ophtalmoscopy. All the data was prospectively compiled on relational databases. **Statistical analysis** Statistical analysis was performed with student's T test x^2 when appropriate, discriminant analysis for defining significant and independent variables for predicting retinopathy was performed using process discriminant. **Results:** 8.5% of patients without retinopathy at the age of 50 developed retinopathy during 4 years of follow-up. These patients are characterized by longer duration and younger onset of diabetes in comparison to the group without retinopathy at 4 year follow-up. Clustering of micro- and macro- vascular complications were noted. Discriminant analysis showed the following factors to be significantly and independently predictors of the development of retinopathy in the elderly :Duration of diabetes, BMI and glucose control. **Conclusions:** Long complication free period does not define elderly patients with type 2 DM patients at lower risk for future development of retinopathy. On the contrary, the increase in disease duration is significantly associated with the development of retinopathy in this age group as described in younger patients.

1975

Association of NIDDM with cataract and retinopathy

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443 consecutive patients of NIDDM were examined by an endocrinologist and an ophthalmologist independently. Apart from routine investigations fundus examination was carried out by direct ophthalmoscope after dilating the pupils. Cataract status was evaluated by slit lamp examination. The mean age of the patients was 47.9 years and mean duration of diabetes was 7.1 years. Of 351 patients having no evidence of retinopathy the mean age was 46.4 years and mean duration of diabetes was 6.0 years, whereas these were 53.1 and 11.6 years respectively in patients showing diabetic retinopathy. Correlations between age of patients with diabetic retinopathy and cataract were statistically significant ($p<0.05$). Similarly correlations of duration of diabetes with retinopathy and cataract were also significant ($p<0.05$). Correlation of retinopathy and cataract was not statistically significant. Drusen bodies (observed in 3.2%) was the only feature where the two observers were not in conformity.

1976

CHANGES OF NONPROLIFERATIVE RETINOPATHY IN NONINSULIN-DEPENDENT DIABETIC PATIENTS.

W-T. Lu, S-S. Yarng, F-H. Liu, H-S. Huang, J-D. Lin and J-H. Juang. Chang Gung Memorial Hospital, Taipei, Taiwan, R.O.C.

To known the nature course of mild to moderate nonproliferative retinopathy in noninsulin-dependent diabetes mellitus, we followed 191 diabetic patients at our metabolic clinic. Twice examinations in one year were performed by using Canon CR-45 UAF non-mydratic fundus photography. Modified Airlie House Classification of diabetic retinopathy was used. There were 97 males and 94 females with the mean age of 57 years and mean duration of 7 years. Among these patients, 13 were treated with insulin, 162 with oral hypoglycemic agents (OHA), 8 with OHA and insulin, and 8 with diet alone. The patients were divided into 4 groups.

	Retinopathy		
Group	Initial	Final	No (%)
A	Negative	Negative	117 (61)
B	Negative	Positive	21 (11)
C	Positive	Negative	9 (5)
D	Positive	Positive	44 (23)

In group B and C, the changes of blood pressure and blood glucose were not related to the changes of retinopathy. The change of glycohemoglobulin (HbA1c) level was lower in group C (0.6 ± 0.1 %) when compared to the patients of group D (1.5 ± 0.3 %) (p=0.009). The differences were analysed by Student's t test. Our data indicate mild or moderate nonproliferative retinopathy may reverse in some cases and the glycemic control may contribute to it.

1977

ABSENCE OF AN ASSOCIATION BETWEEN SERUM SIALIC ACID AND RETINOPATHY IN SOUTH INDIAN NIDDM PATIENTS.

Deepa R, Rema M, Karkuzhali K, Mohan V, M.V. Diabetes Specialities Centre and Madras Diabetes Research Foundation, Madras, India.

An association between serum total sialic acid and various diabetic complications including retinopathy has been reported in European NIDDM patients. We studied serum sialic acid levels in healthy non diabetic controls and in three groups of non insulin dependent diabetes mellitus (NIDDM) patients, namely background diabetic retinopathy (BDR), proliferative diabetic retinopathy (PDR) and those without any retinopathy. Serum sialic acid concentrations were not significantly different between the four study groups: controls (2.27 ± 0.48 mmol/L), NIDDM without retinopathy (2.50 ± 0.46 mmol/L, NIDDM with BDR (2.27 ± 0.50 mmol/L) and NIDDM with PDR (2.43 ± 0.55 mmol/L). These results suggest that there is no association between diabetic retinopathy and sialic acid in South Indian NIDDM patients.

1978

THE FOLLOW-UP RESULTS OF DIABETIC RETINOPATHY WITH A PROPOSAL OF CLASSIFICATION BASED ON A MULTINATIONAL STUDY

E. Miki, Shikata Memorial Miki Clinic, Chiba, Japan

The results concerning diabetic retinopathy of the World Health Organization Multinational Study of Vascular Disease of Diabetes (WHO MSVDD), carried out since 1975, initially in 14 centers in 13 counties, is presented. Based on this data, a simplified yet objective system of recording diabetic retiinopathy suitable for usual medical description and practice seems possible. After a follow-up of 8.4± 2.8 years of WHO MSVDD, 2877 (71.6%) out of 4018 surviving patients in 10 centers of 9 countries participated in funduscopic examination using the same method as in the prevalence study. Simple and objective methods were tried and adoptedin WHO MSVDD, including fundus examination. Retinopathy developed on the average in 47.7% (range: 29.7-76.4%), while the progression to proliferative diabetic retinopathy occurred in 9.7% (range: 3.5- 22.6%). The risk factors for the incidence of retinopathy were: therapeutic regimen, serum cholesterol, systolic blood pressure, and renal disease, while those for the progression to proliferative retinopathy were: therapeutic regimen, renal disease, duration of diabetes, diastolic blood pressure, and age at baseline. Concerning the incidence of retinopathy, the increase or decrease in sensitivity due to different observers (i.e., ophthalmologists vs. internists) seems important besides true changes. In contrast to macroangiopathy, which was found to be very variable among the centers, the progression of diabetic retinopathy seems tobe more uniform. In view of the absence of a simple and objective method of classifying diabetic retinopathy suitable for daily medical practice and description, the experience obtained in this study seems very meaningful for future utilization.

1979

RETINOPATHY AT DIAGNOSIS IN SOUTH INDIAN NIDDM PATIENTS: ASSESSMENT BY RETINAL PHOTOGRAPHY

Rema M, Ponniah M and Mohan V, M.V.Diabetes Specialities Centre and Madras Diabetes Research Foundation, Madras, India.

To assess the prevalence of retinopathy at the time of diagnosis of diabetes, 157 consecutive newly diagnosed (<1 month duration) NIDDM patients attending our centre were studied. Retinopathy was assessed clinically (direct and indirect ophthalmoscopy) and by retinal photography after full mydriasis. Four retinal fields were studied by 35 mm colour transparencies using a 50-VT Topcon retinal camera. Photographs were graded by two independent observers using a modification of the Early Treatment Diabetic Retinopathy Study (ETDRS) classification system. In 5 patients photographs could not be graded due to cataract (2 patients) and poor quality of photographs (3 patients). Eleven of the remaining 152 patients (7.2%) had diabetic retinopathy. All patients had non-proliferative (background) retinopathy but 2 had evidence of maculopathy.The prevalence of retinopathy at diagnosis in South Indian NIDDM patients appears to be lower than that reported in Europeans.

1980

DIABETIC COMPLICATIONS IN NIDDM: RELATIONSHIP TO UPDATED HbA1c.

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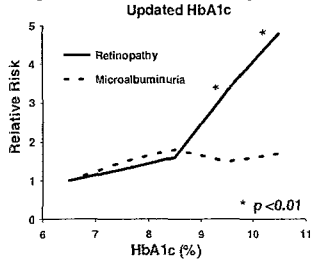
The DCCT reported a smooth relationship between HbA1c (ie mean HbA1c during follow up) and diabetic complications in IDDM. Krolewski et al reported their findings on microalbuminuria (MA) and retinopathy (RET) in IDDM, noting that the risk increased abruptly at an HbA1c threshold of about 8%. The precise relationship between glycaemic exposure and complications in NIDDM is less well defined . Data of 832 NIDDM patients (age: 56.8 ± 10.8 yrs [mean±SD]; duration: 5.7 ± 5.9 yrs; HbA1c: 8.1 ± 1.7% [nr:3.5-6%]) attending our Diabetes Centre for Complications Assessment more than once (2.8 ± 1.8 visits;range: 2-6) over a mean interval of 34 months were analysed. Of those with no RET (n=707) or no MA (30-300mg/L) (n=399) at initial visit, annual incidence of RET was 4.9% (95% CI 4.0-5.8) and MA was 7.0% (95% CI 5.6-8.3). Incidence of developing RET and MA and their relative risk (RR) adjusted for duration of diabetes and the interval between visits were then stratified according to updated HbA1c (Fig 1). We conclude that for NIDDM patients, high glycaemic exposure significantly increases the risk of RET particularly at updated HbA1c of >9% . The corresponding increase in risk of microalbuminuria is more gradual with no clear HbA1c threshold .

Figure 1: Relative Risk According to Updated HbA1c



1981

PREVALENCE, INCIDENCE AND FACTORS CONNECTED WITH DIABETIC RETINOPATHY IN IDDM AND NIDDM PATIENTS

P. Luźniak, A. Czech and J. Tatoń. Department of Internal Medicine and Diabetology, Warsaw Medical School, Warsaw, Poland.

Diabetic retinopathy is the most frequent microvascular sequele of chronic long term IDDM and also very frequent complication of NIDDM. Many studies are pointing now to the possibility of prevention and early treatment of this syndrome from both ophthalmological and diabetological point of view. Therefore early identification of diabetic persons of higher risk of retinopathy development seems to be of crucial importance in practice. The study was undertaken to determine risk factors in 2 groups, that is in IDDM and NIDDM patients. One group comprises 309 IDDM and the other 1334 NIDDM subjects. Morbidity due to all forms of retinopathy was 55.4% in IDDM and 31.4% in NIDDM, the frequency of proliferative retinopathy was respectively 11.0% and 1.3%. The incidence of all forms of retinopathy in 3 - year prospective study was 6.1% per year in IDDM and 3.9% per year in NIDDM. The incidence of proliferative retinopathy was respectively 1.3% per year and 0.6% per year. In the second part of the study the identification of selected factors connected with high risk of development of diabetic retinopathy was undertaken. The following factors were examined: age, sex, diabetes mellitus duration, body mass index, arterial hypertension, total cholesterol triglycerides and creatinine levels, fasting and postprandial glycemia and 24-hour proteinuria. Conclusion. The most significant risk factors connected with development of retinopathy were: diabetes duration, fasting glycemia and proteinuria. On the base of this study, practical algorythms were proposed, with proposals of preventive action.

1982

QUANTUM HEMOTHERAPY IN THE PATIENTS WITH DIABETIC RETINOPATHY.

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We have studied the influence of trancutaneus laser radiation of blood (TLRB) upon ophtalmologic lymphocirculation indices of 68 patients with nonproliterative diabetic retinopathy (DR). Radiation power was 20 mw and wavelength was 630 nm. Fluorescent lymphangiography demonstrated essential changes of bulbar conjunctiva lymph flow. Medium diameter of lymphaticus (DL) has increased to 0,27 ± 0,02 mm and linear lymph flow rate (LLR) has fallen to 4,2 ± 0,12 mm/min compared with age norm (0,19 ± 0,03 mm and 6,9 ± 0,16 mm/min). Volumetric lymph flow rate (VLR) has increased to 1,9 ± 0,09 mm^3/min (norm.: 0,7 ± 0,11 mm^3/min). After the course of TLRB this indices were: DL = 0,24 ± 0,01 mm; LLR = 5,6 ± 0,12 mm/min; VLR = = 1,2 ± 0,11 mm^3/min. This indices were more statistical expressible than in control group (23 patients). Given results confirm pathogenetic directness of TLRB in tritment and prophylaxis of tissue ishemia in DR.

1983

PREDICTIVE FACTORS FOR THE PRESENCE OF DIABETIC RETINOPATHY IN CHINESE NIDDM PATIENTS

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Despite recent improvements in diabetes care and the general acceptance that good glycaemic control reduces the risk of microvascular complications, diabetic retinopathy remains a leading cause of blindness. Even though effective treatment with laser photocoagulation is available, timely intervention is often difficult as sight-threatening stages are often asymptomatic. We randomly selected 607 (Male266;Female341) NIDDM patients with no visual complaints from the University Diabetes Clinic and screened for the presence of any diabetic retinopathy using direct fundoscopy with pupillary dilatation. These patients had a follow up of 10.3 ± 6.6 years since diagnosis, with mean fasting blood glucose of 8.9±3.0 mmol/l, glycosylated haemoglobin (HbA1c) of 7.8± 1.9%, systolic blood pressure 139±18 mmHg and diastolic blood pressure 79±8.9 mmHg (mean ± SD). Their glycaemic control was achieved by diet only (1.3%), oral hypoglycaemic agents (66.2%) or insulin (32.4%). Elevated serum creatinine levels (>120umol/l) was found in 11.6%. Serum albumin level was 42.6 ± 4.2 g/dL. Fasting total cholesterol and triglyceride levels were 5.5 ± 0.05 and 2.1 ± 0.08mmol/l (mean±SE) respectively. Proteinuria was found in 13% of patients (albustix≥ 1+). Peripheral neuropathy (vibration perception threshold >25 on either right or left extremity) was found in 23%. Diabetic retinopathy was found to be present in 19% (116/609) of the patients. (Early Treatment Diabetic Retinopathy Study Classification: mild nonproliferative 68%; moderate nonproliferative 16%; early proliferative 2% and macular oedema 14%). Multiple regression analysis showed that proteinuria ($P < 0.0001$), glycosylated haemoglobin A1c ($P < 0.005$), duration of known disease ($P < 0.005$) and vibration perception threshold values ($P < 0.05$) were predictive of underlying diabetic retinopathy. All other variables tested were not significant. Use of a dipstick test for proteinuria can serve as a simple and economic way of screening for at risk NIDDM patients for further opthalmological examination. This strategy should be an useful alternative in countries where population-based screening for diabetic retinopathy is not feasible.

1984

VISCODELAMINATION DURING VITREOUS SURGERY FOR SEVERE DIABETIC RETINOPATHY

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Viscodelamination technique was employed during vitreous surgery in 217 eyes with taut preretinal fibrovascular membrane and tractional retinal detachment in advanced diabetic retinopathy. As a routine procedure, 1% sodium hyaluronate was injected to separate the epiretinal membrane and the adherent vitreous corfex from the retina. The preretinal membrane was then severed with scissors. Out of all the 217 eyes, anatomical improvement was attained in 155 (72%), unchange in 38 (17%) deterioration in 24 (11%) when evaluated at 25 months on average after surgery. Improved visual acuity was attained in 123 (57%), unchanged in 66 (30%), deterioreted in 28 (13%) out of 217 eyes. Iatrogenic retinal breaks developed in 73 eyes (34%) during surgery which could be treated with intraocular photocoagulation. The viscodelamination technique thus proved to be safe and effective means as adjunct to vitreous surgery to separate epiretinal membrane and the vitreous cortex from the retina.

1985

RISK FACTORS FOR THE PROGRESSION TO PROLIFERATIVE *RETINOPATHY IN JAPANESE NIDDMPATIENTS.*

Nakagami T., Kawahara R., Hori S., Omori Y., Tokyo Women's Medical College, Tokyo, Japan

-Aim- To examine risk factors for the progression to proliferative retinopathy from simple retinopathy in Japanese NIDDM patients. - Subjects- and Methods- 159 NIDDM patients with simple retinopaty first visited our Diabetes Center from 1983-1985. Their age at diagnosis ranged from 30-65 years. The optic fundi were examined at least annually. HbAlc *values of each patients were measured every month. The prevalence of* proliferative retinopathy in the 10th year after registration was compared in 5 groups stratified by mean HbAlc values for ten years, in 2 groups stratified by mean diastolic blood pressure for ten years and in 2 groups strafified by the duration of diabetes at registration. Multiple logistic regression analysis was used to assess the ralationship between proliferative retinopathy and covariate. - Results - The prevalence of proliferative retinopathy was 0%(0/1) in the group with mean HbAlc below 6%, 7.7% (2/25) in the group with mean HbAlc of 6-6.9%, 5.3%(3/57) in the group with mean HbAlc of 7-7.9%, 12.5%(5/40) in the group with mean HbAlc of 8-8.9% and 30.6%(11/36) when the mean HbAlc exceeded 9%. The prevalence of proliferative retinopathy increased with the increase in the mean HbAlc values over 10 years(trend $p<0.005$). The prevalence of proliferative retinopathy of the patients with a duration over 10 years was 2.5 times higher than that of the patients with a duration less than 10 years($p<0.05$). The prevalence of proliferative retinopathy of the patients *with diastolic blood pressure above 90mmHg was 6 times higher than that* of the patients with diastolic blood pressure less than 90mmHg($p<0.005$). Multiple logistic regression analysis revealed that mean HbAlc, diastolic blood pressure and duration of diabetes were the risk factors for the progression to proliferative retinopathy from simple retinopathy. - Conclusion - This data suggests that better control of blood glucose and diastolic blood pressure might decrease the progression to proliferative retinopathy from simple retinopathy in Japanese NIDDM patients.

1986

VISUAL EVOKED POTENTIALS IN PEOPLE WITH IMPAIRED GLUCOSE TOLERANCE

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This study is conducted in order to evaluate the visual pathways from the retina to the occipital cortex in people with impaired glucose tolerance (IGT). We studied visual evoked potentials in 17 patients with IGT, as proposed by WHO's criteria, (34 eye, mean chr. age 50.7±10.5yrs), and in 19 controls with normal oral glucose tolerance (38 eye, mean chr.age 48.9±11.2yrs). Eyes with low vision or any anterior or posterior segment findings are excluded. The test was performed monocularly with two different checkerboard pattern sizes (subtending 30 and 60 minutes of arc). Patients with IGT showed a significant delay in peak latencies of P100 wave (LP100) (in ms) compared to the controls (with pattern size of 30 minutes of arc 114.9±8.5 vs 100.4±5.0, $p<0.001$; with pattern size of 60 minutes of arc 118.5±10.9 vs 101.2±4.4, $p<0.001$). Amplitudes (μV) were also significantly decreased in patients with IGT (pattern size of 30 minutes of arc 2.5±2.2 vs 3.9±1.5, $p<0.05$; with pattern size of 60 minutes of arc 3.0±1.5 vs 4.2±2.0, $p<0.05$). No interocular differences were detected regarding LP100 and amplitudes. As a conclusion, these findings indicate to a subclinical disease and shows that the visual pathway is not spared from involvement in people with IGT.

1987

PERIPAPILLARY RETINAL CAPILLARY FLOW IN DIABETICS USING CONFOCAL SCANNING LASER DOPPLER FLOWMETRY

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<Purpose>: There have been reports that blood flows in the retinal artery and vein varied according to the stage of diabetic retinopathy, but there has been no report to date concerning the flow in the capillary vessels. Here we report the results of our investigation into changes in the peripapillary retinal capillary flow at various stages of diabetic retinopathy. <Subjects & Methods>:We examined 211 eyes of 121 diabetics (56 males and 65 females) aged from 34 to 78 years. By diabetic retinopathy stage there were 83 with non-diabetic retinopathy (NDR), 90 with simple retinopathy (SDR), 26with preproliferative retinopathy (PPDR), and 12 with proliferative retinopathy (PDR). The capillary flow rate was measured by scanning laser doppler flowmetry using a Heidelberg Retina Flowmeter (HRF), which combines the confocal laser scanning technique with laser doppler flowmetry and enables non-invasive two-dimensional mapping of the retinal perfusion. Four areas were measured :- the nasal tempolar region, the upper tempolar region, the lower perpapillary retina region, and the ONH disc. The field examined was 10x2.5 degrees with each field frame consisting of 10x10 pixels. The peripapillary retina and the ONH were scanned by the laser at 795 nm. Data were obtained on 30 points per quadrant of the retina. <Results>:The average capillary flow rate was 465.6 ±2.2 AU for those with NDR, 476.7 ± 1.9 AU for those with SDR, 489.0 ± 3.5 AU for those with PPDR, and 577.8 ± 4.0 AU for those with PDR. <Conclusion>:The capillary flow rate of diabetics were markedly elevated, compared to normal values[1], even at the early stage NDR, and the capillary flow rate then increased with the progression of diabetes stage.

1)Yamana Y et al.,:Motives for ophthalmic Examination of Diabetics.;Prevention and treatment of non-insulin-dependent diabetes mellitus,NIDDM Goto Y ed, 6th Japan-Korea Symposium on Diabetics Mellitus, 363-365. Smith-Gordon London,1992

1988

ASSESSMENT OF RETINOPATHY AND COLOUR VISION IN MATURITY-ONSET DIABETES OF THE YOUNG

BC Lee, M Appleton, D Taylor, J Jacob, AT Hattersley, AC Shore and JE Tooke. University of Exeter, Exeter, UK.

Maturity-onset diabetes of the young (MODY) is a discrete form of NIDDM and it has been suggested that these patients are protected from microvascular complications. Impaired tritan (blue/green) colour vision has been reported in IDDM, NIDDM and in subjects with fasting hyperglycaemia, and is associated with the development of retinopathy. No systematic analysis of the retinal status or function has been made in MODY. We assessed colour discrimination sensitivity by using the Sussex Colour Screener to determine whether there is functional disturbance of colour vision in 18 (9M:9F) subjects with MODY, mean duration of diabetes 13.1 ± 10.4 years (mean ± SD), and age and sex matched normoglycaemic controls (37.5 ± 10.7 v 38.2 ± 11.1 years). The mean glycated haemoglobin of the MODY group (normal range 4.0-6.0%) was 6.8 ± 1.8%. Retinal status was also examined in the patients by using conventional ophthalmoscopy and retinal photography. Retinal photographs were evaluated by an independent ophthalmologist in a single blind study. Tritan discrimination sensitivity was significant reduced in MODY patients (41.07 ± 7.76 v 46.57 ± 7.03 arbitrary units $p<0.05$ Mann-Whitney). Impaired colour vision correlated with degree of retinopathy (R_S=–0.5885, p=0.018). 50% of the MODY patients had background retinopathy, 4 in one eye and 5 in both eyes. Background retinopathy was absent in 4 individuals with diabetes less than 5 years duration. Maculopathy and proliferative retinopathy were not detected. These results suggest that MODY patients are not protected from developing retinopathy or functional abnormality even when reasonable glycaemic control is achieved.

PS 51

Nephropathy – Experimental and Renal Damage

1989

EFFECT OF NITRIC OXIDE SYNTHASE INHIBITOR AND TEMOCAPRIL ON PROGRESSION OF EXPERIMENTAL DIABETIC NEPHROPATHY

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The aim of the study is to clarify the effect of nitric oxide synthase inhibitor : N-nitro-L-arginine (NNA) and temocapril (TP) on the development of diabetic nephropathy in rats previously treated with streptozotocin. Rats were divided into six groups four weeks after the development of diabetes : Group 1drank tap water; Group 2 drank tap water with L-arginine; Group 3 water with NNA; Group 4 water with TP; Group 5 with NNA and TP; Group 6 water with TP and Arg. Control rats drank tap water and water with Arg. Blood pressure, body weight, blood glucose, urine volume and urinary albumin were measured before and at eight, 16 and 21 weeks. Urine albumin increased significantly in diabetic rats. Among them, Group 3 and Group 5 had significantly increased albuminuria more than other diabetic groups at 16 and 21 weeks. Albuminuria at 21 weeks in Group 5 is lower than in Group 3. However it is not statistically significant. Blood pressure also elevated in diabetic rats. In Group 3 and Group 5, that of 8 weeks correlated significantly with albuminuria at 16 and 21 weeks. It decreased after 16 weeks in Group 4 and Group 6. The present study showed the significant effect of nitric oxide synthase inhibitor on the development of albuminuria. Temocapril had significant effect of ameliorating the blood pressure. The result also showed that the elevation of blood pressure may contribute to the development of diabetic nephropathy.

1990

CRONOBIOLOGY OF BLOOD PRESSURE (BP) IN BB-RATS

U. Fischer, S. Berg, P. Heinke and A. Dunger. Gerhardt Katsch Institute of Diabetes, Karlsburg, and Inselklinik, Heringsdorf, Germany.

Alterations in rhythmic BP pattern were reported in human and animal hypertension. This study evaluates these patterns in diabetic (d) and nondiabetic (nd) BB-rats. - Systolic (SYS) and diastolic (DIA) BP, heart rate (HR) and muscular activity (MA) were monitored over 48h at 5min intervals in insulin pellet-treated dBB (diabetes duration 150d), in ndBB, and in age-matched hypertonic (SHR) and normotonic (WOK) controls by means of implanted telemetric BP monitoring system (Data Science Int). - Results: Cross correlation showed excellent synchronisation among all variables. BB-rats had marginally elevated average BP but reduced HR. The amplitudes of all daily rhythms and their % of total variance were elevated, and the acrophases were delayed in BB-rats. Spectral analysis of ultradian periods revealed a shift towards longer periods but no difference in the normalized spectral estimates in BB-rats. - Conclusion: BB-rats exhibit a tendency towards hypertension. The concomitant alterations in the rhythmic patterns of MA, BP and HR appear to be related to diabetes rather than to hypertension.

	means ± SD		d BB (4)	d BB (5)	SHR (5)	WOK (3)
MA	mean	$(5min)^{-1}$	20.7±2.2	12.9±8.1	14.7±4.8	11.2±5.3*
	circadian amplitude		13.5±4.4	7.2±5.0	2.8±1.5*	2.6±0.8*
	ultradian[1)]	h	3.4±0.9	3.9±0.8	3.4±0.7	3.9±1.0
SYS	mean	mm Hg	133±6.0	126±7.0	151±4.0*	122±4.0*
	acrophase[2)]	h p.m.	12.5±1.2	11.5±3.5	10.7±2.8	8.9±2.5*
	circadian amplitude[2)]		5.3±0.6	3.0±1.4	2.0±0.6*	1.6±0.9*
	ultradian[1)2)]	h	3.4±0.0	3.9±0.9	3.0±0.2*	3.7±0.8
HR	mean	min^{-1}	321±13	347±29	337±5*	371±23*
	circadian amplitude		38±5	24±14	8±3*	17±5*
	ultradian[1)]	h	3.4±0.0	4.1±0.8	3.1±0.2*	3.1±0.2*

[1)]dominating period [2)] identical behavior of DIA *p<0.05 vs. dBB and/or ndBB

1991

MESANGIAL CELL AND MATRIX PROTEOGLYCANS: RESPONSE TO GLUCOSE AND ASCORBIC ACID

A.V.McAuliffe, E.J.Fisher, S.V.McLennan, D.K.Yue and J.R.Turtle. University of Sydney, New South Wales, Australia.

Proteoglycans (PG) are highly charged glycosylated proteins attached to mesangial cell (MC) membranes and embedded in mesangial matrix (MM). Expansion of the glomerular mesangium is the most consistent lesion of diabetic nephropathy and abnormalities of PG affecting cell-cell and cell-matrix interactions are thought to play a significant role in this process. AA, an important regulator of extra cellular matrix synthesis, is metabolised abnormally in diabetes. In this study we examined the effects of 0.28mM ascorbic acid (AA) on PG synthesis and its deposition in matrix in rat MC grown in 9mM (LG) or 25mM (HG) glucose for 8 days. PG were measured by labelling with 50μCi of [^{35}S] sulphate present in the culture media and in terms of DPM and precipitablity with 2.5% cetylpyridinium chloride. Activity was corrected for μgDNA (raw data) and [^{35}S] sulphate specific activity (corrected data) and expressed as a % of 9mM glucose values (mean±sem). AA increased ^{35}S-labelled PG in cells and matrix. After correction for [^{35}S] sulphate specific activity this remained elevated in the matrix. HG suppressed the effect of AA in both cells and matrix.

	LG-AA	LG+AA (raw data)	LG + AA (corrected data)	HG + AA (corrected data)
		^{35}S-labelled PG		
Cells	100±5	[a]129±7	108±7	[b]70±6
Matrix	100±11	[a]162±11	[c]142±16	[b]87±9

ANOVA: [a]significantly different to LG-AA, $P < 0.001$.
[b]significantly different to LG-/+AA, $p < 0.0001$.
[c]significantly different to LG-AA and HG+AA, $p < 0.0001$.

These results indicate that HG inhibits the action of AA on sulphation of PG, perhaps by impairing glucosaminyl N-deacetylase activity. This may be of particular importance in diabetes where AA level is already compromised. These findings may be of importance in the pathogenesis of diabetic nephropathy.

1992

INCREASED BLOOD PRESSURE (BP) AND ALBUMINURIA AT DIABETES ONSET IN BB RATS

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Increased arterial BP is the most important risk factor for the progression of diabetic nephropathy. It is not proved whether elevated BP results from or precedes the impairment of kidney function. Therefore diabetes-susceptible BB rats were monitored during the interval when their diabetes usually becomes manifest (DM), using an implanted telemetric BP monitoring system. Systolic (SYS) and diastolic (DIA) BP, heart rate (HR), motor activity (MA) and renal excretion of albumin (AE), N-acetyl-ß-D-glucosaminidase (NAG), creatinine and urea were followed at weekly intervals and evaluated in diabetic (dBB, n=3) rats and in animals not having developed diabetes (ndBB, n=3). Results: Average daily SYS was elevated in d vs. nd rats, and it increased in a time-dependent manner in d animals only. DIA was not elevated at but shortly after DM. The increase in BP at diabetes onset was associated with an increased excretion of albumin and of NAG (0.96±0.7 U/d and 0.22±0.1, at DM vs. before DM). The HR of dBB decreased as diabetes developed and was significantly lower after DM (308±1.4 beats/min) compared to ndBB (342±0.9 beats/min). MA was significantly higher in dBB vs. ndBB at all times of examination but was not related to DM. Conclusion: dBB rats are prone to hypertension which may predict an approaching DM and alteration of kidney function.

MEAN ± SEM °p<0.05 in dependence on time *vs.nd

age weeks	time	PG (mmol/l) # nd	PG (mmol/l) # d	SYS(mmHg) # nd	SYS(mmHg) # d	DIA(mmHg)# nd	DIA(mmHg)# d	AE (μg/d) # nd	AE (μg/d) # d
19	before	5.7	6.6	120.8	126.8	92.2	92.9	5.4	9.7
	DM	±0.1	±0.2	±0.2	±0.2*	±0.2	±0.2	±0.4	±1.5
21	at DM	5.7	17.2	120.6	129,8	91.2	92.9	19.6	119
		±0.1	±1.8*	±0.3	±0.3*°	±0.3	±0.3*	±7.2	±44°
23	after	5.8	17.2	121.3	134,8	93.3	97.4	10.9	208
	DM	±0.1	±2.1*	±0.2	±0.3*°	±0.2 °	±0.2*°	±2.7	±120

1993

RELATIONSHIP BETWEEN CIRCULATING NITRIC OXIDE AND ENDOTHELIN LEVEL IN DIABETIC NEPHROPATHY

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Circulating NO (NO) and ET LEVEL was determined in 30 normal subjects and 63 diabetic patients. Patients were divided into 3 groups based on the urinary albuminexcretion rate (UAER): SDM group (UAER<20ug/min, 20 patients), IDN group (20ug/min≤UAER≤200ug/min, 19 patients), ODN group (UAER>200ug/min, 24 patients). Serum NO level was significantly higher in SDM group (68.66±12.37umol/l) and IDN group (63.43±11.09umol/l) than in normal subjects (44.92±9.04umol/l, p<0.01). Compared with normal subjects NO concentration was descent but no significant difference in ODN group (36.28±10.18umol/l, p>0.05). Plasma ET level was markedly elevatde inSDM group (68.92±11.96ng/l, p< 0.05), IDN group (79.22±19.21ng/l, p<0.01), ODN group (137.16±39.92ng/l, p<0.01) compareed with normal subjects (59.12±11.62ng/l). SerumNO level was negatively correlated with plasma ET leves in 63 diabetic patients. The results suggested that NO may play a poor role in the development of DN, but ET aggravated this bad effect.

1994

EFFECT OF HIGH GLUCOSE ON EXTRACELLULAR MATRIX PRODUCTION BY MOUSE GLOMERULAR EPITHELIAL CELLS

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Extracellular matrix (ECM) plays an important role in structural organization and in biophysical properties of the glomerular filtration barrier. Changes in this structure are involved in the pathogenesis of diabetic nephropathy. Glomerular epithelial cells (GECs) contribute to the ECM component production. In this study we investigated the effects of high and normal glucose concentrations on GEC capacity of synthetizing laminin (LM), fibronectin (FN) and type IV collagen. Mouse GECs were incubated in media containing physiological (5mM) and elevated (30mM) glucose and iso-osmolar (30mM) mannitol concentrations. Intracytoplasmic LM, FN and type IV collagen were evaluated by immunofluorescence. Metabolic labelling with 35S-methionine was performed to demonstrate LM, FN and type IV collagen synthesis by GECs. LM was the extracellular matrix component produced in higher amount in all the three experimental conditions in comparison to FN and type IV collagen. Laminin was completely cell associated in GEC cultured in normal glucose concentrations. The synthesis of this glycoprotein was raised by high glucose and mannitol that induced also a release of about 20% in the cell medium, in comparison to normal glucose. Fibronectin was produced in reduced amount than LM in normal glucose incubated GECs and it was localized in prevalence in the extracellular compartement. Cell associated FN and the release of this protein into the medium were not affected by high glucose and mannitol. Type IV collagen was synthesized in small amount and was almost exclusively cell associated. High glucose did not modify the production and the secretion of this glycoprotein into the medium. These results indicate that GECs are involved in the ECM production and that high glucose can modify the ECM protein concentrations at the glomerular basement membrane level. Osmotic effects may play a role in the increased synthesis and accumulation of these proteins in diabetic nephropathy.

1995

EVIDENCE FOR IMPAIRMENT OF DETOXIFICATION OF 3-DEOXY GLUCOSONE TO 3-DEOXYFRUCTOSE IN DIABETES

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An important factor in the development of diabetic complications is 3-deoxyglucosone (3DG), a key intermediate in the non-enzymatic glycation of proteins. Given the toxic potential of 3DG, cellular processes exist for its detoxification, specifically by reduction at the C-1 to 3-deoxyfructose (3DF). In this study, we have measured the levels of 3DG and 3DF in urine from three groups of volunteers: a) normoglycemics (n=19), b) type I diabetics with little or no complications (n=28) and c) type II diabetics with no renal impairment (n=30). Second-voided fasted urine samples were collected from these individuals and the levels of 3DG and 3DF measured by GC/MS and HPLC, respectively. Both 3DG and 3DF were significantly elevated in type I and type II diabetics relative to normals (see table). While these increases were significant, a far more dramatic difference was observed when the levels of 3DG were correlated with 3DF. The differential increases of 3DF with respect to 3DG (slope), coefficient of regression (R^2) and significance (P value) were calculated from these correlations (see table). These data suggest

	3DG μm/g Cr	3DF μm/g Cr	Δ3DF/Δ3DG (Slope)	R^2	P Value
Normals	2.7 ± 0.9	92 ± 15	39.9	0.72	$< 10^{-6}$
Type Is	4.6 ± 3.6	125 ± 71	9.2	0.27	< 0.004
Type IIs	8.45 ± 10	171 ± 152	9.5	0.39	$< 10^{-4}$

a possible impairment of the reductive detoxification of 3DG in diabetics. This metabolic deficit may be a contributing factor to the development of diabetic complications.

(Supported by NIH grants EY08414 and DK44050)

1996

EFFECT OF GLUCOSE ON GENE EXPRESSION OF TISSUE INHIBITOR METALLOPROTEINASE-1 IN HUMAN MESANGIAL CELLS

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Matrix metalloproteinases and their inhibitors (TIMPs) have been implicated in mediating extracellular matrix accumulation and maybe involved in the mesangium expansion of diabetic nephropathy. In this study we investigated TIMP-1 expression in human mesangial cells exposed to high and low glucose concentrations. TIMP-1 expression is regulated by many factors including a protein kinase C (PKC)-dependent mechanism. To test whether any effect of glucose is mediated by PKC, TIMP-1 mRNA levels were also measured in human mesangial cells grown in high and low glucose and then exposed to a PKC-agonist, phorbol myristate acetate (PMA). Competitive RT-PCR was used to quantitate mRNA levels for TIMP-1 because of the increased sensitivity of this method compared with Northern blot analysis. An internal RNA competitive reference standard which differs from the wild-type mRNA by an 80 base pair deletion was used to control for the efficiency of the RT and PCR steps. Exposure of mesangial cells to high glucose concentrations led to a 57 ± 17% ($p<0.01$) decrease in TIMP-1 mRNA levels relative to the low glucose controls. This differs from previous in vivo and in vitro studies using Northern blot analysis to measure TIMP-1 mRNA levels in rat glomeruli or mesangial cells. A 6 hour exposure of mesangial cells to 10 μM PMA led to a 2.5-fold ($p<0.05$) increase in TIMP-1 mRNA levels in mesangial cells grown in low glucose conditions but this effect was abolished by high glucose concentration. In conclusion, TIMP-1 expression is reduced in a diabetic mileau. High glucose appears to inhibit the effect of PKC on TIMP-1 expression (supported by NH&MRC).

1997

THE MEASUREMENT OF ADVANCED GLYCATION ENDPRODUCT (AGE) SPECIFIC FLUORESCENCE IN CIRCULATION AND URINE

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The late rearrangement products that accumulate by glycation of proteins, advanced glycation endproduct (AGE), have been implicated in the pathogenesis of diabetic complications. It was shown that circulating AGE increase in blood of diabetic patients with end-stage renal disease, and also shown reactive AGE in low molecular weight fraction of their serum, cut-off 10kDa, as AGE peptide (AGE-p). The aim of this study was evaluate the relation of AGE-p levels and diabetic nephropathy. To assess AGE-p in diabetic patients, AGE specific fluorescence (F: excitation 370 nm, emission 440nm) of AGE-p in serum and urine were examined in 101 diabetic subjects with several levels of renal complications; 24 with normoalbuminuria (N), 19 with microalbuminuria (m), 16 with macroalbuminuria (M), 22 with chronic renal failure (C) and 20 with hemodialysis (H). We also assessed age, duration of diabetes, serum creatinine level and HbA1c in these patients. The fluorescent characteristics of AGE-p in serum and urine were similar to those of AGE-BSA. Emission maximum were expressed a single major peak in 440-450nm. F of serum AGE-p in C and H (3.70±0.57 and 16.6±1.55, AU, mean±SE) were significantly higher than N, m and M(1.37±0.08, 1.61±0.35 and 1.41±0.10, $p<0.005$). Also in urine, F of AGE-p in C and H (1.63±0.24 and 1.63±0.13 AU/gCr) were higher than N, m and M(0.56±0.10, 0.65±0.23 and 0.57±0.09, $p<0.05$). F of serum AGE-p and serum creatinine levels were strongly correlated ($r=0.9$, $p<0.001$). These findings suggested that renal function may play a major role for the mechanism of AGE accumulation and excretion on diabetic subjects, and the measurement of AGE specific fluorescence of AGE-p may serve as a useful test to detect circulating reactive AGE levels.

1998

MODULATION OF GALECTIN-3 / AGE-RECEPTOR-3 EXPRESSION BY THE DIABETIC MILIEU IN CULTURED RAT MESANGIAL CELLS

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Advanced glycation end-products (AGEs) have been postulated to mediate hyperglycemia-induced glomerular injury, via binding to cell surface receptors. The 32 kD protein Galectin-3 (Gal-3) has been recently identified as the AGE-receptor 3 (AGE-R3). This study was aimed at evaluating Gal-3 expression in cultured rat mesangial cells (RMC) and its modulation by the diabetic *milieu*. To accomplish this objective, RMC were (a) cultured for 1-4 weeks in media containing normal glucose (5.5mM, NG), high glucose (30mM, HG), or iso-osmolar mannitol (M); or (b) grown for 4 days on dishes previously coated with nonglycated or native bovine serum albumin (BSA), glycated BSA with AGE formation (BSA-AGE), or glycated BSA in which AGE formation was reduced by aminoguanidine (BSA-AM). Gal-3 expression was evaluated by FACS and immunofluorescence using a rabbit anti-rat Gal-3 polyclonal antibody and a swine anti-rabbit IgG-FITC to reveal the reaction. No Gal-3 was demonstrable in RMC cultured in NG (although it became evident after passage 15), whereas cells grown on BSA showed a positive staining with a diffuse (cytoplasmic) fluorescence pattern. Prolonged exposure (3-4 weeks) of RMC to HG, but not to M, as well as growing cells on BSA-AGE and, to a lesser extent, BSA-AM, induced or significantly incresed Gal-3 expression, as assessed by both FACS analysis and immunofluorescence. Moreover, cell cultured under these conditions showed a unique patchy distribution of Gal-3 fluorescence, in addition to the diffuse pattern. Confocal microscopy indicated both a cytoplasmic and cell surface localization of granules compatible with the reported Gal-3 receptor function. These results indicate that (1) Gal-3/AGE-R3 is not expressed under basal conditions in RMC; (2) AGEs induce (or upregulate) the expression of their own receptors; and (3) the effect of AGEs is mimicked by prolonged exposure to HG, possibly due to a time-dependent AGE formation.

1999

Urinary excretion of Glucagon-Like Peptide 1 (GLP1) 7-36 amide: a possible indicator of early tubular dysfunction in type 2 (non insulin-dependent) diabetes.

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The metabolism of GLP1, the most important physiologic insulinotropic factor so far described in man, is unknown. Plasma GLP1 is reported to be markedly increased in uremic subjects, suggesting that the kidney plays a role in the removal of the circulating peptide. Beside this only observation in man, studies in the rat demostrate that the renal clearance of GLP1 involves its glomerular filtration and subsequent tubular degradation. In condition of maintained glomerular function, thus, the negligible urinary excretion of the peptide would confirm the integrity of tubular function. Aim of this study was to investigate the urinary excretion of GLP1 in non insulin-dependent diabetes, evaluating different conditions of renal function. 29 type 2 diabetics and 7 healthy volunteers were studied. On the basis of urinary albumin excretion rate (UAE) 4 groups of subjects were individuated: group 1= control subjects (n=7; UAE=3.4±2.6 ug/ml/min; M±SD), group 2= normo-albuminuric diabetic patients (n=8;UAE=5.2±3.1), group 3=microalbuminuric patients (n=11;UAE=81.7±14.6), group 4=macro-proteinuric patients (n=10; UAE=448.9±94.8). Mean values of urinary GLP1 (RIA; pg/min) resulted significantly different between the groups ($p<0.03$). With respect to group 1 (275.5±132.1) the urinary peptide excretion increased in group 2 (490.4±211.5, $p<0.05$) and further in group 3 (648.6±305,$p<0.01$), whereas no significant difference resulted between macroproteinuric patients (317.9±183.3) and controls. Mean values of glomerular filtration rate (GFR), evaluated as creatinine clearance, was significantly lower in group 4 (57.4±2 ml/min) with respect to both controls and normoalbuminuric diabetic patients (105.6±6.1, 91.2±17.3; $p<0.01$). Considering all subjects examined, a significant relationship resulted between urinary GLP1 and GFR ($p=0.004$). In conclusion: 1) the increase of the peptide excretion observed in normoalbuminuric diabetic patients could indicate an early tubular dysfunction in condition of still maintained glomerular integrity, 2) the tubular defect seems to become more evident with the onset of glomerular involvement, 3) in condition of overt diabetic nephropathy the tubular damage, in terms of urinary peptide excretion, would be masked by the advanced decline of glomerular function.

2000

Role of p21 Ras Signaling Pathway in Cultured Rat Mesangial Cells in High Glucose Media.

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HMG-CoA reductase inhibitor pravastatin, which inhibits farnesylation of p21Ras has the preventive effect on the development of diabetic nephropathy in STZ rats. In order to clarify the role of Ras signaling pathway in the pathogenesis of diabetic nephropathy, we examined the influence of high glucose and pravastatin on the expression p21Ras and TGF-β in culturted rat mesangial cells. Cells were incubated with 5mM(N), 25mM(H), and 25mM glucose in the presence of 500μM pravastatin(P), and after harvesting at 48hrs of the exposure to each culture condition, we examined the expression of H-ras and TGF-β mRNA with Northern blot, p21Ras in the membrane fractions and the ratio of Ras-GTP/GDP with immunoblotting method. There were no changes in the expression of H-ras mRNA among N, H, and P. On the other hand, the expression of p21Ras in the membrane fractions and active form of Ras-GTP was significantly increased in H compared to N, however, these changes were suppresed in P. The expression of TGF-β mRNA was also suppressed by addition of pravastatin into high glucoe media. These data suggest that in mesangial cells in high glucose media, p21Ras is overexpressed in the plasma membrane via translocation from cytoplasm. Pravastatin suppresse the translocation of p21Ras by inhibiting farnesylation and also suppresses the expression of TGF-β, which may result in prevention of diabetic nephropathy.

2001

URINARY KAPPA LIGHT CHAIN AS A PREDICTOR OF EARLY DIABETIC NEPHROPATHY: FIVE-YEARS FOLLOW UP STUDY

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To evaluate whether urinary excretion of kappa light chain(KLC) can be used as a predictor for developement of early diabetic nephropathy, we measured the concentrations of KLC and albumin in first-voided morning urine samples from 107 diabetic patients(NIDDM 103, IDDM 4) without overt proteinuria(urinary albumin creatinine ratio(ACR)< 150mg/gCr). In the patients with increased excretion of urinary KLC creatinine ratio(KCR)(≧5.8mg/gCr), ACR significantly increased both in normoalbuminuric(ACR<20mg/gCr) and microalbuminuric(20≦ ACR< 150mg/gCr) patients after 5 years, whereas in normal KCR (<5.8mg/gCr), ACR showed no significant change both in normo- and microalbuminuric patients. The number of patients with increase in ACR for 5 years was significantly large among the patients with increased KCR. Multiple regression analysis demonstrated that KCR was a significant risk factor for increasing ACR after 5 years. KCR showed no significant change both in normo- and microalbuminuric patients for 5 years. These results suggest that urinary excretion of KLC might provide a useful predictive marker for developement of early diabetic nephropathy.

2002

ROLE OF THROMBOXANE A2 ON RENAL HEMODYNAMICS IN NON-INSULIN-DEPENDENT DIABETIC RATS.

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Thromboxane (TX) A2 plays an important role on renal hyperfiltration in streptozotocine (STZ)-induced diabetic rats, whereas it's role on renal hemodynamics in non-insulin-dependent diabetic (NIDDM) rats remains unknown. To clarify the issue, the effects of intravenous infusion of TXA2 synthetase inhibitor (OKY-046, 6 mg/kg/h) on clearance of inulin and para-aminohippurate (Cin, CPAH) under anesthesia were evaluated in spontaneously NIDDM rats, OLETF (n=8), and control rats, LETO (n=7) at the age of 10 months. OLETF showed obesity, moderate hyperglycemia (12.7± 0.9 mmol/l), and hyperinsulinemia (336±26 pmol/l). Urinary TXB2 excretion (U-TXB2) was slightly higher and the ratio of U-TXB2 to urinary 6-keto prostaglandin F1α (6-kPG) was higher in OLETF (TXB2/6-kPG: 0.22±0.04 vs 0.12 ±0.02, $p<0.05$). OLETF had significantly higher Cin and CPAH than LETO (Cin: 1.1±0.1 vs 0.7±0.1 ml/min/100gBW, CPAH: 3.1±0.2 vs 2.3±0.3 ml/min /100gBW, $p<0.01$). OKY-046 did not restore Cin and CPAH in OLETF although it significantly decreased U-TXB2 and ameliorated TXB2/6-kPG in OLETF. In conclusion, in contrast to STZ-induced diabetic rats, TXA2 was not involved in renal hyperfiltration in OLETF. TXA2 may contribute to renal injuries in OLETF through mechanisms other than hemodynamic injury.

2003

Uric Acid and Blood Sugar Control as Factors Influencing Urinary Transferrin in NIDDM Patients

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This study investigated factors facilitating urinary transferrin excretion comparing with those influencing urinary albumin excretion in NIDDM patients without proteinuria. We examined the correlation between urinary transferrin/ urinary albumin ratio (T/A ratio) and clinical factors including serum uric acid, which was reported to provide an antioxidant defense. Twenty-one male NIDDM patients (Age: 58±13 years old) without proteinuria or pyuria were studied for 1 year. Transferrin was measured by the latex agglutination method and albumin by immunoturbidity in overnight urine. Age, known duration of diabetes, blood pressure, body weight, HbA1c, and serum uric acid were recorded as clinical factors. The T/A ratio in healthy control subjects was 0.052± 0.017 (mean±SD) (n=90). The T/A ratio in patients was significantly higher than that of controls ($p<0.01$). Patients were divided into two groups after the T/A ratio was followed for 1 year. Group A: patients with a decreased T/A ratio (n=10) (T/A: 0.131±0.059→0.082±0.020), Group B: patients with a stable T/A ratio (n=11) (T/A: 0.080±0.044→0.099± 0.034). We found a significant improvement in HbA1c in group A ($p<0.01$), but not in group B. There was a significant correlation between the T/A ratio and serum uric acid in group B ($r=-0.706$, $p<0.02$), but not in group A. We suggest that the excretion of transferrin comparing with that of albumin demonstrated the amelioration of glycemic control and may be linked to an antioxidant defense by uric acid.

2004

THROMBOSPONDIN PROMOTES THE PRODUCTION OF MESANGIAL MATRIX PROTEINS THROUGH ACTIVATING TGF-β IN HUMAN MESANGIAL CELLS

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Thrombospondin (TSP), a multifunctional glycoprotein, is synthesized by mesangial cells (MCs). The role of TSP, however, has not been established in the pathogenesis of diabetic nephropathy. To clarify this, TSP production from human MCs exposed to various concentrations of glucose was measured by ELISA. We also studied the effects of TSP on the production of fibronectin and type IV collagen by ELISA, and on the ability of activating latent TGF-β assessed by the binding capacity to type II receptors, respectively in MCs. Incubating MCs with high glucose media resulted in the increase of TSP in both media and cell layers in a glucose-dose dependent manner. The production of fibronectin and type IV collagen was enhanced by the addition of TSP in a TSP-dose dependent manner. These effects of TSP were blocked by the simultaneous addition of neutralizing antibodies to TGF-β. The activation of latent TGF-β was increased by the addition of TSP to MCs, while the amount of latent TGF-β secreted from MCs was unchanged. These results indicate that TSP production from MCs is enhanced by high concentrations of glucose, and that TSP promotes the production of mesangial matrix proteins through activating latent TGF-β. Therefore, TSP may participate in the pathogenesis of diabetic nephropathy.

2005

THE VALUE OF MICROTRANSFERRINURIA IN EARLY DIAGNOSIS OF DIABETIC NEPHROPATHY

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To evaluate the significance of microtransferrinuria in early diagnosis of diabetic nephropathy, the urinary excretions of albumin (ALB), transferrin (TF) and a1-microglobulin (a1-MG) were measured in samples of 24 hour urine from 25 healthy subjects and 63 diabetic patients (55 with NIDDM and 8 with IDDM). Diabetic patients were grouped according to their ALB excretion (AER) into normal albuminuria (group I, AER <30mg/24h), microalbuminuria (group II, AER 30-300mg/24h) and macroalbuminuria (group III, AER > 300mg/24h). The urinary excretions of TF and a1-MG in all groups exceed those for healthy controls. The urinary excretions of TF and a1-MG were highest in group III subjects, followed by group II and group I subjects. Prevalence of increased values (calculated by using an upper limit of normal range, ie, exceed the 95 th percentile value found in healthy controls) were in 41.03%, 85.76% and 100% of group I, group II and group III patients for TF, in 38.46%, 92.31% and 100% of group I, group II and group III for a1-MG, respectively. The urine excretion of TF and a1-MG was positively related with that of ALB, but there was no relationship between the excretion of TF or a1-MG and FBG or GHbA1. Conclutions: 1.Urinary TF is increased earlier in diabetics than ALB and may be a more sensitive marker for detecting diabetics. The proximal tubular impairment might occur independently on the glomerular alterations in the course of diabetic nephropathy.

2006

TGFβ1 IN DIABETIC NEPHROPATHY - A TRANSGENIC MODEL

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Several findings support a key role of TGFβ in diabetic nephropathy, particularly on the accumulation of glomerular extracellular matrix components (ECM).The aim of the present study was to study the effects of TGFβ1 in the glomerulus *i n vivo*. We have created a transgenic mouse with localized production of TGFβ1 in the kidney. TGFβ1 production is targeted to the juxta-glomerular cells in the kidney by the Ren1c promoter. Microinjection of the DNA construct into fertilized eggs resulted in five transgenic founders out of 25 pups born. We have continued breeding with one transgenic line. Reverse transcription of total RNA followed by PCR with transgene specific primers demonstrated the presence of a transgene specific transcription product. The localization was tested by in situ hybridization. The presence of biological active TGFβ1 in transgenic mice is indicated by increased synthesis of glycosaminglycans as evaluated in cultured kidney slices from transgenic (2044 arbitrary units/mg, n=7) and non-transgenic mice (1061 arbitrary units/mg, n=7) ($p<0.01$). In line 1725 all transgenic mice tested so far (n=15) have accumulations of ECM in their glomeruli. Only in older transgenic mice (6-8 month) or in more advanced cases do we observe interstitial fibrosis. The volume fraction of the glomerular tuft stained with antibodies against the basement components collagen IV and laminin increases in transgenic mice compared to sex- and age matched non-transgenic littermates ($p<0.01$ and $p<0.05$, respectively). The transgenic mice exhibit a variable degree of proteinuria. The youngest mice tested so far are newborn mice. It seems that glomerular changes develop between 1 and 2 month of age. Electronmicroscopy indicates that a very early change in the transgenic mice is a widening of the basmement membrane. In conclusion, we have established a transgenic mouse with localized expression of TGFβ1 in the glomerulus. The model is used to explore the biological effects of TGFβ1 on glomerulus function *in vivo* in relation to diabetic nephropathy. Our transgenic model has several advantages: First, we do not genetically manipulate the cells we actually want to study (the glomerular cells). Second, in contrast to other studies, we do not have any secondary effects induced by failure of other organs due to circulating levels of TGFβ1.

2007

MEASUREMENT OF URINARY IgG IN NIDDM

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In an attempt to evaluate the mechanisms of diabetic nephropathy, we measured the urinary IgG(IgG) in 143 NIDDM patients, as well as urinary transferrin(TF) and urinary albumin(Alb) which were well known as to be predictive for later clinical diabetic nephropathy. Reference data of urinary IgG were obtained from 38 normal glucose-toleranced (NR) and 28 impaired glucose-toleranced persons (IGT). Urinary IgG of NR was 2.81±0.2 mg/gCr and that of IGT was 4.71±0.60mg/gCr, significantly higher than NR. The TF(0.66±0.08 mg/gCr) in IGT was also significantly higher than that in NR(0.43±0.05mg/gCr), but Alb was not (6.06±0.62mg/gCr for IGT, 5.47±0.53mg/gCr for NR). According to reference data for IgG, NIDDM were allocated to 5 groups. The IgG was ≦3.0mg/gCr and TF was ≦1.0mg/gCr in G1 (N=13); IgG was >3.0mg/gCr but ≦5.0mg/gCr, and TF was ≦1.0mg/gCr in G2(N=19); IgG was >5.0mg/gCr, and TF was ≦1.0 mg/gCr in G3(N=22); TF was >1.0mg/gCr, and Alb was ≦20mg/gCr in G4(N=36); Alb was >20mg/gCr in G5(N=53). The IgG showed the significant increase as the group increase (2.31±0.22, 3.94±0.14, 6.85±0.49, 8.94±0.77 25.99±4.93 mg/gCr, respectively), however TF and Alb didn't show the significant increase in G1, G2, and G3; but significantly increased in G3 and G4. The average age, BMI, BP, Cr and NAG in each group showed no difference. The HbA1C and contraction period tended to increase as the group increase. No patients in G1 showed the diabetic retinopathy, 6 patients in G2, 8 in G3, 6 in G4, and 15 in G5.**[Conclusion]** IgG seems to be filtrate from the kidney early stage in diabetes, possibly earlier than TF or Alb.

2008

GROWTH FACTORS AND DIABETIC NEPHROPATHY: AN EXPERIMENTAL AND CLINICAL STUDY.

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The aim of this work was to investigate if some growth promoting polypeptides have a relation to the diabetic renal disease, relying on studying their distribution in the renal tissue, both in experimental and clinical situations. The experimental part included 50 Wistar rats with streptozotocin (STZ)-induced diabetes mellitus (day 0) and 20 sham animals. A group of 5 STZ- and 2 sham animals were sacrificed on days 0, 2, 4, 7, 9, 14, 30, 60, 120, and 150. The kidneys were examined for evidence of renal growth and prepared for immunohistochemical and pathological studies. In the clinical part, renal biopsies from 5 diabetic patients were studied. All renal tissues were stained for routine microscopical evaluation as well as for immunohistochemistry for the following growth factors: Epidermal growth factor (EGF), EGF-receptor, transforming growth factor beta (TGFb), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and interleukin-6 (IL-6). Tubular lesions in the form of vacuolations were noticed early in the time course of diabetes with stuffing of some tubular cells with glycogen and fat. Immunostaining for growth factors was demonstrated in consistent patterns in different stages of diabetic renal disease both in animals and in humans. Some of the growth factor immunostaining patterns were co-localized with the tubular lesions early in the disease, others with the interstitial expansion late in the disease. Glomerular staining for PDGF was shown to be related to the advanced cases of glomerulosclerosis. The localization and distribution of growth factors in the diabetic kidneys might suggest a role in pathogenesis of diabetic renal disease.

2009

INCREASED PLASMA AND URINARY CONCENTRARTIONS OF EXTRACELLULAR GLUTATHION PEROXIDASE (eGPx) IN NON-INSULIN-DEPENDENT-DIABETIC (NIDDM) PATIENTS

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It is well known that diabetic patients have high risk of microangiopathy and atheroscrelosis. Recently, Oxygen radicals is considered to play an important role in the production and development of atherosclerosis. GPx that is one of the major antioxidant enzymes, catalyses the reduction of H_2O_2 to alcohols. Therefore in NIDDM, we determined plasma and urinary concentrations of eGPx, and examined its relationship with diabetic control and complications, especially nephropathy. Eighty NIDDM subjects(46 male and 34 female) and 30 healthy subjects were investigated. Plasma and urinary concenrations of eGPx were measured with the ELISA method (Clin.Chim.Acta. 236: 93; 1995). Diabetic patients were allocated to three groups according to their levels of urinary albumin excretion: group 1 (30 mg/g.Cr), group 2(30-300), Group 3(>300). Urinary NAG levels were also measured. Plasma eGPx level in diabetics was significantly higher than that of controls (17.8 to 23.0 μ g/ml, $p<0.05$). Plasma eGPx levels were not correlated with fasting plasma glucose and HbA1c levels. There was no difference in plasma eGPx levels among three groups. Urinary levels of eGPx in group 3 increased significantly as compared with those in group 1 and group 2 (group 1: 44.6 ng/ml, group 2: 42.6, group 3: 280.6). Moreover, urinary eGPx levels were correlated with urinary NAG levels ($rS=0.357$, $p<0.01$). In diabetic patients, plasma eGPx levels increased as compared with normal subjects. Moreover, urinary eGPx levels with clinical macroalbuminuria were higher than those without nephropathy. The increased rate of eGPx synthesis in proximal tubular epitherial cells may contribute to the elevated levels of plasma eGPx found in diabetic patients. Urinary eGPx levels might be a marker of nephropathy in NIDDM.

2010

TRASFORMING GROWTH FACTOR-β_1 MODULATES INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-3 IN KIDNEY ENDOTHELIAL CELLS

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We previously reported that TGF-β_1 and IGF-I differentially modulate in a dose response manner the level of IGFBP-3 decreasing and increasing, respectively, its presence in human kidney endothelial cells (GEN). The aim of our study is to investigate the role that this BP-3 regulation could have on GEN growth control. Conditioned medium (CM) from GEN was harvested after 48 h serum-free culture and electrophoresed on SDS-PAGE and then incubated with [^{125} IGF-II]. The TGF-β_1 effect on BP-3 GEN probably involves a non-enzimatic activity since the addition of 10 nM protease inhibitors (aprotinin, EDTA and PMSF) did not block the effect of TGF-β_1 Our preliminary results seem to confirm that both TGF-β_1 and IGF-I could regulate the BP-3 level in controlling the expression of specific GEN BP-3 mRNA. Since the two growth factors modulate the level of IGFBP-3 which may have both inhibitory or stimulatory actions, we examined the effect of TGF-β_1 on GEN mitogenesis. At this purpose we mesasured GEN cell growth in regular medium with TGF-β_1 alone or in combination with IGF-I at different times by a haemocytometer count. TGF-β_1 significantly reduced cell growth starting from 12 hours even if a significant increase of the IGF-I level was detected by a specific commercial radioimmunoassay. Moreover the addition of IGF-I in presence of TGF-β_1 did not modify this inhibitory effect. Finally, the policlonal antibody against BP-3 added to the regular GEN cell growth medium dramatically decreased the mitogenesis of these cells. We conclude that: i) the classic inhibitory effect of TGF-β_1 on endothelial cell growth could be, in part, explained by the decreased BP-3 level; ii) the mitogenic effect of IGF-I observed in endothelial cells could explain instead with the BP-3 level increased; iii) the addition of an Ab-BP-3 reduces mitogeniesis; iv) the increased IGF availability induced by TGF-β_1 is not able to stimulate cell growth due to the lack of a simultaneous BP-3 increase. These results suggest the primary role played by BP-3 in the regulation of endothelial cell growth.

2011

URINE MICRO-FDP(FIBRIN/FIBRINOGEN DEGRADATION PRODUCTS) -A CANDIDATE FOR EARLIER MARKER FOR DIABETIC NEPHROPATHY-

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We have developed a highly sensitive assay method for the detection of FDP in urine specimens and explored the possibility that urine micro-FDP can be an earlier marker for diabetic nephropathy. We examined 196 NIDDMs, 15 hyperlipidemics, 25 hypertensives and 142 non-diabetic healthy volunteers (N). Urine (early morning) FDP was measured essencially by chemiluminescent enzyme-linked immunoassay with dioxetane derivative substrate for the labelled alkaline phosphatase. The detection limit is 1 ng/ml, which is approximately 1/100 of conventional turbidimetric method based on latex aggregation. The CV was 5.5%. Abnormally high level of urine FDP (above mean+2SD of group N) was found in 68% (45/66) of NIDDMs with normo-albuminuria. More than 90% (30/33) of NIDDMs with micro- or macroalbuminuria and 69% (39/57) of those with elevated urine transferrin levels had abnormally high urine FDP levels. There were no significant differences in either plasma FDP or D-dimer levels between NIDDMs with normo-albuminuria and group N. Only 20% (6/30) of non-diabetic hypertensives had elevated urine FDP, while all hyper-tensive NIDDMs showed abnormally high urine FDP. Even within NIDDMs with normoalbuminuria, elevation of HbA1c had significant effect on increase in urine FDP. Neither hyperlipidemia nor smoking had any significant effect on urine FDP levels. Thus, urine micro-FDP can be a new candidate for an earlier marker for diabetic nephropathy.

2012

THE IGF SYSTEM IN EARLY DIABETIC NEPHROPATHY IN THE SAND RAT.

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Early increase in kidney IGF-I secondary to increased IGFBP1 was shown to correlate with the increase in renal perfusion and size in streptozotocin (STZ) induced hypoinsulinemic diabetes in the rat (animal model for IDDM). The increase in renal IGFBP1 and IGF-I in this model was related to the hypoinsulinemic state induced by STZ. We studied the influence of hyperglycemia in the setting of normoinsulinemia on renal perfusion and size and on the IGF system in hyprglycemic normoinsulinemic sand rats (animal model for NIDDM). Induction of diabetes by special diet 40 days after weaning resulted in average blood glucose of 270±14 mg% within 2 days as compared to 82±6 mg% in the control group. A 50% increase in GFR was seen two weeks after induction of diabetes ($P<0.05$), however 24 hour urine albumin excretion was similar in both groups. Kidney size was similar after 5 days of diabetes, but was increased by 15% after 15 and 30 days of diabetes while heart size was unchanged. Serum IGF-I, IGFBP1,2,3 and 4, and GH were unchanged after 5, 15 and 30 days of diabetes. Renal IGF-I mRNA, IGF-I receptor mRNA and IGFBP1 mRNA were unchanged by the diabetic state. Renal IGF-I and IGFBP1,2,3 and 4 levels were unchanged after 5, 15 and 30 days of diabetes. In conclusion, kidney hyperperfusion and enlargement is characteristic of the normoinsulinemic hyperglycemic state, but tends to be less pronounced and to develop at a later stage. In this model, renal hypertrophy and hyperperfusion developed devoid of any change in the IGF system.

2013

PLASMA AND URINARY ENDOTHELIN-1 (ET-1) AS MARKER OF DIABETIC NEPHROPATHY.

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The vasoconstrictive peptide ET-1 can be found in both human blood and urine. Aim of this study was to verify the role played by ET-1 as marker of early renal damage. We evaluated plasma and urinary ET-1 levels in 27 lean, normotensive non-insulin dependent diabetic (NIDDM) patients (16 males and 11 females, mean age 59±8 years, HbA1c <6%, 18 normo and 9 microalbuminurics), and in 12 age and sex-matched healthy subjects. Statistical analysis was conducted by Student's t-test for paired and unpaired data. Compared to controls, a significant increase of plasma ET-1 in NIDDM patients (respectively 0.6±0.1 vs 1.72±0.38 pg/mL, $p<0.001$) was found. Among these latter, plasma ET-1 levels were significantly higher in microalbuminuric than in normoalbuminuric patients (1.97±0.58 vs 1.59±0.14, $p<0.02$). Urinary ET-1 values were higher in controls than in diabetic patients (70.1±15.2 vs 44.0±20.8 pg/min, $p<0.05$) and no difference was noted between micro and normoalbuminuric patients (48.5±20.1 vs 40.8±21.6 pg/min). Since filtered ET-1 is destroyed by the neutral endopeptidases of proximal tubulus, urinary ET-1 is believed to be distally produced. According to our results, plasma ET-1, as microalbuminuria, is expression of a generalized endothelial dysfunction, and can be used also as marker of glomerular damage. On the contrary, urinary ET-1 levels reflect mainly tubular function and can be used in early diagnosis of subclinical tubular damage.

2014

THE IGF SYSTEM IN EARLY DIABETIC NEPHROPATHY IN NORMOINSULINEMIC HYPERGLYCEMIC RATS.

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A marked increase in renal perfusion and size is characteristic of STZ induced hypoinsulinemic hyperglycemic state in the rat (animal model of IDDM). Early increase in kidney IGF-I secondary to increased IGFBP1 was shown to correlate with the increase in renal perfusion and size in this model of IDDM. However, since the increase in renal IGFBP1 in this model was related to hypoinsulinemia, we studied the influence of hyperglycemia in the setting of normoinsulinemia on renal perfusion and size and on the IGF system in hyperglycemic normoinsulinemic rats (animal model of NIDDM). Injection of 50 mg/Kg of STZ to normal rats resulted in normal glucose on fast with non fast hyperglycemia. Fast and postprandial insulin levels were similar to those in non diabetic control rats. Induction of STZ diabetes resulted in 30% increase in GFR ($p<0.05$) within the first week of diabetes. Kidney size was similar after 10 days of diabetes but was increased by 33% and 36% after 30 and 48 days of diabetes respectively. Serum IGF-I and IGFBP3 decreased significantly after 10 days of diabetes. GH and 30 KDA Bps (BP1 + BP2) were unchanged. Renal IGFBP1 mRNA was unchanged after 10, 30 and 48 days of diabetes. 30 KDA Bps protein were significantly increased in the medulla after 30 and 48 days of diabetes. IGFBP3 also significantly increased in the medulla and cortex of the diabetic rat after 30 and 48 days. We conclude that kidney hypertrophy is similar in normoinsulinemic hyperglycemic state when compared to hypoinsulinemic hyperglycemic state, but tends to develop at a later stage. Increase in renal IGFBP1 and IGFBP3 and decrease in serum IGFBP-3 might play an important role in the development of renal hypertrophy in rats with hyperglycemic normoinsulinemic state.

2015

URINARY GLYCOSAMINOGLYCAN EXCRETION (GAGe) IN NIDDM AND IDDM PATIENTS

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In diabetes mellitus, the quantity and quality of proteoglycan and its GAG chains are modified in different vascular and tissular components. The aim of this work was to evaluate the clinical characteristics, the degree of metabolic control (HbA_1), 24-hr urinary GAGe by the carbazol method, and microalbuminuria (MA) by RIA in 35 IDDM, 27 NIDDM, and 32 non-diabetic subjects (C).

	n	GAGe mmol/creat. mol	GAGe mg/ 24 h
Controls	32	4.39 ± 0.47	10.2 ± 0.9
Total diabetic population	62	6.17 ± 0.60*	15.7 ± 1.4***
IDDM without AH	26	7.06 ± 1.15**	16.6 ± 2.3∧
NIDDM with AH	17	5.47 ± 0.68	17.0 ± 2.6**
IDDM without MA	30	6.93 ± 1.02*	16.2 ± 2.1**
NIDDM with MA	10	6.36 ± 0.99	19.3 ± 3.4**
IDDM HbA_1 > 10%	21	7.39 ± 1.10**	18.8 ± 2.6∧
NIDDM HbA_1 > 10%	21	5.73 ± 0.77	16.0 ± 2.6*

vs. C = ∧: $p < 0.005$; ***: $p<0.01$; **: $p < 0.02$; *: $p< 0.05$

We found that diabetic subjects had a significantly higher GAGe, particularly uncompensated diabetics (HbA_1 > 10%), compared to C. The increase in GAGe preceded the development of MA in IDDM. That was not the case in NIDDM, in which such increase was more frequent in hypertensive patients (AH). The increase in GAGe could be an early indicator of dysmetabolism of these compounds and therefore of the development of diabetes chronic complications.

2016

Role of growth hormone-induced IGF-1 in the modulation of renal haemodynamics in normoalbuminuric insulin-dependent diabetic patients

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An increase in glomerural filtration rate (GFR) has been proposed as a risk factor the development of diabetic nephropathy but it is still unclear whether physiological levels of GH and IGF-1 play a role in these haemodynamic changes. We investigated in a dose-response study the role of GH induced IGF1 in the modulation of renal haemodynamics in normoalbuminuric, normofiltering insulin-dependent diabetic patients We measured GFR and renal plasma flow (RPF) by inulin and para-aminohippurate renal clearances at basaline and 23 hrs after the injection of different doses of GH (0.1, 0.2, 0.4 U/Kg of body weight) in 7 normoalbuminuric, normofiltering insulin-dependent diabetic patients during sustained euglycaemia. Age of patients was 27 yrs (22-34), duration of diabetes: 8 yrs (5-12), creatinine clearance 118±12 ml/min (112-128) and albumin excretion rate: 10 micr/min (2-14). Plama levels of GH increased significantly after 1 hr (from 0.6±0.2 at baseline to 16±4, 30±8, 130±12 ng/ml for 0.1, 0.2 and 0.4 U/kg respectively). Plasma levels of IGF-1 reached a peak 22-24 hrs after GH injection (from 160±15 at baseline to 232±10 ($p<0.05$), 260±64 ($p<0.05$), 410±70 ($p<0.01$) ng/ml for 0.1, 0.2 and 0.4 U/kg of GH respectively). Basal values of GFR and RPF were 122±12 and 550±106 ml/min respectively and did not changed after the injection of 0.1 and 0.2 U//Kg of GH. They significantly increased to 170±22 ml/min and 620 ml/min ($p<0.05$ for both) after the somministration of 0.4 U/kg of GH. The increase coincided with the IGF-1 peak. These results demonstrate that physiological and supraphysiological increase of IGF-1 have no effect on renal haemodynamics. Only pharmacological levels of growth hormone-induced IGF-1 appear to modify renal haemodynamics. These casts doubts on the role of IGF-1 in the pathophysiology of renal disfunction in diabetics.

2017

URINARY HEPARAN SULFATE IN NORMOALBUMINURIC DIABETIC PATIENTS. Bonavita CD, Elbert A, Paglione AM, Bragagnolo J, Mainetti H, Ruiz M, Clinical Biochemistry Depart.Faculty of Pharmacy and Biochemistry. Diabetology Division . Hospital de Clinicas. Buenos Aires. Argentina.

The decrease of the heparan sulfate (HS)in the glomerular basal membrane was described as a lesion in diabetic nephropathy. This decrease should be produce by less synthesis because of the deficient action of the N-Glusaminil Deacetilase enzyme or the lost of the HS by the increase in the catabolism or in excretion. We compare the urinary HS excretion in normoalbuminuric diabetics and in a control group. We studied 42 diabetics :17males (6IDDM, 11NIDDM, age range 20-83 years), and 25 females (6 IDDM,19NIDDM, age range 17-83 years). We compared with a control group: 12 males(age range 25-76years)and 12 females (age range 18-73years). The urinary HS dosage was done by a precipitation with Br-Cetiltrimetilamonio, hydrolisis and quantification of glycosamine by the Smith-Gilkerson reaction. The results obtained (expressed in mg. of glycosamine/24 hours) were:

	Diabetics (mean ±SD)	Controls (mean ±SD)
Males	0.93 ± 0.50*	0.43 ± 0.16
Females	0.55±0.31#	0.28±0.17^

***vs Male controls P<0.001,#vs. Females controls P<0.001,^vsMales controls**

Conclusions: 1. Males excrete more urinary HS than females,

2. Diabetics excrete more urinary HS than controls

2018

INCREASED ACTIVITY OF GLOMERULAR CYTOSOLIC PHOSPHOLIPASE A2 IN NIDDM RATS. Y. Furuya, S. Tagami, H. Yoshimura, T. Horida, S. Sakaue, K. Aoki, J. Ishii, A. Hasegawa, J. Hirokawa and Y. Kawakami. First Department of Medicine, Hokkaido University, Sapporo, Japan.

Cytosolic phospholipaseA2 (cPLA2) is a rate-limiting step in eicosanoid biosynthesis and is potentially involved in glomerular hyperfiltration which leads to diabetic nephropathy. In an attempt to clarify the temporal relationships of plasma levels of glucose, the activity of glomerular cPLA2, creatinine clearance and histological changes in glomeruli, we used Otsuka Long Evans Tokushima Fatty (OLETF) rats that spontaneously develop NIDDM. Groups of 5-6 OLETF rats were compared to control rats at the ages of 8, 30 and 46 weeks. The plasma level of glucose was significantly higher in OLETF rats than in control rats at 30 and 46 weeks of age (OLETF vs. control; 160±28 vs.111 ± 11, 201 ± 91 vs.109 ± 21 mg/dl, respectively). Although pathological changes in glomeruli of OLETF rats appeared only at 46 weeks of age, the activity of cPLA2 was significantly higher in OLETF rats than in control rats at 30 weeks (2138±502 vs.1297±199 dpm, $p<0.001$), but not at 46 weeks. The creatinine clearance had a tendency to increase again only at 30 weeks in OLETF rats. Furthermore, the activity of cPLA2 at 30 weeks had significant correlations with plasma levels of glucose ($r=0.67$; $p<0.01$) and with creatinine clearance ($r=0.52$; $p<0.05$) in OLETF rats. We then conducted an additional in vitro experiment using mesangial cells and found that cells cultured with a high concentration (27.5 mM) of glucose for 5 days exhibited a marked increase in the activity of cPLA2 compared to those cultured with a physiologic concentration of glucose (1473±391 vs. 742±251 dpm, $p<0.01$). These data suggest that increased activity of glomerular cPLA2 and glomerular hyperfiltration occur prior to the development of morphological changes in OLETF rats, presumably due to an elevation in the plasma level of glucose.

2019

RELATION AMONG GLOMERULAR BARRIERS, TUBULAR PROTEINS AND PROGNOSIS OF DIABETIC NEPHROPATHY. M.Satoh, N.Kunii, K.Manome and K.Izumi.Fukushima workmen's compensatory hospital. Fukushima, Japan.

This study was performed to clarify the relation between glomerular basement membrane (GBM) and tubular derangements and prognosis of diabetic nephropathy . In 49 patients with NIDDM, clearances of creatinine (Ccr), IgG (CIgG) and transferrin (Ctrf), urinary excretion of albumin (UAE), N-acetyl-β-D-glucosaminidase (NAG) and β_2-microglobulin (β_2MG) were examined. These patients were followed 24 months. Twenty subjects were examined as control. In diabetics, mean Ccr 92.4, CIgG 15.7 x 10^{-4} , Ctrf 102.3 x 10^{-4} ml/min, UAE 103.3 mg/g cr., NAG 4.1 U/l, β_2 MG 524.3 mg/l and CIgG/Ctrf ratio was 1.12. Renal function was deteriorated in 7 diabetics. In these cases, UAE, NAG * and β_2 MG were elevated. Ctrf*, CIgG/Ctrf ratio* were decreased and CIgG was elevated (*:P<0.05). In poor prognostic diabetic nephropathy, derangement of size barrier and selectivity of GBM is prominent. Charge barrier is also destructed, however, mechanisms of urinary excretion of trf and IgG may be not identical. Tubulointerstitial damage will disturb renal function.

2020

HIGH GLUCOSE-INDUCED TRANSFORMING GROWTH FACTOR ß1 PRODUCTION IS MEDIATED BY THE HEXOSAMINE PATHWAY

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Previous studies revealed that exposure of mesangial cells to high glucose concentrations induces the production of matrix proteins mediated by transforming growth factor ß1 (TGF-ß1). It was the aim of our study to elucidate the molecular mechanism by which elevated glucose levels induce TGF-ß1. Production of matrix proteins and TGF-ß protein was tested by ELISA. Bioactive TGF-ß was determined by the antiproliferative activity in the mink lung epithelial cell assay. Expression of TGF-ß1 was estimated by non-radioactive *in situ* hybridization. First, we tested if structural analogues of D-glucose may mimic the high glucose effect in cultured mesangial cells. We found that D-glucosamine was strikingly more potent than D-glucose itself in enhancing the production of TGF-ß protein and subsequent production of the matrix components heparan sulfate proteoglycan and fibronectin in a time- and dose-dependent manner. Since neutralizing antibodies to TGF-ß abolished the glucosamine-induced effects on mesangial matrix production glucosamine act through induction of TGF-ß. D-glucosamine also promoted conversion of latent TGF-ß to the active form. Therefore, we suggested that the hexosamine biosynthetic pathway the key enzyme of which is glutamine: fructose-6-phosphate amidotransferase (GFAT) contributes to the high glucose-induced increase in TGF-ß1 production. Inhibition of GFAT by the substrate analogue azaserine or by inhibition of GFAT protein synthesis with antisense oligonucleotide prevented the high glucose-induced increase in TGF-ß1 expression and bioactivity and subsequent effects on mesangial cell proliferation and matrix production. Overall, our study indicates that the flux of glucose metabolism through the GFAT catalyzed hexosamine biosynthetic pathway is involved in the glucose-induced mesangial production of TGF-ß leading to increased matrix production.

2021

GLOMERULAR FILTRATION OF IMMUNOGLOBULINS ALTERS THE PATTERN OF TUBULAR FUNCTION IN DIABETIC PATIENTS WITH NEPHROPATHY. S.B.Solerte,M.Fioravanti,S.Severgnini,N.Cerutti,M.A.Netti, M.Locatelli and E.Ferrari , Department of Internal Medicine University of Pavia,Piazza Borromeo 2,27100 Pavia(Italy)

A role of tubular component in the mechanism of proteinuria is not excluded in diabetic patients with nephropathy. In this context,the tubular marker $\alpha_1$microglobulin(UM) was evaluated(by nephelometry)in relation to the urinary excretion rates of albumin(UAER),IgG(UIgGER)and IgA(UIgAER) and to glomerular filtration rate(GFR,51-Cr EDTA)in diabetic patients without nephropathy(NNDP,n=97) and with incipient (INDP,n=45)and overt nephropathy(ONDP,n=26).Urinary UM was below the upper normal limit(10 mg/L)in NNDP and hyperfiltering normoalbuminuric patients(7±1.2 SD mg/L and 6.7±1.1 mg/L).Fifteen INDP(34%)showed UM in normal limits,whereas 30 INDP(66%)presented UM above 10 mg/L;the highest UM values were associated to UAER>50 ug/min and to UIgGER>11 ug/min. Lower UIgGER(<9 ug/min)was found in INDP with normal UM, than in INDP with UM>10 mg/L. All ONDP showed UM above 10 mg/L(mean=30±9 mg/L);the highest UM values were associated to UIgAER>40 ug/min and to UIgGER>150 ug/min. No significant correlations among UM,GFR and glycated haemoglobin levels were found in the three groups of patients.A tubular component of proteinuria might be suggested in overt nephropathy and in the late stage of incipient nephropathy. In particular,the tubular overload of filtered immunoglobulins might be associated with the progression of proteinuria and of renal microangiopathic derangement.

2022

COMPARISON OF THE EFFECTS OF AMINOGUANIDINE, ENALAPRIL, VITAMIN C AND VITAMINS C+E COMBINATION ON DIABETIC NEUROPATHY AND NEPHROPATHY IN STREPTOZOTOCİN-DIABETIC RATS

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The effects of aminoguanidine (AG), enalapril (ENA), vitamin C (VITC) and vitamins C+E combination (VITC+E) on diabetic neuropathy and diabetic nephropathy was compared in streptozotocin- diabetic rats. For this purpose, 40 diabetic adult male Wistar albino rats that were randomly divided into five groups and 8 non-diabetic rats as a normal control group were selected for this study. After induction of diabetes, group 1 (n=8) was treated by the AG in the drinking water (500mg/L), group 2(n:8) was treated by ENA (50mg/L) in the drinking water, group 3(n:8) was treated by VITC (150mg/L) in the drinking water, group 4(n:8) was treated by vitamin C (150mg/kg) in the drinking water, plus vitamin E (500mg/kg) in normal rat chow for 4 months and group 5 (n:8) was a non-treated diabetic control group. Somatosensorial evoked potential (SEP) latency was measured by stimulating via caudal nerve and recording via cortex and nerve conduction velocity (NCV) was measured from caudal nerve every month. Urinary protein excretion and urine volume were also measured during the study. Moreover, fractional kidney weight (FKW) and, neural levels of TBARS were measured at the end of the study. Diabetes caused deficits in SEP and NCV ($p<0.05$ vs. non-diabetic control rats, respectively). All treatment modalities restored SEP latencies ($p<0.05$ vs. non-diabetic control group),but most significant improvement was observed in VITC+E treated group. VIT C and especially VITC+E and AG restored NCV during the study, but ENA has no effect on NCV. FKW was found to be significantly greater in diabetic-control-, VITC-, and VITC+E- treated groups when compared to in normal control group ($p<0.05$). However, slight , but not significant, decrease in FKW was detected in ENA- and AG-treated groups. Neuronal TBARS levels were decreased in all groups but not in ENA-treated group. The most significant decrease in urinary protein excretion and urine volume was found in ENA- and AG- treated groups ($p< 0.05$ vs. diabetic control group). Weight and the glucose level were not influenced by the treatments. Our results suggest that VITC and especially VITC+E combination , and AG have beneficial effects on both central and peripheral neuropathies, but ENA has beneficial effects only on central neuropathy. Moreover, ENA and AG are more effective treatment than VITC and VITC+E for diabetic nephropathy. These beneficial effects are not associated with the regulation of glicemia, but may be related in part with prevention of lipid peroxidation.

2023

URINARY ENZYMES AS A MARKER OF TUBULOINTERSTITIAL CHANGES IN NIDDM AND IGT SUBJECTS

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It is proposed that tubulointerstitial changes due to peritubular microangiopathy may even predate glomerular changes in diabetic patients. Several urinary-enzymes have been used as marker of tubulo epithelial damage. N-acetyl-β-D-glucosaminidase (NAG) is the most commonly used marker. More specific tubular markers have been developed by the University of Antwerp, Belgium, which are human-intestinal-alkaline-phosphatase (h-IAP), restricted to the brush border of the S3 segment, and tissue-non-specific-isoenzyme (TNAP) ,excreted mainly in the S1 and S2 tubular segments. The purposes of this study was to identify the role of NAG, h-IAP and TNAP in detecting early tubulointerstitial changes in NIDDM , IGT and normal-glucose-tolerance subjects. 176 NIDDM and 108 IGT subjects whose diagnosis was confirmed with Oral-Glucose-Tolerance-Test (OGTT), participated in this study. To obtain a general picture of the basic values in the population, 53 participants living in the same area who have a normal glucose tolerance after OGTT were used as the *control group*. The urinary enzyme assays were performed on second morning urine samples. The values of the urinary enzymes (h-IAP,TNAP and NAG) were corrected to creatinine urine (U/gram creatinine). The mean values and 95% of confident interval in Control, IGT and NIDDM for h-IAP(U/g) were 1.07(CI=0.64-1.51), 1.68(CI=1.31-2.04), 2.61(CI=2.09-3.14) respectively, for NAG(U/g) were 3.65(CI=3.06-4.2), 5.94(CI=5.35-6.54), 10.7(CI=8.69-12.7), respectevely whereas for TNAP(U/g) were 1.49(CI=1.26-1.69), 0.19(CI=0.06-0.31), 1.15(CI=0.65-1.48) respectively. It is clearly shown that the mean values of h-IAP and NAG increased in a stepwise pattern from the lowest value in the control group to the highest value in the NIDDM group, wheras TNAP did not follow this pattern. The percentage of subjects with high h-IAP increased from 11.3% in the control group to 21.3% in the IGT group and to 40.3% in the NIDDM group. The NAG showed a more prominent stepwise pattern which were 15.1%, 50.9% and 72.3% respectively. On the other hand, the TNAP did not follow this pattern. There are not sufficient references to explain the correlation between the levels of glucose tolerance and h-IAP and NAG. However, glucose tolerance states reflects insulin resistancy and blood glucose levels. It can be speculated that the tubulointerstitial changes,specifically the S3 segment, which was shown by the increases of h-IAP and NAG assay, are associated with the glucose tolerance status of the patient.

2024

PROGRESSION OF NEPHROPATHY IN SPONTANEOUS DIABETIC RATS IS PREVENTED BY OPB-9195, A NOVEL INHIBITOR OF ADVANCED GLYCATION.

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Irreversible advanced glycation endproducts (AGEs) formation is considered to be a factor contributing to the complications of diabetes. OPB-9195, a novel inhibitor of advanced glycation, effectively inhibited AGEs-derived cross-linking and the formation of AGEs in dose-dependent manner in vitro. To evaluate the therapeutic effect of OPB-9195, we investigated the pathological findings of kidneys as well as serum levels of AGEs in spontaneous diabetic rats, Otsuka-Long-Evans-Tokushima-Fatty (OLETF) rats. OLETF rats were randomized into a non-treated group (n-Tx: n=12) and OPB-9195-treated group (Tx: n=12). OPB-9195 had been administrated to Tx rats from 24 weeks of age, and the rats in the two groups were killed at 24, 44 and 56 weeks of age. Serum levels of AGEs in Tx were significantly lower than those in n-Tx at 44 and 56 weeks of age (5.3±0.6 v.s.10.0±2.5, 6.9±0.9 v.s.15.1±2.0U/ml, P<0.01). PAS-positive nodular lesions and exudative changes that were present in n-Tx sections at 56 weeks of age were practically nil in Tx sections, and the percentage of sclerotic glomeruli was significantly ameliorated in Tx at 44 weeks of age in microscopic examination (3.1±1.0 v.s. 9.7±4.1%, P<0.05). Sclerosis index and urine albumin excretion were also significantly lower in Tx than those in n-Tx even at 56 weeks of age (0.54±0.01 v.s. 0.71±0.01, 7.5±6.2 v.s.18.6±4.8mg/mgCr, P<0.05). Immunohistochemical staining of AGEs deposition in glomeruli of Tx was markedly diminished compared to n-Tx at 56 weeks of age. In conclusion, OPB-9195 prevents the progression of diabetic nephropathy by lowering serum levels of AGEs and attenuating AGEs deposition in glomeruli in OLETF rats.

2025

ELEVATED URINARY ENZYMES LEVELS ASSOCIATED WITH HIGH BLOOD GLUCOSE LEVELS

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Diabetes mellitus and the associated glycosuria may lead to tubular dysfunction. The excretion of urinary enzymes such as N-acetyl-β-D-glucosaminidase (NAG) and other proteins may be increased early in the diabetic process and be independent of the excretion of albumin. Recently, studies on alkaline phosphatase, which are ectoenzyme localized on the brush border of proximal tubular cells, have given promising results as markers of early tubular involvement. Human-Intestinal-alkaline phosphatase (hIAP) is exclusively localized in the S3-segment while tissue-non specific isoenzyme (hTNAP) is present all along the proximal tubule. The purposes of this study was to identify the increase of NAG, h-IAP and TNAP levels in association with high glucose levels. 176 NIDDM , 108 IGT and 59 subjects whose diagnosis was confirmed with OGTT, participated in this study. Regardless of their diagnosis or treatment they were classified according to their fasting blood glucose of 80-120, 120-140,140-200 and > 200 mg/dl, and 2 hours after glucose load blood sugar levels of 80-160, 160-180, 180-240, and >240 mg/dl. The cut-off points for the elevated urinary enzymes levels were: IAP ≥ 2 U/gr creatinine, NAG ≥ 5 U/gr creatinine and TNAP ≥ 2.5 U/gr creatinine. This study revealed that the higher the blood glucose level is associated with higher percentage of subjects with high urinary enzymes, as can be seen in this following table.

Elevated Urinary Enzymes	FASTING BLOOD SUGAR <120 n=205	120-140 n= 30	140 - 200 n =47	> 200 n=61	2-HOURS AFTER LOAD <160 n=93	160-180 n=38	180-240 n=87	>240 n=123
IAP %	19.5	26.7	42.6	57.4	11.8	28.9	32.2	50 5
NAG %	41	66.7	80.9	86.9	31.2	36 8	60 9	78.9
TNAP %	3.9	13.3	19.1	36.1	4.3	5.3	5.7	25 2

It can ,therefore, be speculated that the renal tubular cells, specifically the S3 segment is impaired ,which associated to high blood glucose levels.

2026

RENAL PROTECTIVE EFFECT OF PERINDOPRIL IN EXPERIMENTAL DIABETIC RATS

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The purpose was to observe the renal protective effect of perindopril in STZ-induced diabetic rats. Methods: the blood glucose levels were maintained > 16.7mmol/L in 64 diabetic rats. The DP group (n=32) was treated with perindopril (1mg/Kg/d) and the DC group (n=32) was was diabetic control. 20 rats were normal controls (NC group) . 24h urinary protein excretion (PRO) were measured at 1, 3, 6 months, The left kidneys were processed for morphological examination. Mean glomerular plana area (MGPA), mean glomerular volume (MGV) were calculated using sterological method. Results: during the study, PRO in both diabetic groups were higher than that of DC group. At 6 month, proteinuria in DP group was decreased remarkedly (DC and DP vs NC: P<0.01, respectively, DC vs DP: P<0.05). After treatment, the MGPA and MGV in DP group were smaller significantly than that of DC group (MGPA: DC 7351.1 ±167.5, DP: 6828 ±159.5um², P<0.01, MGV: DC 814.7 ±26.9, DP: 706.45 ±25.1 um³, P<0.01). DC and DP group showed significant mesangial expansion, glomerular basement membrance (GBM) thickness and fusion of epithelial cell foot process, But thickness of GBM were alleviated to a certain extent in DP group. Conclusion: perindopril could decrease proteinuria and alleviate the pathological changes of kidney in diabetic rats.

2027

URINARY EXCRETION OF PANCREATIC STONE PROTEIN IN DIABETIC NEPHROPATHY

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Urinary pancreatic stone protein (PSP) levels were measured in 68 diabetic patients and 170 healthy controls to investigate the relationship between the progression of diabetic nephropathy and PSP excretion. Urinary albumin, N-acetyl-β-glucosaminidase (NAG), α1-microglobulin, creatinine clearance, and the blood PSP level were also determined in the diabetic patients. The urinary glucose level and glycemic control did not influence the urinary PSP level. In patients with normoalbuminuria (urinary albumin < 20 mg/gCr, n=31), microalbuminuria (20-200 mg/gCr, n=19), and macroalbuminuria (>200 mg/gCr, n=18), the mean urinary PSP level was 347, 507, and 860 μg/gCr, respectively. These levels were significantly higher than the level in normal volunteers (168 μg/gCr, $p<0.01$). A significant positive correlation was observed between the urinary PSP level and the NAG or α1-microglobulin levels ($p<0.01$). There was a stronger correlation with α1-microglobulin. Blood PSP levels were also elevated in patients who had renal impairment with a decreased creatinine clearance. In conclusion, urinary PSP excretion was increased from the initial stage of diabetic nephropathy and this increase became more marked as nephropathy progressed. Increased PSP excretion may reflect renal tubular dysfunction.

2028

RENAL IMPAIRMENT AND PATHOLOGY ARE PREVENTED BY PERINDOPRIL TREATMENT IN DIABETIC TRANSGENIC REN-2 RATS.

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Diabetic microvascular disease responds readily to angiotensin converting enzyme inhibition suggesting that an amplified tissue renin-angiotensin system (RAS) may be causative in diabetic pathology. The transgenic (TG) Ren-2 rat which by phenotype exhibits enhanced tissue renin and high plasma prorenin provides an opportunity to test this hypothesis. Six week old female, Sprague-Dawley (SD) and heterozygous TG rats were given either streptozotocin (STZ, 55 mg/kg) or citrate buffer (control) by tail vein injection and sacrificed at 4(SD,TG), 12(TG) and 24(SD) weeks. A further group of diabetic TG received perindopril (P, 2mg/L) in their drinking water for 12 weeks. Plasma glucose (diabetic >18mmol/L) was measured weekly. Systolic BP in 12 week TG rats was unchanged with diabetes (229±7mmHg) but decreased to normotensive levels with P (149±11mmHg). Body weights of diabetic rats and diabetic + P were significantly lower than controls ($p<0.01$). Plasma electrolytes were normal except in 12 week diabetic TG rats which were hyperkalaemic (6.7±0.3 mmol/l). Renal impairment was apparent in these diabetic TG rats and was corrected by P; GFR fell from 3.5±0.1 to 1.6±0.3 ml/min ($p<0.05$), while with P the decline was largely prevented (2.4±0.2 ml/min, $p<0.05$). Albumin excretion rate (AER) was elevated with diabetes (0.3x/÷1.4 to 1x/÷1.4mg/24 hours, $p<0.05$) but ameliorated with P (0.7x/÷1.3mg/24 hours). In 12 week diabetic TG kidneys, but not controls, glomeruli displayed twice as much sclerosis (glomerulosclerotic index, $p<0.01$), tubules were vacuolated and contained glycogen and efferent arterioles were hyalinized. In kidney medulla, interstitial fibrosis and inflammatory cells were present and tubules were dilated and degenerated. These changes were markedly reduced with P. At 12 weeks, diabetic TG kidneys displayed increased renin immunolabelling in juxtaglomerular cells (JG) compared with controls or diabetic SD, and renin was also present in the cytoplasm of proximal tubules. With P, renin immunolabelling in JG cells was more intense while it disappeared from proximal tubules. Plasma total renin collected from the tail vein of conscious diabetic SD and TG rats was unchanged despite an increase in kidney renin content in diabetic TG from 4 to 12 weeks (0.52±0.1 to 2.1±0.68GU/kidney, $p<0.01$). The TG Ren-2 rat displays rapid onset diabetic renal impairment with florid glomerular and tubulointerstitial pathology. The preservation of renal function and structure with perindopril provides further evidence that the renal RAS is related causally to the development of severe diabetic renal disease.

2029

INCREASED URINARY EXCRETION OF BIOLOGICALY ACTIVE TUMOR NECROSIS FACTOR α IN DIABETIC NEPHROPATHY.

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In a follow up study of 9 months we investigated the diagnostic value of TNFα in diabetic microangiopathy (samples obtained in intervals of 6 weeks). Plasma and urinary TNF bioactivity were detected by using the L929 cell cytotoxicity assay in the following groups. 59 patients with NIDDM (group 1), 16 patients with IDDM (group 2) and 30 matched healthy controls (group 3). Recombinant human TNFα (Sigma) served as a standard. Monoclonal neutralizing TNFα antibody (Boehringer) was applied to detect the TNFα cytotoxicity in the samples. A significant elevation of the plasma TNFα levels was observed both in NIDDM and IDDM patients with diabetic microangiopathy (retino-, nephro-, neuropathy, alone or in combination, TNFα concentration x±SE pg/mL group1: 139±21, group 2: 95±19) as compared to group 3 (22±8) or to the patients of group 1 (62±9) and group 2 (27±7) without microangiopathy. The urinary TNFα bioactivity was detectable only in diabetic nephropathy (microalbumin excretion >30mg/day, TNFα x±SE in group 1 and 2: 24±3 pg/mL) as compared to patients without nephropathy and controls (below the detection limit). According to our observation, TNFα may play a role in the pathophysiology of diabetic microangiopathy. Urinary TNFα bioactivity can serve as a diagnostic marker for diabetic nephropathy.

2030

ALOND® REDUCES HIGH URINARY ALBUMIN EXCRETION BUT NOT BLOOD PRESSURE IN CONSCIOUS DIABETIC RATS.

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Aldose reductase inhibitor (ARI) zopolrestat (Alond®) (AL) reduces elevated urinary albumin excretion (UAE) in non-insulinized diabetic rats (e.g., *Pharmacology,* 52:292, 1996) by a mechanism that is unknown. The present aim was to determine if an AL dose effective vs. UAE alters 24-hr mean arterial blood pressure (MAP), a potentially important determinant of UAE, in conscious chronically diabetic rats. Radiotelemetry pressure units (Data Sciences International) were surgically implanted in ~160 g SD rats. After 12 days, streptozocin (STZ)-diabetes was induced, and MAP was monitored continuously in unrestrained rats for 15 weeks. AL (~100 mg/kg bw) was added to the diet at ~12 weeks and was removed from the diet at ~14 weeks, with monitoring continued for 1 more week. Results: During 12±2 weeks of untreated diabetes, 24-hr MAP gradually declined from ~100 mm Hg to a stable baseline of 84.7±7.7 mm Hg (N=13 rats), the average (avg) of the 7 days preceding AL treatment. Addition of AL to the diet had no detectable effect on MAP, either initially or during 14±4 days. That is, avg MAP on AL was 84.9±7.4 mm Hg; ΔMAP vs. preceding 7-day baseline = +0.2±2.5 mmHg (NS, paired t-test, N=13). When AL was discontinued, MAP the following week was unchanged, 84.4±8.0 mm Hg; ΔMAP vs. preceding time on AL = -0.5±3.7 mmHg (NS, N=13). We conclude: 1) Arterial hypotension develops in chronic non-insulinized STZ diabetic rats; 2) under conditions previously described where ARI AL reduces UAE, AL has no detectable effect on systemic MAP; therefore 3) it is likely that AL reduces UAE independently of any effect on systemic MAP, most likely by normalizing renal microvascular pressure, flow, and/or capillary permeability.

2031

Diagnostic Significance of Urinary Immunoglobulin G in Diabetic Nephropathy

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We evaluated the diagnostic utility of urinary immunoglobulin G (IgG) in patients with diabetic nephropathy by comparing findings with those of renal biopsy speciments. A total of 55 diabetic patients were divided into 4 groups, D_0, D_I, D_{II} and D_{III-IV} according to the severity of diffuse glomerular lesions using Gellman's criteria. Twenty-five non-diabetic volunteers were used as controls. Using 24-hour urine specimens, IgG was measured by an enzyme-linked immunosorbent-assay (ELISA). In addition, urine samples were assayed for albumin, α_1-microglobulin, β_2-microglobulin and N-acetyl-β-D-glucosaminidase. The urinary excretion of IgG was significantly increased in diabetic patients as compared with the healthy controls. Among diabetic patients, IgG excretion was significantly higher in the D_I, D_{II} and D_{III-IV} groups than in the D_0 group, and in D_{III-IV} when compared to either D_I or D_{II}. Urinary IgG levels above the 95 percentile for healthy controls indicate the presence of significant urinary IgG levels. Diabetic patients showing the significant urinary IgG were significantly increased in D_I and D_{II} groups compared to those showing microalbuminuria. There was no correlation between the IgG excretion and other laboratory indices evaluated. These findings indicate that urinary IgG may be useful in detecting the early stage of diabetic nephropathy.

2032

AN ALDOSE REDUCTASE INHIBITOR (EPALRESTST) PREVENTS GLUCOSE-INDUCED UP-REGULATION OF TGF-β IN CULTURED HUMAN MESANGIAL CELLS

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TGF-β has been thought to be a key factor in the pathogenesis of diabetic nephropathy. This study was performed to clarify the effect of epalrestat (EP), an aldose reductase inhibitor, on glucose-induced up-regulation of TGF-β in cultured human mesangial cells (MCs). Human MCs were cultured with RPMI-1640 medium supplemented with 5 and 33 mmol/l glucose in the presence or absence of 10^{-5} mol/l EP. TGF-β secreted into the medium was measured by ELISA. The amounts of fibronectin in the medium and cell layer were also determined by ELISA. Protein kinase C (PKC) activities in the cytosolic and membrane fractions were measured by a electrophoresis method using a fluorescent protein substrates. The amount of TGF-β was greater in 33 mmol/l glucose than that in 5 mmol/l glucose. The production of fibronectin was simultaneously enhanced by a high concentration of glucose. The increases in both TGF-β and fibronectin were completely prevented by the addition of EP to high glucose media. PKC activities were increased in membrane fractions of MCs exposed to high glucose media. Increased PKC activities in membrane fractions were also abolished by EP. These results indicate that EP prevents glucose-induced up-regulation of TGF-β, presumably by suppressing PKC activation in human MCs.

2033

DIRECT ASSAY OF URINARY TYPE IV COLLAGEN IN DIABETICS WITH A SENSITIVE ENZYME IMMUNOASSAY

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Aim. In order to evaluate clinical significance of urinary type IV collagen (uCL-IV) in diabetic nephropathy, we have developed a sensitive enzyme immunoassay (EIA). Methods. Concentrations of uCL-IV were directly determined with a one-step sandwich EIA, utilizing two monoclonal antibodies to different sites of human type IV collagen. 142 diabetics were divided into 4 groups according to renal functions. Group I normoalbuminuria n=76, Group II microalbuminuria n=31, Group III overt albuminuria n=18, Group IV chronic renal failure n=17. Urinary transferrin , α1-microglobulin , β2-microglobulin were also determined. Results. Concentrations of uCL-IV (mg/gCreatinine, Mean $\pm$ SD, reference value below 3.74) in group I, II, III and IV were 3.43 $\pm$ 3.81, 12.3 $\pm$ 30.1, 16.5 $\pm$ 17.9, 36.3 $\pm$ 21.5, respectively ($p<0.01$ between every two group by unpaired t-test).
Conclusion. The evaluation of uCL-IV by direct assay gives information on the activity of structurally altering glomerular processes, and will contribute to better management from the early stage of diabetic nephropathy.

2034

COMPLICATIONS IN A NON-HUMAN PRIMATE MODEL OF IDDM AND THE EFFECTS OF AMINOGUANIDINE.

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Aminoguanidine (AG) affects the formation of advanced glycated endproducts and nitric oxide which are thought to alter cellular function in diabetes. The aim of this study was to assess diabetic complications and the effects of AG in a non-human primate model of diabetes. Twenty-five male baboons (*Papio hamadryas,* 8 diabetic, 5 AG treated diabetic, 5 AG treated control and 7 control) were used in this study. The motor and sensory nerve conduction velocity (MNCV, SNCV) were measured using standard recording techniques and presented as % change from the mean value of the age-matched controls. Renal function was measured by glomerular filtration rate (GFR, ^{3}H-inulin). Glomerular basement membrane (GBM) thickness and mesangial volume (MV) were determined by morphometry at yearly biopsies.

	HbA1c	GFR	GBM	MV	MNCV	SNCV
	%	ml/min/kg	nm	%	%	%
Control	4.9±0.7	2.0±0.2	387±10	18±2	-	-
IDDM	8.9±0.3*	2.8±0.6*	444±73*	20±3	-16.3*	-8.4
IDDM/AG	11.2±0.5*	-	-	-	-20*	-6.7
Control/AG	4.4±0.5	-	-	-	-6.1	+4.2

* Significant at $P < 0.05$ level using analysis of variance with Bonferroni test. Renal structural and functional changes occur early in baboons with IDDM. Treatment with AG did not abolish slowing of the nerve conduction velocity. The results of other parameters in the AG treated animals are pending. (Supported by JDFI and The Rebecca Cooper Foundation)

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Nephropathy – Genetics, Epidemiology and Risk Factors

2035

THE EPITHELIAL SODIUM-POTASSIUM CHANNEL GENE (ENaC) IN RELATION TO HYPERTENSION AND DIABETIC NEPHROPATHY

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ENaC consists of three subunits (α, β and γ), and is expressed in the distal convoluted tubules in the kidney. We and others have found mutations in the gene coding for the C-terminal parts of the β and γ subunits of ENaC in patients with Liddle's syndrome which is characterized by hypokalemic hypertension with low renin and aldosterone values because of endogenous hyperactivity of ENaC. In this study we tested whether the gene is involved in the developement of primary hypertension (HT) or diabetic nephropathy (DN) as salt retention has been suggested to play a major role in the pathogenesis of these disorders. The C-terminal parts of the β- and γ-subunit genes were screened for polymorphisms using SSCP and DNA sequencing in 105 Swedish HT patients (64 males/41 females, age 55.7±14.1y, BMI 27.6±4.5kg/m^2, SBP 149.8±18.4mmHg, DBP 88.3±10.8 mmHg) and in 20 Finnish IDDM patients with overt DN(10m, 10f)/50 NIDDM patients with incipient or overt DN (20m/30f, age 68.3±11.7y, BMI 28.5±5.2kg/m^2, AER 199.4±525.6μg/min). All the HT patients were on antihypertensive medication. Incipient DN was defined as AER>20μg/min and overt DN as AER >200μg/min. 109 Swedish normotensive subjects (60m/49f, age 58.9±10.2y, BMI 25.5±3.3kg/m^2, SBP 126.7±14.7mmHg, DBP 74.3±8.7mmHg) and 80 Finnish nondiabetic subjects (42m/38f, age 59.7±8.5y, BMI 26.1±3.1 kg/m^2, AER 3.6±2.2μg/min) served as controls. In the γ-subunit, a common nonsense mutation was found in codon 650 (CTC->CTG). To test if this polymorphism is associated with HT or DN the genotype frequencies in the HT- and DN-groups were compared with the frequencies in the corresponding control groups. The genotype frequency distribution of the codon 650 polymorphism did not differ between HT patients (CC 65.7%, CG 33.3%, GG 1%) and control subjects (CC 66.0%, CG 31.1%, GG 2.8%) nor between the DN patients (CC 62.9%, CG 32.9%, GG 4.3%) and control subjects (CC 63.8%, CG 35.0%, GG 1.2%). Conclusions: We found a novel polymorphism in the ENaC gene which can be used for further studies evaluating the role of this gene in HT and DN. Preliminary studies did not find support for a role of the ENaC gene in HT or DN.

2036

DIABETIC NEPHROPATHY IN IDDM PATIENTS IN SPAIN. ESTUDIO DIAMANTE. Esmatjes E, de Alvaro F for the Spanish Diabetic Nephropathy Study Group.

The **aim** of this cross-sectional study was to establish the prevalence of renal involvement and to identify associations with its most impor-tant possible determinants in a large group of patients with IDDM.

Patients and methods 1822 patientes (50.5% males, mean age 30.5±9.7 years, diabetes duration 14.1±9.2 years) controled in Endocrinology Units of 18 spanish hospitals were studied. Urinary albumin excretion, plasma creatinine, lipid profile, HbA_{1c}, as well as, familiar history of hypertension and nephropathy tobacco consumption, arterial blood pressure and BMI was evaluated.

Results The prevalence of microalbuminuria, clinical nephropathy (macroalbuminuria + renal failure) and hypertension were, respectively 14.1%, 8.5% and 11.3%. Risk factors associated with greater renal affection were male sex (p=0.003), age (p=0.000), diabetes duration (p=0.0001), arterial hypertension (p=0.00000) BMI (p=0.012) and familiar history of nephropathy (p=0.0017). The prevalence of smokers (former+current) was higher in male patients with microalbuminuria (65%) and clinical nephropathy (68%) than in patients with normal renal function (54%, p<0.05). Moreover, HbA_{1c} (p=0.001), total colesterol (p=0.000) and triglycerides (p=0.000) levels were higher in renal affected patients.

Conclusions The prevalence of diabetic nephropathy Type 1 diabetics patients in Spain is similar to that observed in other european countries. The association between diabetic nephropathy and hypertension, lipid abnormalities, metabolic control and male sex is confirmed and tobacco consumption appears as an aditional risk factor to be consider in diabetes care.

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SODIUM-LITHIUM COUNTERTRANSPORT PREDICTS INCIDENT NEPHROPATHY IN WOMEN BUT NOT IN MEN WITH IDDM

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Some but not all cross-sectional studies have suggested that sodium-lithium countertransport (NaLiCT) could be a useful marker for diabetic nephropathy. In this study we examined the ability of NaLiCT to predict the incidence of nephropathy in IDDM. Study subjects were from the Epidemiology of Diabetes Complications Study, an ongoing 10-yr prospective study of childhood onset IDDM. Participants were clinically examined at baseline and biennially thereafter. Microalbuminuria (MA) was diagnosed as an albumin excretion rate (AER) between 20-200 μg/min, and overt nephropathy (ON), AER >200 μg/min in 2 of 3 timed urine samples. NaLiCT was measured in 122 subjects who were free of MA, and in 237 subjects, free of ON at baseline, and who also provided 6 years followup data. During the 6 years, 32 (15 males) developed MA, and 16 developed ON (7 females). Mean (SD) (mmol Li/l RBC/hr) baseline NaLiCT by 6-yr incidence of nephropathy are shown in the table:

	MA-	MA+	ON-	ON+
Males	.39(.16)	.40(.08)	.40(.15)	.36(.06)
Females	.33(.10)	.46(.17)*	.39(.14)	.53(.19)†

* P = .02; † P=.05

NaLiCT was not significantly correlated with HbA_1 or IDDM duration, but positively correlated with triglycerides. Using .40 mmol NaLiCT as a cutoff, the rates of MA or ON were similar between low and high NaLiCT groups in males; however, in females, those with high NaLiCT showed an increased risk of developing MA or ON (43% vs. 19% for MA, p=.04; 15% vs. 3% for ON, p=.02). Multivariate analyses show that the independent predictors of MA are HbA_1, waist to hip ratio, and triglycerides for females, and HbA_1 for males; the independent predictors of ON are NaLiCT and hypertension for females, and hypertension for males. The results suggest that NaLiCT is a strong predictor of incident diabetic nephropathy in women but not men IDDM, and the association is independent of hypertension.

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ASSOCIATION OF NEUROCLINICAL AND ELETROPHYSIOLOGICAL FINDINGS AND MICROALBUMINURIA IN BRAZILIAN IDDM PATIENTS.

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The aim of our study was to investigate the association between the neurological examination,eletrophysiological study (ES) and microalbuminuria (MICRO).The neurological symptoms were scored as present or absent.The tactil sensory testing was performed with the Semmes-Weinstein monofilaments.The vibratory and the pinpricking pain of the fingers and toes was scored as normal,decreased or absent.The reflexes were scored using the NIMDS Myothatic Reflex Scale.The eletrophysiological assessment were done on the Nihon Kohden-Neuropack2.The routine nerve sensory conduction(SC) studies were done on the median(M),radial(R),ulnar(U) and sural (S)nerves.The motor nerve conduction velocity(MC) were performed on the median and peroneal..From 52 IDDM patients (22male) aged 24.6±7.1 years with diabetes duration of 9.1 ± 6.1 years, 36 were submitted to the above examination and collected three timed overnight albumin excretion (OAER) on three non-consecutive days.Albumin concentration was determined by radio-immunoassay (DPC,LA).Micro (2 out of 3 OAER ≥ 20 ug/min and < 200 ug/min) was found in 6 (16,6%) and Normo in 30 (83,4%) . Statistical analysis was done by Mann-Whitney and Fisher exact test. Neurological symptoms were present in 12 (33%) patients, with no association with sex, HbA1c and systemic blood pressure, but was asssociated with smoking habit (p= 0.04).There is a association between the Micro and the presence of neurological signs and symptoms (p<0.05). The ankle reflex was absent in 26 (72,2%) ,8 (25%) had pinpriking anesthesia, and 3 (8,3%) tactil anesthesia. A decrease in SC on the right M and right U was observed in Micro vs Normo 46(0-51)vs 51(0-60) p=0.05, and 44(0-22)vs 50(41-64) p=0.05,respectively.The MC on the right M was decreased in Micro vs Normo 50(47-52)vs57(38-96) p=0.008 respectively.In conclusion neuropathy was associated with Micro and the SC and MC were more affected on the right side in this group of patients.

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ABNORMAL FUNCTION OF MULTIPLE MEMBRANE TRANSPORT SYSTEMS IN FAMILIES OF TYPE 1 DIABETICS.

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To search for recurrent features which distinguish type 1 insulin-dependent diabetes mellitus (IDDM) and its familiar background, we studied 52 type 1 diabetics (33±12 y; disease duration 14±9 y; 13 with microalbuminuria, 14 with retinopathy) and 54 parents of IDDM patients (55±9 y), 43 siblings (34±9 y), and 47 healthy control subjects (44±14 y, range 23-77) by measuring hemato-urinary analytes and erythrocyte Na/H antiport.

IDDM patients had higher Na/H antiport activity than healthy controls (8.10±3.25 vs 6.27±2.45 mmol/L h, $p<0.01$), apart from the presence of diabetic nephropathy; unexpectedly, retinopathic diabetic patients showed the highest exchange rates. Parents had overactive Na/H antiport (9.02±3.85 vs 6.94±2.20, $p<0.05$); except 6 of them, who had impaired glucose tolerance, remaining parents had 24 h-glycosuria lower than controls (0.4±1.5 vs 0.7±0.5 mmol/24h, $p<0.001$). Parents of retinopathics showed the highest exchange activity (10.39±3.88) associated with higher mean arterial blood pressure (101±6 vs 92±6 mmHg, $p=0.01$) than controls. Siblings had overactive Na/H exchange (8.41±3.60 vs 6.26±2.50, $p<0.01$) and lower urinary glucose output (0.2±0.5 vs 0.6±0.6, $p<0.001$); also urinary uric acid output, measured in 26 of them, resulted lower than in controls (2.5±0.8 vs 3.3±1.2 mmol/24 h, $p<0.05$).

We suggest the presence of a primary abnormality in Na/H antiport activity in families of IDDM patients, associated with secondary involvement of those transport systems which are influenced by transmembrane electrical/chemical H^+ gradient (such as glucose and urate uptake in the proximal tubule).

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Microalbuminuria and retinopathy among prepubertal children and post-pubertal Brazilian insulin dependent diabetic patients.

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The aim of our cross-sectional study was to determine the frequency and clinical variables associated with retinopathy and microalbuminuria(Micro) among prepubertal children(PPC) and post-pubertal(PPB) IDDM patients.For this purpose we have studied 78 IDDM patients (37 male) aged (mean±SD) 17.9 ±8.3 years with diabetes duration of 5.1±5.2 years. There were 64 PPB and 14 PPC which collected three timed overnight albumin excretion (OAER-10h) on three non-consecutive days. Albumin concentration was determined by radio-immunoassay (DPC,LA).Statistical analysis was performed by Fisher exact and Mann-Whitney tests and data were expressed as median(range).For stepwise multiple regression analysis OAER was log transformed. Mean intraindividual coefficient of variation for OAER was 60%. Micro (2 out of 3 OAER ≥20μg/min and <200μg/min) was found in 8 (10.3%) and normoalbuminuria (Normo-OAER<20μg/min) in 70 (89.7%) patients. Retinopathy was noted in 4(5.3%) and was more frequent in Micro than Normo patients, $p=0.03$. An increase in OAER was observed in PPB in comparison with PPC, respectively: 8.6(0.4-105.2) vs 2.4(0.3-18.2)μg/min,$p=0.002$.A stratified analysis has shown that this difference was only significant in patients with <5 years of diabetes duration with an OAER of 5.5(0.4-52.4)μg/min and 0.9(0.3-18.2)μg/min, $p=0.009$ for PPB and PPC respectively.The stepwise multiple regression analysis performed in the pooled diabetic group with mean of the three OAER samples and the following independent variables:age,diabetes duration, body mass index, HbA1, and systolic(sBP) and diastolic blood pressure has shown that age and sBP were significant ($r=0.47$, $p=0.0004$; $r=0.50$, $p=0.0001$) respectively. In conclusion our study has observed that Micro and retinopathy were more frequent in PPB than in PPC IDDM patients. sBP and age were the most important clinical variables associated with microalbuminuria.

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HIGH GLUCOSE INCREASES FOS & JUN PRODUCTION BY MESANGIAL CELLS:A LINK TO HIGH TGF-β_1 EXPRESSION?

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Glomerulosclerosis and diabetic nephropathy are triggered by exposure to high glucose (HG). TGF-β_1 is a factor thought to mediate this process. Therefore the cellular events that link exposure to HG with increased TGF-β production is of great importance. Activator protein-1 (AP-1; a heterodimer of c-fos and c-jun) is elevated in response to HG and we hypothesise that AP-1 increases TGF-β expression through the AP-1 transcription initiation sites in the TGF-β gene promoter. Therefore this study investigates the changes of fos and jun in response to HG. Cultured human fetal mesangial cells were harvested directly into SDS sample electrophoresis buffer after exposure to HG in serum free conditions for 24 and 48h. Further comparisons were made between cell exposed to HG for 24 & 48h and then incubated in LG for a further 48h (24/48;48/48). Densitometry was carried out on western blots probed with specific antibodies and detected with ECL (Amersham). Results are expressed as a ratio of HG/LG.

	24	48	24/48	48/48
c-jun	1.52±0.19*	1.80±0.09*	1.46±0.28*	1.81±0.38*
c-fos	1.99±0.16*	1.53±0.02*	1.20±0.10*	1.31±0.01*

* indicates significant difference from a ratio of 1.0 $p<0.05$

These results confirm that HG causes significant elevation of the c-fos and c-jun levels in mesangial cells which persist even after the HG has been removed. These results support the hypothesis that HG may elevate TGF-β levels through the AP-1 sites of the gene.

(Supported by Diabetes Australia)

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MICROALBUMINURIA IN PREGNANCY COMPLICATED BY DIABETES.

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The aim of the study was to investigate the effect of pregnancy in patients with IDDM (insulin dependent diabetes) and GDM (gestational diabetes) on renal function and to evaluate microalbuminuria during pregnancy and after delivery. The study included 22 pregnant women with IDDM and 28 with GDM. All the patients were treated with human insulin by the intensive insulin therapy method. Five GDM patients required insulin treatment whereas the other 23 were on a diabetic diet only. In all the patients microalbuminuria, glomerular filtration, serum creatinine concentration, urin examination, becteriuria, glycohaemoglobin concentration (HbA1c) and eye fundus examination were performed at each trimester of pregnancy and after delivery. All the patients had normal creatinine concentrations (mean - 0.65mg/dl) and blood pressure. No significant bacteriuria was noted. A marked microalbuminuria (>15ug/min) developed in 6 IDDM patients. During pregnancy microalbuminuria values ranged from 17.7 to 364.6ug/min (mean 86.9ug/min). After delivery, normalization of albuminuria (mean 10.46ug/min) was noted in all the IDDM patients. In the patients with microalbuminuria the HbA1c level (mean 6.8%), glomerular filtration (mean 154.26ml/min) and weight of the newborn (mean 3831g) were significantly higher than in the patients without microalbuminuria. In 5 GDM patients a marked microalbuminuria - mean 75.3ug/min (19.4 - 280.0ug/min) was also observed. In this group too a higher level of HbA1c (mean 6.5%), glomerular filtration (mean 155.6ml/min) and a greater newborn weight (mean 4028g) than in the group without microalbuminuria was observed. The values of microalbuminuria in this group also became normal after pregnancy (mean 12.3ug/min).

Conclusions: 1) Glycaemic control had a marked effect on microalbuminuria during pregnancy. 2) The microalbuminuria occuring in IDDM and GDM patients without renal failure was of a transient nature. 3) Pregnancy did not essentially impair kidney function in diabetic patients.

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LACK OF RELATION BETWEEN RENAL KALLIKREIN GENE POLYMORPHISM AND DIABETIC NEPHROPATHY. F. Torremocha*, R. Maréchaud*, M. Marre, P. Passa, M. Rodier, F. Alhenc-Gelas and X. Jeunemaitre for the GENEDIAB group. *Poitiers, France and Belgium. The kallikrein-kinin system is involved in the renal blood flow regulation, which is altered in diabetes. We have previously identified two mutations (R53H,Q121E) on the renal kallikrein gene (hKLK1) showing that R53H is associated with a low urinary kallikrein excretion rate. We tested the relationship between these two polymorphisms and renal disease in 489 insulin dependent diabetes mellitus (IDDM) subjects exposed to microangiopathy (IDDM before age of 35 years, affected by a proliferative retinopathy), who were recruited in 17 diabetic clinics in France and Belgium (GENEDIAB study). These subjects (276 men and 213 women, 44±12 year-old, diabetes duration of 29±10 years) were staged from case records as follows: 1 : no nephropathy, 2 : incipient (microalbuminuria), 3 : established (proteinuria), 4 : advanced nephropathy (plasma creatinine > 150 µmol/l or renal remplacement therapy). The R53H and Q121E polymorphisms were determined by the Allele Specific Oligonucleotide (ASO) hybridation technique.

Polymorphisms	Stages				*p*
	1 (n=156)	2 (n=105)	3 (n=124)	4 (n=104)	
allele frequency					
R53H	.044	.024	.056	.038	*0,69*
Q121E	.301	.342	.326	.394	*0,05*

The R53H polymorphism is not associated with the diabetic nephropathy. The is a slight, but not significant, association between Q121E and the nephropathy stage. These data suggest that both R53H and Q121E polymorphisms are not major factors contributing to the pathogenesis of diabetic nephropathy.

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DOES NEPHROMEGALY PREDICT EARLY NEPHROPATHY IN NORMOALBUMINURIC DIABETICS ?

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It has been suggested that glomerular filtration rate and kidney size increase before microalbuminuria develops. In this study, we investigated whether nephromegaly in normoalbuminuric period would be an early sign of nephropathy. Thus glomerular filtration rate, kidney size and albuminuria were measured in 30 normoalbuminuric (Group I), 25 microalbuminuric (Group II) and 30 macroalbuminuric (Group III) diabetic patients (Age:48±10; 53 ±12; 55±12 years). Glomerular filtration rate was estimated from creatinine clearence; kidney size was measured by ultrasonography and albuminuria was detected by immunoturbidometric method. Glomerular filtration rate values were found 130±67 ml/min; 98±50 ml/min and 48±29 ml/min, respectively ($p<0.05$ in all groups) right and left kidney sizes were found 110±9/113±10 mm, 103±9/108±8 mm and 87±13/94±14 mm, respectively ($p<0.01$ in all groups) albuminuria levels were found 0.85±0.27 mg/dl; 6.83±4.46 mg/dl and 40±12 mg/dl respectively. Significant inverse correlation was found between kidney size and albuminuria. ($r=-0.71$, $p<0.05$ in Group I; $r=-0.68$, $p<0.05$ in Group II and $r=-0.69$, $p<0.05$ in Group III) Significant correlation was found between glomerular filtration rate and kidney size in all groups ($r=0.46$; $r=0.52$; $r=0.56$ respectively, $p<0.05$). In conclusion, demonstration of increase in kidney size by ultrasonography during normoalbuminuric period may practically show decrease in renal functions.

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EFFECT OF ACE GENE ON DIABETIC NEPHROPATHY IN NIDDM PATIENTS WITH INSULIN RESISTANCE

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We aim to evaluate the effect of angiotensin converting enzyme (ACE) gene on the onset and/or progression of diabetic nephropathy in NIDDM with significant insulin resistance. Insertion/deletion (I/D) polymorphism of ACE gene was determined by PCR method in 49 NIDDM subjects who had normal renal function and were not medicated with ACE inhibitor. Mean glucose-infusion-rate (M-value) was calculated under euglycemic hyperinsulinemic clamp. Mean M-value in 115 NIDDM patients was 6.18mg/min/kg. The patients whose M-value was more than the mean value were classified into control group (n=15). The patients whose M-value was less than the mean value were classified into 3 groups . Group A: albumin excretion rate (AER) was less than 15µg/min (n=14), Group B: AER was from 15 to 70 µg/min (n=11), Group C: AER was more than 70 µg/min (n=9). Duration of the disease, BMI, blood pressure and glycemic control were not different among the groups. The frequency of D-allele in Group B and C was significantly higher than that in the control and Group A (0.591, 0.722, 0.233 and 0.214, respectively) ($p<0.01$). This suggests that ACE genotype (D-allele) have effect on the onset and/or progression of diabetic nephropathy in NIDDM subjects with significant insulin resistance.

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PREVALENCE AND CLINICAL FEATURES OF HYPERTENSION IN AN OUTPATIENT DIABETES CLINIC

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Aim of this study was to observe the prevalence of hypertension in a large,unselected diabetic population attending an outpatient clinic and to assess its relationship with some demographic,metabolic and clinical parameters. 668 consecutive diabetic patients were studied:53 had IDDM (21 males,mean age 31.5±13.4,mean diabetes duration 13.1±10.2 years) and 615 had NIDDM (285 males,mean age 61.7±9.9,known diabetes duration 11.3±8.8 years).Hypertension was defined by WHO criteria (blood pressure) 160/95 mmHg as a mean of 3 readings after a 5-min resting or the patient being on treatment).Hypertension was observed in 7/53 (13.2%) IDDM patients and was associated with increased age (52±12 vs 28±10,$p<0.0001$) and diabetes duration (19±12 vs 12±8,$p<0.001$) and with higher urinary albumin (72±88 vs 8±15 µg/ml,$p<0.0001$) and serum creatinine (1.2±.6 vs .8±.2 mg/dl,$p<0.005$) levels. 54% (332/615) of NIDDM patients were hypertensive; with respect to the normotensive ones they were older (65±8 vs 58±10,$p<0.0001$) and had a longer diabetes duration (13±9 vs 10±7,$p<0.005$), a greater body mass index (BMI,30±5 vs 27±7,$p<0.005$),higher serum cholesterol (213±43 vs 201±42 mg/dl,$p<0.001$) and uric acid (4.6±1.4 vs 3.8±1.1mg/dl,$p<0.0001$) levels and an increased prevalence of retinopathy (139/332 vs 78/283 patients,$p<0.01$) and coronary heart disease (CHD,85/332 vs 47/283,$p<0.05$).Also in this group microalbuminuria (32±64 vs 12±21,$p<0.0001$) and serum creatinine (1.1±.7 vs .9±.2,$p<0.0001$) were higher in hypertensive than in normotensive patients.These results indicate that,in an outpatient diabetes setting,there is a high prevalence (more than 50%) of hypertension,which is closely related to NIDDM. Age,diabetes duration and impaired kidney function seem to be risk factors for hypertension both in IDDM and in NIDDM;moreover in NIDDM hypertension is associated with other features of the insulin-resistance syndrome (BMI,cholesterol,uric acid) and with an increased morbidity for vascular complications (CHD and retinopathy).

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RENAL RESPONSE TO HYPERGLYCÆMIA AND ANGIOTENSIN I CONVERTING ENZYME DELETION (D) ALLELE IN IDDM.

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The Angiotensin I Converting Enzyme (ACE) Insertion/Deletion (I/D) polymorphism is a candidate gene for risk of nephropathy in IDDM patients. Facing to generalized capillary vasodilation provoked by hyperglycæmia, constitutively low (ACE II genotype) or high (ID or DD genotypes) ACE levels would produce low/high renal Angiotensin II and subsequently affect glomerular hydrostatic pressures. We studied Glomerular Filtration Rate (GFR) and Effective Renal Plasma Flow (ERPF) (^{125}I-Iodothalamate and ^{131}I-Hippurate infusion of 27 normotensive, normoalbuminuric IDDM patients during two consecutive 90-min periods of near-normoglycæmia (5.7±1.8 mmol/l), then hyperglycæmia (14.9±2.2 mmol/l), according to ACE D allele absence or presence. Nine patients with II genotypes were matched with 18 (1 case/2controls) others with ID (n=8) or DD (n=10) genotypes for age (34±10 years), sex (6 M/3 F), and IDDM duration (14±9 years). Plasma ACE were different in patients with II genotype vs others: 374±90 vs 541±138 µg/l (p=0.0032). During normoglycæmia, patients with II genotypes did not differ from others for GFR (146±22 vs 138±16 ml/min/1.73m^2) or ERPF (607±93 vs 632±68 ml/min/1.73m^2). During hyperglycæmia, GFR of patients with II genotype did not vary (-6.6±11.5% of initial values), while it increased in others (+4.3±6.7%, p=0.0045); ERPF varied similarly: II genotype -8.2±12.8% vs others +6.7±11.2% (p=0.006). Thus, ACE D allele can lead to changes in renal hæmodynamics able to produce nephropathy in response to hyperglycæmia.

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MICROALBUMINURIA IS ASSOCIATED WITH DIABETES COMPLICATIONS IN AFRICAN TYPE 2 PATIENTS.

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The relationship between microalbuminuria, chronic complications and cardiovascular risk factors was studied in 55 black African type 2 diabetic patients (27 microalbuminuric and 28 normoalbuminuric) matched for age, BMI, and known duration of diabetes. Microalbuminuria was diagnosed as urinary albumin excretion > 20µg/min on an overnight urine sample. Blood pressure measurements, serum lipids dosage, fundoscopy and effort testing were performed in all the patients. Echocardiography was used to evaluate left ventricular mass and ejection fraction. The two subgroups were compared with regards to the variables studied using t-test and χ^2 test with Yates correction where necessary. Results were as follows:

	Normoalbuminuria (n = 28)	Microalbuminuria (n = 27)	p-value
Systolic blood pressure[1]	133 ± 21 mm Hg	145 ± 23 mm Hg	0.04
Diastolic blood pressure[1]	81 ± 9 mm Hg	90 ± 15 mm Hg	0.009
Retinopathy	11.1 %	67.9 %	0.00006
Neuropathy	37.0 %	64.3 %	0.04
ST segment depression	7.4 %	10.7 %	
Left ventricular mass[1]	125 ± 50 g/m^2	158 ± 40 g/m^2	0.04
Ejection fraction[1]	52 ± 9 %	47 ± 10 %	
Total cholesterol[1]	4.4 ± 1.7 mmol/l	4.1 ± 1.4 mmol/l	
LDL cholesterol[1]	3.2 ± 1.7 mmol/l	2.6 ± 1.5 mmol/l	
HDL cholesterol[1]	1.0 ± 0.5 mmol/l	1.2 ± 0.5 mmol/l	
Triglycerides[1]	1.1 ± 0.7 mmol/l	1.5 ± 0.8 mmol/l	

[1] Mean ± standard deviation

We conclude that microalbuminuria is associated with increased blood pressure, left ventricular hypertrophy, diabetic neuropathy and retinopathy in the population studied. Ischaemia, serum lipids and left ventricular systolic dysfunction were not associated with microalbuminuria.

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POLYMORPHISM OF THE ANGIOTENSIN CONVERTING ENZYME (ACE) GENE IN PATIENTS WITH DIABETIC NEPHROPATHY.

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Diabetic nephropathy appears in about 10 to 30% of insulin-dependent (IDDM) patients. It is supposed that genetic predisposition to hypertension may be responsible for the development of diabetic nephropathy. Several studies on the ACE gene polymorphism have demonstrated that DD genotype is associated with an increased risk of cardiovascular diseases. The aim of this study was the determine wheater there is a potential association between ACE polymorphism and diabetic nephropathy in IDDM patients. The study was carried out in two groups. Group I consisted of 145 normotensive non-diabetic men (twenty-four hour ambulatory blood pressure) whose age was 23±2 years, mean blood pressure 127/77 mmHg. Group II consisted of 64 IDDM patients with incipient or overt diabetic nephropathy. Their age was 38±8 years, the duration of diabetes was 19±7 years, the mean blood pressure was 139/83 mmHg. The examination of the insertion/deletion I/D polymorphism in intron 16 of ACE gene was performed using the polymerase chain reaction. The following results were obtained:

Genotype	Group I n=145	Group II n=64
DD	38 (26%)	26 (41%)
ID	74 (51%)	31 (48%)
II	33(23%)	7 (11%)
D allele frequency	0.52	0.67

Conclusion: It seems that the presence of the D allele of the ACE gene can be a marker of diabetic nephropathy.

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RELATIONSHIP BETWEEN URINARY EXCRETION OF ALBUMIN, TRANSFERRIN, AND DIABETIC RETINOPATHY IN NIDDM.

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In order to study the relationship between the early change of retina and microproteinuria, we measured urinary transferrin (uTf), urinary albumin (uAlb), and a simple latex agglutination test (SLAT) of uAlb in 245 non-insulin-dependent diabetes. We divided the subjects by the levels of albuminuria by radioimmunoassay; the lower group (less than 30mg/l) and the higher group (more than 30mg/l in uAlb), and compared the frequency of positive cases in SLAT. In all subjects uAlb was 22.7 ± 1.5 (mg/l, mean± SE) and uTf was 2.91 ± 0.27 (mg/gCr). UTf significantly correlated (r=0.74, p<0.001) with uAlb. UAlb and uTf of the subjects with no diabetic retinopathy (NDR, n=157) were 20.3± 1.8 and 2.62 ± 0.34, those with background retinopathy (SDR, n=57) were 27.9± 3.3 and 3.40± 0.52, and those with proliferative retinopathy (PDR, n=31) were 25.6 ± 4.0, and 3.47 ± 0.83. UAlb of the subjects with SDR were significantly higher than those with NDR (p<0.03). UTf of the subjects with SDR and PDR were significantly higher than those with NDR (p<0.04, p<0.05, respectively). In the subjects with SDR, the frequency of positive cases in SLAT was higher (n=11, 78.6%) in patients with more than 30mg/l in uAlb and more than 1.0mg/gCr in uTf (n=14, 24.6%). In the study of the subjects with PDR, the frequency of positive cases in SLAT was higher (n=7, 100%) in patients with more than 30mg/l in uAlb and more than 1.0mg/gCr in uTf (n=7, 22.6%). We concluded that the SLAT was a useful study to check the uAlb excretion for a lot of out-patients of diabetics as the first screening test and the measurement of uTf would be an indicator of an early sign of diabetic vascular complications.

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MICROALBUMINURIA, HYPERTENSION AND ANGIOTENSIN CONVERTING ENZYME GENE POLYMORPHISM IN IDDM

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Recent studies yielded conflicting results concerning the predictive role of insertion/deletion (I/D) polymorphism of angiotensin-converting-enzyme (ACE) gene for the development of diabetic nephropathy (DN) in IDDM. However, the DN in DD homozygotes seems to progress more rapidly compared with II and ID subjects. The aim of our study was to evaluate the frequency of ACE genotypes and alleles in three groups of patients with the long-lasting IDDM: 1 - with incipient nephropathy (IN, n=19), 2 - normoalbuminuric normotensive (NN, n=11), 3 - normoalbuminuric hypertensive (NH, n=6). The I/D polymorphism was assessed by PCR method.

Genotypes distribution in the whole study group was in Hardy-Weinberg equilibrium. Genotypes frequency: IN (4 DD, 9 ID, 6 II), NN (4 DD, 7 ID, 0 II), NH (0 DD, 3 ID, 3 II) - χ^2=7.27, p < 0.122. No significant difference in the frequency of ACE genotypes has been found among three groups. However, we observed the lack of DD homozygotes in NH and the lack of II homozygotes in NN. Alleles frequency: IN (17 D, 21 I), NN (15 D, 7 I), NH (3 D, 9 I) - χ^2=6.28, p < 0.043. There was a significant difference in the alleles frequency in the study groups with reduced representation of D allele in NH patients. These preliminary results failed to confirm the II genotype as a marker for reduced risk of microalbuminuria and hypertension in IDDM.

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RISK FACTORS FOR MICROALBUMINURIA AND MACROPROTEINURIA IN IDDM AND INSULIN-TREATED NIDDM PATIENTS

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Aim of the study was: analysing the rate of 24 hr albumin excretion, its change during the last two years, the prevalence rates of retinopathy and hypertension and their correlation with albuminuria in 445 IDDM (189 males) and 456 insulin-treated NIDDM (131 males) patients who were cared at our Outpatient Department. The diabetes duration of the two groups of patients (13,8±10,4 vs. 13,7±7,7 yr.) and their actual HbA_{1c} level (7,8±1,5 vs. 7,7±1,4%) were identical The prevalence rate of microalbuminuria among IDDM patients was lower (18 vs. 27%, p<0.01), but that of macroproteinuria was similar (5 vs. 6%). Prevalence rate of all forms of retinopathy was also similar (38 vs. 41%). Hypertension joined with 17% of IDDM and with 54% of NIDDM patients (p<0.001). Positive correlations were found between hypertension and microalbuminuria and between hypertension and macroproteinuria only in IDDM patients (p<0.001) but between hypertension and retinopathy (p<0,01) and between albuminuria and retinopathy (p<0.01) in both groups of patients respectively. Analysing the change in rate of albuminuria during the last two year period we found, that 30% of both IDDM and NIDDM patients with microalbuminuria in 1996 had normal albumin excretion in 1994 and 30% of IDDM and 66% of NIDDM patients with macroproteinuria in 1996 had microalbuminuria two years before. No correlation was found between progression of albuminuria and the prevalence of hypertension in either group of patients. On the basis of these data we have to pay particular attention both to the increased risk of nephropathy in insulin-treated NIDDM patients, and to the importance of effective treatment of hypertension in diabetes mellitus.

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AGE, SEX AND DRIFT OF ANGIOTENSIN I-CONVERTING ENZYME GENE ALLELES: IS THIS RELEVANT TO DIABETIC ANGIOPATHY?

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Previously, we reported high frequency of D (deletion) allele of angiotensin I-converting enzyme (ACE) gene and DD homozygosity in general population, and protective effect of I (insertion) allele in IDDM patients with diabetic nephropathy (DN) and NIDDM patients with myocardial infarction (MI). DN and MI may dramatically reduce life expectancy in diabetes mellitus. Now we tested interference of ID/ACE alleles and genotypes with age and sex in mixed cohort of healthy donors (n=168) and IDDM/NIDDM patients (n=194) as a model of interaction of genetic and non-genetic risk factors influencing survival. All subjects (n=362) were divided in 3 groups: 1 – "young" (mean age 28.8±6.6 yr.; range 17-40 yr.; n=140); 2 – "middle" (mean age 50.2±6.2 yr.; range 40-60 yr.; n=155); 3 – "elderly" (mean age 65.7±3.8 yr., range 61-77 yr.; n=73). Clear though non-significant ID/ACE allele and genotype drifting was observed: I and D allele frequencies in 1, 2 and 3 groups were 0.371, 0.403, 0.425; and 0.629, 0.597, 0.575, respectively. Prevalence of D allele-containing ID/ACE genotypes also changed: II – 19.2, 18.7, 19.3%; whereas ID – 35.7, 43.2, 46.6%; and DD – 45, 38.1, 34.2%, respectively. When total mixed group was considered as males (mean age 41.8±14.9 yr.; n=163) vs. females (mean age 40±15.2 yr.; n=199), evident but non-significant displacement of these markers was also observed: I allele – 0.377 vs. 0.407; D allele – 0.623 vs. 0.593; II genotype – 17.2 vs. 20.1%; ID genotype – 41.1 vs. 41.2%; DD genotype – 41.7 vs. 38.7%, respectively. Since current data on mortality rate and distribution of other risk factors in different age strata of both diabetic and general Moscow populations were unavailable, our preliminary results should be interpreted carefully. We, however, conclude that ID/ACE alleles and genotypes may interfere with age and sex thereby providing a balance of various survival factors. Therefore, it should be taken into account in clinical interpretation of ACE genotyping results.

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SERUM SIALIC ACID LEVELS IN NIDDM PATIENTS WITH AND WITHOUT DIABETIC NEPHROPATHY. IS IT A RISK FACTOR?

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PURPOSE: Sialic Acid is one of the acute phase reactants, with a very important role in maintaining the negative charge of renal glomerular basement membrane. Increased serum levels of sialic acid are recently mentioned as a high risk factor for cardiovascular disease. Additionaly, there are studies that support that patients with NIDDM and albuminuria have 20-40 times higher death rates from cardiovascular disease than those with NIDDM without albuminuria. We are examining the possible relation between serum levels of sialic acid and diabetic nephropathy.

MATERIAL-METHODS: We measured serum levels of sialic acid, cholesterol, tg, creatinine and HBA_{1C} in three groups of patients with NIDDM.

GROUP A: 26 patients with NIDDM without albuminuria.
GROUP B: 18 patients with NIDDM with microalbuminuria.
GROUP C: 28 patients with NIDDM with macroalbuminuria.
GROUP D: 18 adult subjects as control group without D.M.

We also examined the correlation between serum levels of sialic acid and albuminuria in patients with NIDDM.

We used the photometric method for measurement of total serum levels of sialic acid and we refined as microalbuminuria the daily excretion of albumin from 30 mg/24h to 30 mg/24h. All groups were comparable to age (61.6 - 61.3 - 64.5 - 50.8 yrs) sex, B.M.I. (28.5 - 29.12 - 30.06 - 29.01), smoking frequency (81% - 33% - 35% - 30%), duration; of diabetes mellitus (11.3 - 12.1 - 12.8).

RESULTS: Group A had higher serum levels of sialic acid than group D 70.61 to 64.15 (p<0.05). In comparison, group A had higher sialic acid levels (82.9±13, p<0.001 and 79.41, p<0.001) groups B and C.

The multifactorial analyses between serum levels of sialic acid and the other parameters was statisticaly significant for serum levels of triglycerides in group A, for serum levels of total cholesterol and triglycerides in group B and finally for serum levels of triglycerides and G.F.R. in group C.

CONCLUSION: Serum levels of static acid are significantly higher early and later stages of diabetic nephropathy.

We could consider sialic acid as a risk factor for the development and progression of diabetic nephropathin NIDDM.

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RELATIONSHIP BETWEEN ACE GENE POLYMORPHISM AND NEPHROPATY IN TURKISH DIABETIC POPULATION.

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Angiotensin-converting enzyme (ACE) is involved in the metabolism of two major vasoactive peptides, convertion anjiotensin I into anjiotensin II and inactivation bradykinin. The insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene is related to susceptibility to nephropathy in IDDM and macrovascular complications in NIDDM, although some investigation do not support this association. Ethnic/race-related variance in ACE gene polymorphism is known. Therefore we have examined the relationship between this ACE gene polymorphism and presence of nephropathy in Turkish subjects with IDDM and NIDDM. ACE genotyping was performed using the polymerase chain reaction(PCR). We studied 108 subjects with NIDDM and 28 subjects with IDDM. Twenty-five percent of IDDM patients and 50% of NIDDM patients had microalbuminuria or overt proteinuria. The DD, ID and II genotypes were present in 25%, 57.1% and 17.9% in IDDM subjects, respectively. The DD, ID and II genotypes were present in 15.7%, 65.7% and 18.5% in NIDDM subjects, respectively. The frequency of ACE I/D genotype was similar to subjects with IDDM and NIDDM. There was no difference in genotype distribution between NIDDM patients with microalbuminuria or overt nephropathy and those with normoalbuminuria: 16.6%/70.4%/13.0% vs. 14.8%/61.1%/24.1% had DD/ID/II genotypes, respectively. According to our results, ACE genotype frequency does not seen to be a useful marker in determining genetic susceptibility to diabetic nephropathy.

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MICROALBUMINURIA, PROTEINURIA AND LIPID RISK FACTORS IN NIDDM

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Main aim of the study was to evaluate concentrations of various lipids, lipoproteins /including Lp(a)/ and apoproteins in NIDDM subjects and their relationship to the presence of microalbuminuria and proteinuria. We compared 145 NIDDM patiens (35 of them without microalbuminuria, 83 with microalbuminuria and 27 with proteinuria or renal insufficiency) with a control group of 58 healthy subjects. All NIDDM subgroups had significantly higher total serum triglycerides, total cholesterol/HDL-C ratio and apo B and lower HDL-C than the control group. Microalbuminuria correlated significantly and positively with creatinine ($r=0.3731$, $p<0.001$) and serum triglycerides ($r=0.2498$, $p<0.05$) and negatively with HDL-C ($r=-0.2622$, $p<0.05$) (Spearman rank correlations). The risk concentrations of Lp(a) >30 mg/dl were significantly more prevalent in microalbuminuric group than in the control group (36.2% vs. 17.2%, $p<0.05$). Newetheless median Lp(a) concentrations in these subgroups were not significantly different, as well as other lipid parameters. The group with albuminuria or renal insufficiency had significantly higher total cholesterol (TC) (6.79±2.73 vs. 5.53, $p<0.0001$), TC/HDL-C (8.03±6.61 vs. 5.52±1.75, $p<0.001$) and LDL-C/HDL-C (3.64±1.29 vs. 2.91±1.06, $p<0.001$) ratios and C-peptide (3.19±1.35 vs. 2.44±0.99, $p<0.05$) than the patients without microalbuminuria. In conclusion, proteinuria in NIDDM is associated with a very atherogenic lipid profile. Minor lipoprotein changes start to be apparent even in microalbuminuria.

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Relationship Between Angiotensin I converting Enzyme Gene polymorphism and diabetic nephropathy

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Diabetic nephropathy (DN) of non- insulin dependent diabetes mellitus (NIDDM) is probably diagnoged by renal hemodynamic abnormalities and by a genetic predispositon. Angiotensin I converting enzyme (ACE) regulates renal circulation through angiotensin II formation and kinins metabolism. An Insertion/ deletion polymorphism of the ACE gene is strongly associated with the level of ACE. We used PCR to detect the polymorphism of the ACE gene and studied the relationship between this polymorphism and DN : We compared 85 unrelated NIDDM individuals and 40 healthy compared 85 unrelated NIDDM individuals and 40 healthy controls, ACE genotype distribution was hot different; compared 46 NIDDM with DN petents to 39 NIDDM without DN subject, an imbiance of genotype distribution was observed, the frequencies of DD genotype and D allele are significantly higher in patients with DN ($P<0.05$), the D allele is associated with DN, a relative risk for the D allele was observed (odds ratio 2.8, $P< 0.05$). Identification of NIDDM patients with DD gentype would help early detection and treatment of DN.

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ASSESSMENT OF MICROALBUMINURIA IN TAIWANESE DIABETIC PATIENTS — COMPARISON BETWEEN 24-HOUR, EARLY MORNING SPOT URINE AND SEMIQUANTITATIVE DIPSTICK TEST.

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Purpose: To determine which methods as early morning spot urine Albumin/Cr ratios and semiquantitative dipstick Micral-test could be alternatives to 24-hr urinary collections for assessment of microalbuminuria.

Materials & Methods: We measured 159 NIDDM Taiwanese outpatients (85 female, 74 male, age 57.4±11.6), collected urinary samples at the same day included (1)24-hr collections (2)early morning spot ruine, assessed albumin (immunonephelometry), creatinine, and by semiquantitative Micral-Test (Boehringer Mannheim).

Results & Disscussions:

1. Overall prevalence of microalbuminuria (>30mg/24-hr) in 159 Taiwanese NIDDM outpatients is 34.6%(24-hr), 33.9% (spot urine Albumin/Cr), 36.5%(Micral-Test), comparable to other's investigations.
2. There are strong correlations between 24 hr urine and spot urine Albumin/Cr ($R=0.49\sim0.89$, $p=0.0001$) when less or more than 30 mg/24-hr separatively, also good correlations between 24-hr urine and spot urine Micral-Test ($R=0.31\sim0.69$, $P=0.0001$) although less stronger.
3. Take 24-hr urine collections of microalbuminuria (>30mg) as standard, early morning spot urine Albumin/Cr ratios showed good sensitivity 89%, specificity 93%, and positive predictive value of 87%, false positive rate 6.6%, so early morning spot urine can be choosed as screening test of microalbuminuria instead of timed urine collections.
4. Semiquantitative Dipstick Micral-Test had an overall sensitivity 94%, specificity 58%, positive predictive valure 54%, negative predictive value 95%, false positive results 42% which suggested positive Micral-test should be confirmed by timed collections or spot urine method, and negative test could be accepted and retested annually.

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No association of the polymorphisms of angiotensin-I converting enzyme (ACE) and angiotensinogen (AGT) genes with diabetic nephropathy in Japanese long-standing IDDM patients.

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Aim: The aim of this study is to investigate whether the polymorphisms of the ACE and AGT genes are associated with diabetic nephropathy in IDDM patients. **Subjects and Methods:** 137 Japanese IDDM patients (M/F=58/79) with a duration of over 15 years were selected in terms of matching in current age, onset age and glycemic control of normo(N), micro(M), and macro(A) albuminuria groups. Plasma ACE activity and angiotensin II were measured, and DNA from PBMC was amplified by PCR using specific primers of ACE and AGT genes. Each PCR product was electrophoresed through 10% PAGE following ethidium bromide stain-ing. Chi-square test was used for statistical analysis. **Results:** The number of (N), (M) and (A) groups were 75/36/25. Plasma ACE activity and angiotensin II levels in these groups were 16.0±5.2, 16.1±6.6 and 13.1±6.1 IU/L/37℃ (NS), and 98.3±93.0, 90.5±107.8 and 137 ±161.7 pg/ml (NS). The ACE DD/DI/II genotype in these groups were 8/40/27, 7/16/13 and 3/10/12 (NS). Plasma ACE activity level was significantly higher in DD genotype group among other ACE genotypes (P=0.0008), but DD genotype was not related with nephropathy. The AGT TT/TM/MM genotype in these groups were 47/17/2, 16/13/0 and 17/4/3 (NS). ACE activity and angiotensin II levels were not related to AGT gene polymorphism. **Conclusion:** The development of diabetic nephropathy in Japanese long-standing IDDM patients was not related to ACE or AGT gene polymorphism and these activity or levels.

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ETHNIC INFLUENCES ON NEPHROPATHY IN PATIENTS WITH NIDDM FROM A MULTI-ETHNIC URBAN AUSTRALIAN COMMUNITY

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Cross-sectional data from the first 1,000 patients with NIDDM recruited to the Fremantle Diabetes Study (a prospective study of ethnic differences in care, control and complications in diabetics drawn from a population base of 118,000) were used to identify variables associated with diabetic nephropathy. The logarithm of the urinary albumin/creatinine ratio (lnACR) was determined for each patient and an estimate of the glomerular filtration rate (eGRF) was calculated from serum creatinine using the Cockroft-Gault formula corrected for body surface area. Ethnicity was assessed from self-perception of ethnic background and other demographic data, with Anglo-Celts forming 63%, Southern Europeans 20%, Asians 3% and Aborigines 1%. There was an overall significant inverse correlation between lnACR and eGFR (r=-0.19; P<0.0001). Multiple logistic regression analysis revealed that age, sex, diabetes duration, treatment type, waist-hip ratio, supine systolic and diastolic blood pressure, fasting plasma glucose, serum creatinine and fasting serum triglycerides were significantly and independently associated with lnACR (P<0.03). After adjusting for these variables and adding ethnic background to the model, only Aboriginal ethnicity was associated with lnACR (P=0.003). The variables significantly associated with eGFR, once those used in its calculation (age, sex, weight and height) had been excluded, were fasting plasma glucose, and fasting serum HDL-cholesterol and triglycerides (P<0.01), with only Aboriginal ethnicity predictive of eGFR when ethnic background was included (P=0.02).These data suggest that lnACR and eGFR are related indices of renal function which identify Aboriginal ethnicity as a risk factor for nephropathy. Whilst eGFR is associated primarily with indices of metabolic control, urinary albumin excretion is a more hybrid variable which reflects other factors not necessarily related to renal disease or diabetes.

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ANALYSIS OF ACE POLYMORPHISM IN NIDDM SIB-PAIRS CONCORDANT FOR NORMAL OR INCREASED BLOOD PRESSURE VALUES AND ALBUMIN EXCRETION RATE.

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Nephropathy risk in diabetes appears increased by essential hypertension. Some case-control studies have indicated an association between allele D of angiotensin-converting enzyme (ACE) polymorphism and renal complication in diabetes, while others have not. We have evaluated the possible relation of ACE genotype (identified by PCR amplification of genomic DNA extracted from peripheral leukocytes) with nephropathy and hypertension in a population of NIDDM sib-pairs from North-East of Italy and Sardinia with and without hypertension and abnormal albumin excretion rate. We used the non parametric test of Bishop et al. with X^2 test to compare the observed allele frequencies with the expected values under no linkage. We studied 46 sibships concordant for NIDDM and microalbuminuria (MA+); 116 sibships concordant for NIDDM and normoalbuminuria (MA-); 76 sibships concordant for NIDDM and hypertension (H+) and 38 sibships concordant for NIDDM and normal blood pressure values (H-). There were not significant differences in the four groups of sib-pairs concerning either allelic frequencies (MA+ expected: DD 18, ID 46, II 28 vs observed: DD 21, ID 40, II 31; MA- expected: DD 82, ID 112, II 38 vs observed: DD 77, ID 122, II 33; H+ expected: DD 55, ID 73, II 24 vs observed: DD 56, ID 70, II 26; H- expected: DD 28, ID 36, II 12 vs observed: DD 33, ID 27, II 16) or number of affected sib pairs sharing 0, 1 or 2 alleles IBS respect to expected values under no linkage (MA+ expected: X_0 1, X_1 16 and X_2 29 vs observed: X_0 0, X_1 18 and X_2 28; MA- expected: X_0 3, X_1 40 and X_2 73 vs observed: X_0 6, X_1 32 and X_2 78; H+ expected: X_0 2, X_1 26 and X_2 48 vs observed: X_0 2, X_1 24 and X_2 50; H- expected X_0 1, X_1 13 and X_2 24 vs observed X_0 1, X_1 15 and X_2 22, all p=n.s.). Our results suggest that the presence of allele D is not associated with hypertension or micro-macroalbuminuria in NIDDM sib-pairs, and that ACE genotype does not contribute to the genetic susceptibility to nephropathy and hypertension in NIDDM.

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THE PREVELENCE OF MICRO AND MACRO ALBUMINURIA IN NIDDM PATIENTS IN NELLORE, ANDHRA PRADESH, INDIA

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The purpose of the present study is to determine the prevelence of micro and macro albuminuria in NIDDM patients and their relationship with some risk factors.

2086 NIDDM patients who attended to our clinic between 1993 to 1995 were investigated for Albumin Excretion Rate (AER).

The prevelence of micro albuminuria (AER 20 to 200 micro grams/litre) is 32.5%, macro albuminurea (AER greater than 200 micro grams/litre) is 17.6%. In comparision with normo albuminuric subjects both micro and macro albuminuric diabetic subjects had significantly longer duration of diabetes, high levels of blood pressure both systolic and diastolic, Fasting blood glucose, HbAlc and Serum triglycerides.

This study showed high prevelence of micro and macro albuminuria in NIDDM subjects who were ar risk for other long term complications of diabetes like Nephropathy and Cardio Vascular complications.

NO.OF NIDDM PATIENTS	NIDDM WITH MICRO ALBUMINURIA	NIDDM WITH MACRO ALBUMINURIA
2086	679 (32.5%)	367 (17.6%)

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RELATIONSHIP BETWEEN I/D ACE POLYMORPHISM AND LEFT VENTRICULAR HYPERTROPHY (LVH) IN DIABETIC PATIENTS TREATED BY HAEMODIALYSIS (HD).

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Previous studies have shown that in subjects with DD ACE genotype plasma angiotensin II level is higher then in patients with I/D and I/I ACE genotype. On the other side studies have shown that DD ACE genotype predisposes patients with heart disease, no normal individuals, to left ventricular hyperthrophy (LVH). LVH is known single risk factor of cardiac death among patients with chronic renal failure (CRF) treated by haemodialysis (HD). Main factor of death haemodialyzed CRF subjects with diabetic nephropathy is cardiovascular complications. Aim of our study was to assess relationship between I/D ACE polymorphism and LVH in diabetic subjects with CRF treated by HD. We studied 44 diabetic subjects on regular bicarbonate HD (mean HD time >12 months) in age 51,5 (median; 25-75% 43-60 years). Left ventricular mess index (LVMI), relative wall thickness (RWT), ejection fraction (EF) and E to A wave flow (E/A) were estimated using echocardiography Doppler Scan (Siemens 450 SI). ACE I/D polymorphism in intron 16 was genotyped using the PCR method. Statistical analysis was performed using Kruskal-Wallis test and correlation tests. Selected results are presented in the table (median; 25%, 75%).

	LVMI g/m²	RWT I/I	EF %	E/A I/I
I/I ACE (N=6)	121 (120, 173)	0,67 (0,48, 0,75)	39 (35, 52)	0,70 (0,65, 0,71)
I/D ACE (N=12)	116 (84, 138)	0,57 (0,48, 0,61)	55 (43,56)	0,87 (0,76 0,87)
D/D ACE (N=26)	130 (108,148)	0,53 (0,48, 0,55)	51((47,59)	1.00 (0,78, 1.10)
Kruskal-Wallis test	NS	NS	NS	NS

Conclusion :
There are no significant relationship between I/D ACE polymorphism and LVH in diabetic patients treated by HD.

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URINARY ALBUMIN EXCRETION IN NEWLY DIAGNOSED NIDDM

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The study was aimed to determine the prevalence of albuminuria in newly diagnosed indian NIDDM patients. 168 (114 male,54 female) newly diagnosed diabetes with NIDDM in the age group 35-80 were selected for urinary albumin excretion studies along with 105 controls. The patients were initially screened for albuminuria by Micral Test and those who were borderline or positive had 24 hour urinary albumin studies. In all 15 (8.9%) had significant proteinuria,and 51 (30.4%) had micro-albuminuria, giving a total of 66 (39.3%) who had elevated urinary albumin excretion as compared to only 6 of the controls; this was strongly significant (p=0.0005).There was a slightly increased prevalence of proteinuria in 52 hypertensives; 25 had proteinuria of some degree. Patients with albuminuria were older (average age 57.03 against 43.05 in patients with normo-albuminuria). However,none of these differences were statistically significant. 12patients had retinopathy and 6 had evidence of peripheral neuropathy. This indicates that increased albumin excretion is common in Indian NIDDM patients at diagnosis and may occur without associated risk factors and other micro-vascular complications.

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MICROALBUMINURIA: EVALUATION OF SAMPLING AND DETECTION METHODS

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Objective: to compare urinary albumin excretion rate (AER) results obtained with different sampling and detection methods. Design and method: 220 diabetic patients were studied after macroalbuminuria or urinary infection were excluded: in 110 AER was compared as measured by immunoturbidimetry (MAU) and by Micral II (Mic2) strips (gold-labeled antialbumin monoclonal antibodies) in samples collected simultaneously during 24hs and at random; in another 110 AER was measured by MAU and Mic2 in simultaneous 24hs and first matinal micturition (FMM) samples; albumin/creatinin (Alb/Cr) excretion ratio was compared in all 24hs, random and FMM samples. AER normal maximum value was considered 20 mg/l; Alb/Cr normal value must not exceed 3mg/mM. Since MAU results are quantitative and Mic2 results are semiquantitative, comparison was made by classifying them as concordant or discordant according to agreement or not in relation to normal or abnormal values. Concordance and discordance were then statistically analyzed by Pearson Chi² test. Results: AER: MAU 24hs vs. MAU random: 75.5% concordant ; MAU 24hs vs. MAU FMM: 91% concordant ($p<0.05$). Mic2 24hs vs.Mic2 random: 82% concordant; Mic2 24hs vs. Mic2 FMM: 94.5% concordant ($p<0.05$). MAU 24hs vs. Mic2 random: 78% concordant; MAU 24hs vs. Mic2 FMM: 93.6% concordant ($p<0.05$). MAU 24hs vs.Mic2 24hs: 96.8% concordant. Alb/Cr: 24hs vs. random: 71.8% concordant; Alb/Cr: 24hs vs. FMM: 91.8% concordant ($p<0.05$). Conclusion: urine collected at random was not a representative sample of AER during 24hs while FMM urine was representative both with MAU or with Mic2. Need of at least two AER abnormal results out of three consecutively spaced urine samples is stressed for the diagnosis of microalbuminuria. Alb/Cr ratio measured in FMM urine yields results comparable to 24hs samples..

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THE PREVALENCE OF ALBUMINURIA AMONG PATIENTS WITH TYPE 2 DIABETES MELLITUS IN CENTRAL ANATOLIA, TURKEY

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Microalbuminuria is an early marker of renal injury among the patients with diabetes mellitus. As demonstrated previously, poorly regulated blood glucose, high blood pressure and duration of diabetes increases urinary albumin excretion (UAE) and after manifest proteinuria ensues, renal functions deteriorates irreversibly. By the treatment of hypertension and tight glycaemic control the progression of nephropathy may be prevented during the microalbuminuric period. The present study was performed to determine the prevalence of diabetic nephropathy in Kayseri, Central Anatolia, where type 2 diabetes prevalence is high, 6.9%. Six hundred-eleven patients with diabetes, who were diagnosed and followed-up in Erciyes University Hospital between January 1993- September 1996, were included in the study. Blood pressure, HbA1c values, renal functions, UAE and duration of diabetes were recorded. Patiens were divided into three groups according to their UAEs. Among 611 patients, 292 (47.7%) were men, 319 (52.3%) were women. The frequency of normoalbuminuria, microalbuminuria and macroalbuminuria was 49.8%, 25.5% and 24.7% respectively. The prevalence of hypertension was 12.2% in normoalbuminuric group, 36.5% in microalbuminuric group and 72.8% in macroalbuminuric group. Creatinine clearance was significantly ($p<0.01$) lower in macroalbuminuric group (46.9± 2.4 ml/min.) than normoalbuminuric group (101± 0.1 ml/min) as determined by student-t test. The average duration of diabetes was 80.9± 3.9 months in normoalbuminuric group and 160± 17 months in macroalbuminuric group. Results are expressed as means± SD. In conclusion, we have found that the prevalence of albuminuria is rather high among the patients with type 2 diabetes mellitus in Kayseri, Central Anatolia.

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SCREENING FOR MICROALBUMINURIA WITH MICRAL-TEST II

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Microalbuminuria is a recognised marker for diabetic nephropathy. Current screening methods often involve a laboratory assay, which is expensive. This Study compared near patient testing, using Micral-Test II (Boehringer-Mannheim), with a laboratory (immunoprecipitation) method. 150 early morning diabetic clinic urines were tested, ranging from Albustix (Bayer Diagnostics) negative (70), trace (52), one plus (20) to two plusses (8). 66 urines measured < 20 mg/litre on laboratory assay, of which 65 were also read as < 20 mg/litre on Micral-Test II (specificity 98.5%). 84 samples measured 20 mg/litre or more on laboratory assay, of which 3 were < 20 mg/litre on Micral-Test II (sensitivity 96.4%). Comparing Micral-Test II to laboratory albumin/creatinine ratio reduced specificity to 71%, but sensitivity remained almost unchanged at 94.4%. 101 samples were tested with Micral-Test II in poor artificial light, among which there was one false positive and one false negative. 49 samples were tested in daylight, among which there were two false negative results. Micral-Test II is a reliable, cost-effective method of screening for microalbuminuria, detecting all values above 30 mg/litre in this study. The reading appears equally reliable in artificial light. Laboratory assay can be reserved for those screening positive on Micral-Test II

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MICROALBUMINURIA AND CLINICAL FEATURES AMONG BRAZILIAN NON-INSULIN-DEPENDENT DIABETIC PATIENTS

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The purpose of our study was to investigate the frequency and clinical variables associated with microalbuminuria(Micro)among non-insulin-dependent diabetic patients.We studied 59 patients(38female,21male)aged59.6± 9.2 years(mean±SD)with diabetes duration of 11.6 ± 7.9 years. The patients collected three overnight urine for albumin excretion (OAER-10h) on three non-consecutive days and had blood sample collection for analysis of BFG and lipid profile (total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides). Albumin concentration was determined by radio-immunoassay (DPC,LA) and lipid profile by colorimetric method (COBRA/MIRAS, ROCHE). Statistical analysis was performed by Fisher exact and Mann-Whitney tests and data were expressed as median (range). For univariate regression analysis OAER was log trasformed. Mean intraindividual coefficient of variation for OAER was 64.4%. Retinopathy was assessed by fundus examination after pupillary dilation. Neuropathy was assessed by neurological symptoms and clinical examination and coronary artery disease (CAD) by history of cardiac disease. Micro (2 out of 3 OAER ≥20μg/min and ≤200μg/min) was found in 23 (39%) and normoalbuminuria (Normo-OAER <20μg/min) in 36 (61%) . Micro was associated with clinical neuropathy (p-0.03), but not with retinopathy, CAD,systolic (sBP)and diastolic blood pressure (dBP),diabetes duration or smoking habits. The coefficient correlation between the mean of the three OAER and sBP was r=0.36, p<0.001. Retinopathy was noted in 34 (57.6%) and was associated with sBP(p-0.05)and diabetes duration 8 (0.3-20) vs 14 (1-30)years (p-0.003). Neuropathy was present in 46(80.7%) and associated with smoking habits (p-0.04) and diabetes duration 13 (1-30) vs 4 (0.3-15)years.
We concluded based in our data that there is no association between any single clinical or laboratorial abnormalities and diabetes chronic complications.

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EVALUATION OF RANDOM URINE SCREENING FOR MICROALBUMIN-URIA WITH MICRAL-TEST II

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Obtaining serial overnight or timed samples of urine from diabetic patients is often problematic and inconvenient. The aim of this study was to assess the clinical utility of Micral-Test II (Boehringer Mannhein) for detection of microalbuminuria using random urine samples. Urinalyses were performed on 108 diabetic patients, comprising 69 NIDDM (37M mean age 57 years, 32F mean age 58 years) and 39 IDDM (14M mean age 44 years, 25F mean age 50 years), who presented electively to a provincial hospital Diabetes Clinic. Within 1-2 days, 24 hour urine protein collections were achieved in the same subjects. Documentation of microalbuminuria was carried out using a standard reference method. The results showed that, using a Micral-Test II cut-off of 20 mg/L for microalbuminuria-positive urines, the test sensitivity was 89%, specificity 61%, positive predictive value 53% and negative predictive value 92%. Using a higher cut-off of 50 mg/L, the above parameters were 64%, 90%, 77% and 83%, respectively. With a lower cut-off of 10 mg/L (estimated on strip), no improvement in any parameter occurred (89%, 51%, 48%, 90%) compared to the 20 mg/L cut-off level. In conclusion, Micral-Test II yields a reliable semi-quantitative estimate of microalbuminuria via conveniently-obtained random urine samples from diabetic patients, regardless of potential confounding variables that may affect protein concentration in such samples.

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RACE AND DIABETIC NEPHROPATHY IN SALVADOR, BRAZIL

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Diabetic nephropathy (DN) is the main cause of end stage renal disease in the western developed countries. It is commonest in black people and other racial minorities.In Brazil, diabetes mellitus (DM) is the 3rd cause of dialysis, specially in white men. There is no racial difference on the population prevalence of DM, nevertheless it is unknown any relation among race and DN. The aim of the study was determining possible racial association to DN, and looking for racial differences related to gender, average ages at time of diagnosis, duration of disease and insulin therapy. Medical reports of post-mortem diabetic patients (natural death) were examined at the Bahia Federal University Hospital in Brazil, for the 1949-1994 period. Variables related to the objective, the causes of death and renal micorscopy were registred . The criteria for DN were pre-defined and the patients classified in a double-blind manner as; with DN, without DN and with possible DN. The race/color were described by the pathologists as: white, mulatto and black. Frequency ratios (R), χ^2 test for association and t test for average differences were performed for the statistical analysis. Of the 200 autopsied diabetic patients 16 were excluded by secondary diabetes or no registered race; 174 autopsied patients were assessed according to the charts where non-insulin-dependent diabetes mellitus (NIDDM) and insulin-dependent diabetes mellitus (IDDM) were taken into consideration. The other 10 probably had DN. Of the 174 diabetic patients, 71(40,8%) had DN. Diabetic nephropathy was more frequent in blacks than in whites in spite of a p>0,05, but with a R of 1,6 for black men as compared to whites and mulattos. The frequency of DN in black men and women was similar (R female/male=1,2). Black men with DN were diagnosed on an average 11 years after the whites. The black men patients with and without DN had the shortest duration of disease (about 2 years). In relation to the women, those non white without DN also had a short duration of disease. Black and mulatto NIDDM women with DN received insulin therapy more frequently than the white women. The authors pointed out several possible differences in gender and race in the natural history and/or clinical course of diabetes.

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ANALYSIS OF FIRST MORNING URINE SAMPLE AS SCREENING TEST FOR MICROALBUMINURIA BY RECEIVER-OPERATING CHARACTERISTICS CURVE

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The aim of our study was to determine the efficacy of urinary albumin concentration ([ALB] - µg/ml) and albumin to creatinine ratio (ALB/Cr mg/mmol) in first morning urine sample (FMUS) for screening of microalbuminuria (MICRO). 50 IDDM and 30 NIDDM outpatients collected OAER and FMUS concomitantly on three non-consecutives days (T1,T2,T3). Timed overnight urinary albumin excretion (OAER-10h) was considered the gold standard. The data were analysed by receiver-operating characteristics (ROC) curve. MICRO (2 out of 3 OAER ≥ 20ug/min and < 200ug/min) was found in n=21 and normoalbuminuria (OAER < 20ug/min) in 59 patients. [ALB] was determined by radioimmunoassay (DPC, L.A.) and creatinine by Jaffé's method. The correlation coefficients between OAER and [ALB] were 0.75 (T1 - p<0.0001)), 0.77 (T2 - p<0.0001) and 0.69 (T3 - p<0.0001) and between OAER and ALB/Cr were 0.69 (T1 - p<0.0001)), 0.78 (T2 - p<0.0001)) and 0.75 (T3 - p<0.0001). The areas under ROC curves for [ALB] were T1=0,7180 (SE=0,016), T2=0,7238 (SE=0,0166) and T3=0,7256 (SE=0,0186) and for ALB/Cr were T1=0,7341 (SE=0,068), T2=0,7225 (SE=0,0164) and T3=0,7137 (SE=0,0189). No differences were found between the areas under ROC curves for [ALB] and ALB/Cr, respectively: T1 (p=0.24), T2 (p=0.48), T3 (p = 0,33), as well as when comparing the three FMUS for [ALB] and for ALB/Cr. The cut-off values for [ALB] with best sensitivity (Se) and specificity (Sp) were respectively; T1=13.75 (Se=91.7% and Sp=89.3%); T2=14 (Se=81.8% and Sp=86.2%); T3=14 (Se= 80% and Sp=84.6%); and for ALB/Cr were T1=2.25 (Se=70.8% and Sp=87.5%); T2=3.11 (Se=95.5% and Sp=87.9%); T3=2.84 (Se=86.7% and Sp=83.1%). In conclusion we noticed a good reproductibility for [ALB] and ALB/Cr and, although false positive and negative cases for MICRO could not be excluded, the cut-off points determined by ROC curves for [ALB] and ALB/Cr in FMUS had a good Se and Sp in predicting an OAER ≥ 20 µg/min.

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INCIDENCE AND DETERMINANTS OF MICROALBUMINURIA IN KOREAN SUBJECTS WITH NIDDM.

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The incidence of diabetic nephropathy in NIDDM differs widely according to the race. Although clinical proteinuria is reportedly more common in NIDDM patients in the Far East Asians than in the Caucasians, data on the incidence of microalbuminuria are not available. This study was undertaken to know the incidence and the determinants of microalbuminuria in Korean NIDDM patients. A cohort of 188 Korean NIDDM patients with initial normoalbuminuria were followed prospectively for 5.5 ± 0.9 yrs. Of the 146 patients who finished the study (63 men, 83 women), 37 showed persistently elevated urinary albumin excretion (UAE; > 20 ug/min) during follow-up, giving an incidence of 52/1,000 person-years. When we compared the characteristics of the progressors and the non-progressors, age (65 ± 11 vs. 59 ± 10 yr, P < 0.05) and duration of diabetes (16.7 ± 7.9 vs. 11.1 ± 5.7 yr, P < 0.001) were higher in the progressors than in the non-progressors. The baseline UAE was significantly higher in the progressors (8.7 ± 4.3 vs. 6.7 ± 4.0 ug/min, P < 0.05). More patients in the progressors had retinopathy at baseline (51 % vs. 14 %, P < 0.001) and at the completion of follow-up (76 % vs. 28 %, P < 0.001). The mean FBS and HbA_1 values during follow-up period were significantly higher in the progressors than in the non-progressors (9.6 ± 2.1 vs. 8.7 + 0.9 mM, 11.1 ± 2.4 vs. 9.9 ± 2.0 %, P< 0.05 respectively). The mean systolic and diastolic blood pressures were also significantly higher in the progressors than in the non-progressors (146 ± 15 vs. 135 ± 16 mmHg, 88 ± 7 vs. 83 ± 8 mmHg, P < 0.001 respectively). Multiple logistic regression analysis revealed that duration of diabetes, mean FBS and mean systolic blood pressure are independent variables having a statistically significant influence on the development of microalbuminuria. Our data show that the incidence of microalbuminuria in Korean NIDDM patients is lower than that reported in Pima Indians (84/1,000 patients-year) but is as high as that in Caucasian IDDM patients (30 - 48/1,000 patients-year). It is suggested that poor glycemic control and high blood pressure are risk factors for development of microalbuminuria in NIDDM.

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MICROALBUMINURIA IN IDDM : IT´S AND SOME ASSOCIATED RISK FACTORS ARE DECRESED ?

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General prevalence of microalbuminuria (ma) in IDDM patients (IDDM-P) in Europe countries is known and in similar ranges. The influence of specialized care would be play some beneficial roles in diabetic nephropathy (DN) after some intervention programs. **AIMS :** reanalized ND prevalences in IDDM-P under long-term clinical observation period in order to stablish posibilities of clinical regression of ma and asociated metabolic risk factors. **PATIENTS AND METHODS :** 319 IDDM-P between 1970 and 1995 was observed. 51 % was male, 49 % female and age range between 1 and 42 y. Evolution time of disease was 12.8 ± 9.7 y. All treated with three-four daily insulin inyections and HbA1c, Cholesterol (CHO), TGL, HDL-CHO, Blood pressure (BP) as sistolic (SBP) and diastolic (DBP) studied and habitual biochemical parameters of neprophathy considered. Stadistical studies content variables for bivariant and multivariant analisys and as end-point target we search the acumulative incidence of DM and ma for one year interval-period after first examination.We use a SPSS Satd Pack for social sciences (Chicago, 1990) under a PC-computer system. **RESULTS :** we found ma in 29.5 % of IDDM-P. High BP was present in these patients : 29 % with SBP>140 and 40 % with DBP>90 mmHg. Tabac as cigarettes/day had a positive and stadistical correlation to ma excretion 10.2 ± 12.1 (p < 0.01) with stablish DN (p< 0.05). Mean of HbA1c was superior in ma patients : 8.2 ± 1.79 (p<0.02) and positive familiar antecedents of nephropathy was frecuently associated between IDDM with ma compared with non-ma IDDM-P (p<0.05). CHO was superior in ma group 31%, (p<0.01). A stadistical and significant reduction in prevalence cumulated ratios of ma was found between the diferent cohorts and the "time observation period" in years (p<0.01). **CONCLUSIONS :** Our rates of ma and asociated metabolic and cardiovascular risk factors are coincident with others european studies. We observed during last decade a reduction in we sugest that cumulated incidence of ma and the clinical intervention of specialist care at Diabetes Units must play some special role in our IDDM-P.

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PREVALENCE OF DIABETIC NEPHROPATHY IN PRIMARY CARE AND HOSPITALS OF AN URBAN AREA

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In a screening project for diabetic nephropathy inpatients and outpatients independent from age, sex, duration and type of diabetes or diabetes therapy were screened for micro- or macroalbuminuria unless one of 8 exclusion critera was present. After giving informed consent, the patients performed self-tests for microalbuminuria in the first morning urine on three days using dip-stick tests. The same urine samples were retested in the office or the hospital, in hospitals aliquots were additionally quantitatively analyzed. In case of >20 mg/l albumin on at least two days retest was assumed as positive and a urinalysis using the Combur-9 Test ® followed to rule out confounders and to differentiate between micro- and macroalbuminuria. In 58 randomly selected doctor`s offices in a large German city 647 diabetic patients were included (38 IDDM patients: age 36.0±15.1 yrs, duration of diabetes 15.1±10.1 yrs; 543 NIDDM patients: age 66.3±10.7 yrs, duration of diabetes 9.5±7.1 yrs; 33 patients had secondary diabetes and in 33 cases the type of diabetes was not documented. In 5 hospitals 361 diabetic patients were included: 141 IDDM patients: age 42.8±16.0 yrs, duration of diabetes 17.8±11.8 yrs; 194 NIDDM patients: age 65.5±11.0 yrs, duration of diabetes 9.7±8.1 yrs; 9 patients had secondary diabetes and in 17 cases the type of diabetes was not documented. In doctor`s offices (hospitals) following prevalences of an elevated urinary albumin concentration were observed: IDDM patients: microalbuminuria 19.9 % (13.5 %) macroalbuminuria 0 % (6.9 %), micro-/macroalbuminuria plus pathologic urinalysis 3.0 % (6.3 %). In NIDDM outpatients with clinical duration of diabetes <5y., 5-15y., >15y. prevalences were: microalbuminuria 17 %, 17 %, 28 %, macroalbuminuria 8 %, 13 %, 23 % and micro-/macroalbuminuria plus pathologic urinalysis 5 %; 4 %, 8 % respectively. Because of the high prevalences of previously unknown micro- and macroalbuminuria in diabetic patients, both in primary care and in hospitals, a regular screening for microalbuminuria should be performed.

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EFFECT OF PUBERTY ON URINARY ALBUMIN EXCRETION IN DIABETIC CHILDREN AND ADOLESCENTS;

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The aim of this prospective study was to examine the influence of puberty on the progression of urinary albumin excretion rate (AER) in peripubertal diabetic patients. Three groups of insulin-dependent diabetic patients (age>6 yrs, diabetes duration>2 yrs, AER<20 µg/min) matched for diabetes duration were selected on the basis of Tanner staging (T_{1-5}) and were followed up for 3 years: *prepubertal* (n=20, age: 7.5±1.2 yrs, T_1 throughout the study), *pubertal* (n=28, age: 11.0±0.5 yrs, T_2 at entry and T_4 at exit) and *postpubertal* (n=26, age: 15.2±0.7 yrs, T_5) groups. The average HbA_{1c} over the study period did not differ significantly in the study groups. At 6 monthly intervals, 24-h urine collection was used to determine AER by an immunonephelometric method. AER increased significantly over 3 years in the pubertal [median (IQ range): 4.9 (2.8-7.6) vs. 14.8 (12.0-18.6) µg/min; p=0.014] and postpubertal [4.6 (2.6-7.7) vs. 8.5 (6.2-12.3) µg/min; p=0.045] groups but not in prepubertal subjects [4.8 (2.5-8.1) vs. 5.1 (2.7-8.9) µg/min; p=0.901]. The annual progression of AER was significantly higher in the pubertal than in the prepubertal or postpubertal groups [3.3 (3.1-3.7) vs. 0.1 (0.1-0.3); p=0.012 and 1.3 (1.2-1.5) µg/min/yr; p=0.035, respectively]. None of the prepubertal subjects but 6 of pubertal and 2 of postpubertal patients developed microalbuminuria (AER>20 µg/min on two consecutive occasions) over 3 years. Multiple logistic regression analysis showed that the risk of development of microalbuminuria was increased in pubertal patients as compared with the prepubertal [adjusted OR (95% CI): 3.7 (1.8-9.1)] or postpubertal [adjusted OR: 1.8 (1.2-4.2)] subjects. In conclusion, higher progression rate of AER occures in pubertal years than before or after this period. This suggests that the pubertal milieu leads to an accelerated process of early kidney damage in diabetes.

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CHANGE OF GFR AND URINARY ALBUMIN EXCRETION RATE IN NIDDM PATIENTS WITH MICROALBUMINURIA

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Microalbuminuria is a well-established marker for incipient nephropathy in inulin-dependent diabetes mellitus (IDDM). In contrast, the meaning of microalbuminuria in non-insulin-dependent diabetes mellitus (NIDDM) is complex. The presence of microalbuminuria in NIDDM patients is closely linked to cardiovascular disease and mortality. Microalbuminuria progresses to overt proteinuria in NIDDM patients, but not as consistently as it does in IDDM patients. We previously suggested that microalbuminuria in the presence of retinopathy may represent a state of real incipient diabetic nephropathy in NIDDM with declining glomerular filtration rate (GFR), while the significance of microalbuminuria in the absence of retinopathy may be more heterogenous. To further test this hypothesis, we followed the changes in GFR and urinary albumin excretion rate (UAE) in microalbuminuric NIDDM patients with and without retinopathy for 3.3 ± 0.6 years. Among 52 NIDDM patients with initial microalbuminuria (UAE; 20 - 200 ug/min), 31 had retinopathy from the baseline (group A), while 18 patients did not have retinopathy throughout the study (group B). UAE significantly increased in group A (66.2 ± 43.0 to 416.1 ± 729.3 ug/min, $P < 0.05$) but not in group B (53.0 ± 40.3 to 78.3 ± 69.0 ug/min, NS). GFR significantly decreased both in group A (116.2 ± 26.5 to 84.8 ± 24.2 ml/min/1.73 m^2, $P < 0.001$) and group B (134.8 ± 27.7 to 119.3 ± 27.7 ml/min/1.73 m^2, $P < 0.001$), but the magnitude of changes in GFR was significantly higher in group A than in group B (9.7 ± 6.6 vs. 4.6 ± 4.8 ml/min/1.73 m^2/ year, $P < 0.01$). Twenty six percent of group A patients (8/31) progressed to overt proteinuria in group A, while 11 % (2/18) of group B patients developed overt proteinuria. These results again suggest that the meaning of microalbuminuria in NIDDM may be complex. Microalbuminuira in the presence of retinopathy predicts aggravation of albuminuria and decline in GFR. In contrast, the renal function in microalbuminuric NIDDM patients in the absence of retinopathy may remain stable for years.

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PROGRESSION FROM MICROALBUMINURIA TO DIABETIC NEPHROPATHY

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An uncontrolled study has suggested that microalbuminuria is a poor predictor of progression to diabetic nephropathy in IDDM of long duration (>15 years). Our aim was to evaluate putative predictors, including duration of diabetes, for the progression from persistent microalbuminuria (30-300mg/24h) to diabetic nephropathy. 181 IDDM patients with persistent microalbuminuria (>300 mg/24h) were identified in 1984. They were followed with yearly examinations for up to 10 years (mean (SD) 7.9 (2.6) years. 45 % of patients with a duration of <15 years and 26 % of patients with a duration of ≥15 years of diabetes progressed to overt nephropathy (p=0.01). The yearly incidence of nephropathy (% per year) according to duration of diabetes are given in the table:

Duration of diabetes (years)	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45
Incidence of nephropahty	7.6	10.2	9.5	4.3	2.7	2.9	2.6	4.5

At baseline there were significant differences ($p<0.05$) between non-progressors vs progressors in albuminuria (geometric mean (antilog SE)) 77 (1.06) vs 116 (1.09) and duration of diabetes (mean (SE)) 24 (1) vs 20 (1.5) years, whereas there were no differences in arterial blood pressure 139/83 (1.7/0.9) vs 137/84 (2.2/1.2) mm Hg, haemoglobin A_{1c} 9.2 (0.18) vs 9.4 (0.3) %, s-creatinine, height, BMI, age, insulindose, retinopathy, antihypertensive medication, smoking or socio-economic status. A stepwise Cox regression analysis with the above mentioned baseline variables as independent variables revealed that elevated albuminuria and short duration of diabetes were the only factors associated with progression to nephropathy. Omission of albuminuria from the analysis made no difference. At baseline 15% with short duration and 17% with long duration of diabetes were on antihypertensive medication, and at end of follow-up it was 77 and 75% respectively (mainly ACE inhibition). This renoprotective treatment probably explains the lower rate of progression compared to previous studies dealing with the natural history. In conclusion albuminuria and short duration of diabetes are risk factors for progression to diabetic nephropathy in microalbuminuric IDDM patients. However long duration does not exclude progression.

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CHARACTERISTICS OF DIABETIC NEPHROPATHY IN NIDDM PATIENTS

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Contrary to the well-known features of diabetic nephropathy (DN) in IDDM patients (pts) the prevalence, course and risk factors of DN in NIDDM pts are not clear. The aim of our study was to assess the prevalence of microalbuminuria (MA) and macroalbuminuria (MAA), their relationship with some known risk factors and with other diabetic complications in 200 NIDDM pts (100 females and 100 males). 68 pts (33 %) were normalbuminuric (NA), 55 (26 %) MA and 77 (41 %) MAA. There was no significant difference among the three groups in the age, BMI, known duration of diabetes and hypertension.The BMI (kg/m2) was high in each group (28.8±5.29, 28.0±5.2, and 29.8±4.6, mean±SD). 65% of pts had hypertension in NA, 77% in MA and 81% in MAA group. The GFR (ml/min/1.73m2) was 71.9±26.8 in NA pts, 82.3±36.8 in MA pts and 56.3±32 in MAA pts. There was no significant relationship between the urinary albumin excretion (UAE) and glycemic control, cholesterol, HDL cholesterol, but the UAE was significantly positively correlated with triglicerides, (P<0.01), uric acid (P<0.01), serum creatinine (P<0.01) and negatively with GFR (P<0.01). There was no significant relationship between the UAE and kidney size. Diabetic retinopathy occurs already in NA pts (27 %) and 51 % of MAA patients were without retinopathy. 56 % of NA pts, 57 % of MA pts and 93 % of MAA pts had macroangiopathy. We conclude that (1) there is already impaired renal function in NIDDM pts with NA and MA (2) well-known cardiovascular risk factors seem to be the risk factors of DN (3) renal lesion of NIDDM pts may have caused by other diseases, than diabetes (atherosclerosis, hypertension e.g.) (4) unlike in IDDM, where the strict glycemic control is the main preventive factor of DN, in NIDDM the control of hypertension, hyperlipidemia, obesity, hyperuricemia may have priority.

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VASCULAR RISK FACTORS IN PARENTS OF PATIENTS WITH INSULIN DEPENDENT DIABETES AND NEPHROPATHY

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Parents of patients with diabetic nephropathy (DN) have an increased risk of vascular death suggesting a common familial risk factor, proposed to be hypertension. We have examined cardiovascular risk factors in parents of diabetic patients with (PN) and without nephropathy (PC).

65 patients with IDDM and DN and matched controls were interviewed and 87% of parents identified either as alive or the cause of death ascertained (PN114, PC 113). For all parents, a previous diagnosis of hypertension was obtained in 22% of PN and 14% of PC (Fisher's exact test, P= 0.17) and 23% of PN and 12% of PC of those parents who were still alive (PN 65, PC 74: P=0.11).

If both parents were living (PN, n=26 pairs, PC, n=35 pairs) they were invited to attend for 24 hour ambulatory blood pressure monitoring (ABPM) and measurement of cardiovascular risk factors. Parents with known angina were excluded as medication could not be withdrawn (PN 5 pairs, PC 11 pairs), while others were unwilling to take part (PN 9 pairs PC 12 pairs). Antihypertensive medication was withdrawn in 25% of PN and 17% of PC. In 2 parents(PN) antihypertensive medication had to be restarted due to rises in blood pressure and these pairs were excluded.

Age was similar in both parent groups (PN 66± 2 years, 10 pairs *vs* PC 65± 1years, 12 pairs) as was mean 24-hour systolic ABP (PN 133± 5 mmHg *vs* PC 131± 4mmHg. P=0.79), diastolic ABP (77± 2 mmHg in both groups). total cholesterol, body mass index and waist hip ratio.

While suggesting an increase in the prevalence of hypertension in parents of patients with DN, in those with two living parents the familial predisposition to DN in offspring is not explained by blood pressure alone.

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URINARY ALBUMIN EXCRETION RATE AND CARDIOVASCULAR DISEASE IN SPANIARD TYPE 2 DIABETIC PATIENTS.

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To assess the frequency of urinary albumin excretion abnormalities and their associations with cardiovascular disease or its classical risk factors in type 2 diabetes mellitus, 1348 clinic-proceeding patients have been studied retrospectively. The overnight urinary albumin excretion rate, blood pressure, smoking, ophthalmic and cardiovascular status, current therapies, estimates of glycemic control, plasma lipids, serum creatinine and uric acid have been ascertained. 767 (56.8%) patients were found normoalbuminuric, 461 (34.1%) microalbuminuric and 120 (8.9%) macroalbuminuric. In bivariate analyses, the urinary albumin excretion rate had statistically significant ($p<0.05$) relationships with age, duration of diabetes, male sex, waist-to-hip ratio, systolic and diastolic pressure, coronary heart disease, cerebrovascular disease, peripheral vascular disease, hypertension, antihypertensive therapy, laser-treated retinopathy, need of insulin therapy, smoking habit, fasting glycaemia, HbA_{1c}, creatinine, uric acid, triglycerides, HDL-cholesterol and apolipoprotein B. Almost statistically significant ($p<0.1$) relationships were found with hypolipidaemic therapy, insulin dose, non-HDL-cholesterol, apolipoprotein A_1 and lipoprotein (a). In a multivariate stepwise logistic regression model, HbA_{1c}, creatinine, hypertension, male sex, age, diastolic blood pressure, coronary heart disease and hypolipidaemic therapy were sequentially selected as variables independently associated with the UAER category. Micro and macroalbuminuria are frequent abnormalities associated with poorly controlled and complicated disease, with surrogate markers of insulin resistance, with overt cardiovascular disease and with the male sex.

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PREDISPOSITION TO VASCULAR DEATH IN PARENTS OF PATIENTS WITH INSULIN DEPENDENT DIABETES AND NEPHROPATHY

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To further examine the previously reported increased prevalence of vascular disease in parents of IDDM patients with diabetic nephropathy (DN) we have ascertained ages and causes of death in the parents of cases with DN and controls, in a background population with a high rate of vascular disease.

65 IDDM patients with DN (serum creatinine >120μmols/l and ACR >20 $mg.mmol^{-1}$) were identified from our database and matched for age, sex, and duration of diabetes to control patients without diabetic nephropathy. 87% of all parents were identified as either living or the cause of death ascertained by retrieval of death certificates.

Kaplan-Meier survival analysis revealed that parents of patients with diabetic nephropathy (PN) had an increased rate of death from vascular causes (PN 49% of all deaths *vs* PC 33%, P<0.05) and particularly stroke (PN 18% of all deaths *vs* PC 3% P<0.01). While the total number of deaths was not significantly different between groups (PN 43% dead vs PC 34% , P=0.07), living parents of IDDM patients with DN were younger than those of controls (PN 67± 1 years *vs* PC 70± 1, P<0.05).

We conclude that parents of patients with nephropathy are more likely to die from vascular disease, especially stroke, supporting the hypothesis that a common familial factor may predispose both to vascular disease and to the development of diabetic kidney disease.

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GLOMERULAR HYPERFILTRATION IN JAPANESE NIDDM PATIENTS WITH NORMO- AND MICROALBUMINURIA.

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The aim of this study was to investigate the significance of glomerular hyperfiltration(HF) in the pathogenesis and progression of renal impairment in Japanese NIDDM patients with incipient nephropathy. GFR(4hr iohexol clearance) and urinary albumin excretion were measured in 112 nortensive, normo- and microalbuminuric NIDDM patients(DM) of age 44.7+9.6 years and 44age-matched normal control subjects(NC). In NC, GFR showed a linear decline with age in the regression equation of Y=-0.84+127.9(r=-0.503). Based on this, the cut-off values of GFR($ml/min/1.48m^2$) for HF in each decade of age were determined as 140 in 20s, 130 in 30s, 120 in 40s, 110 in 50s. GFR in DM was higher than NC throughout all ages. HF was found in 18.8% of DM. There were no significant differences in duration of diabetes, HbA1c,blood pressure and UAE between normo- and hyperfiltrating DM. In microalbuminuric DM, the decline rate of GFR, as assessed from the regression equation , was significantly higher than in normoalbuminuric DM. In conclusion, 1) GFR in healthy adult Japanese declines at the rate of 0.84 $ml/min/1.48 m^2$/year, and 2) based on the age-adjusted cut-off values of GFR, HF was present in 19% of cases and HF may be a risk factor of progression of diabetic nephropathy in normo- and microalbuminuric Japanese NIDDM patients.

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FACTORS ASSOCIATED WITH ABNORMAL BLOOD PRESSURE PATTERN IN NORMOTENSIVE NORMOALBUMINURIC IDDM PATIENTS

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The aim of this study was to analyse the autonomic function in normoalbuminuric IDDM patients with a blunted fall in nocturnal blood pressure (BP). Thyrty-five normotensive, normoalbuminuric [albumin excretion rate (AER)<20 μg/min] with normal cardiovascular autonomic tests were studied. Glomerular filtration rate (GFR) was measured by ^{51}CrEDTA, extracellular volume (ECV) by the ^{51}CrEDTA distribution volume and AER by RIE. 24h ambulatory BP was measured by a portable monitor (Del Mar Avionics, auscultatory technique) at 10min intervals during the day and 15min intervals during the night. 24h ECG (Holter Analyser-Del Mar Avionics, model 750A) was recorded and heart rate variability (HRV) was calculated in time domain [mean of normal RR interval, standard deviation (SD) of normal RR intervals in 5 min, mean of SD of normal RR intervals in 5 min, SD of normal RR intervals, root square mean of successive differences of adjacent RR intervals and percent of successive differences between adjacent RR intervals > 50 ms] and in frequency domain [power spectral analysis: low frequency (LF) and high frequency (HF) component, in a 256 sec in a sitting position and during sleep]. Patients with diastolic BP night/day (DBP N/D) ratio >0.9 were considered non-dippers (0.96 ± 0.67; n=12) and the others dippers (0.84 ± 0.37). GFR was higher in non-dippers than in dippers (148.8 ± 24.9; 129.3 ± 23.1 ml/min/1.73m^2; p=0.03). ECV tended to be higher in non-dippers (22.7 ± 3.8 l/1.73m^2) than in dippers (20.4 ± 3.0 l/1.73m^2 ; p=0.058). HRV indexes in time domain did not differ between the groups. Non-dippers presented a higher LF component than dippers in the sitting position [0.38 ± 0.12 normalized units (NU); 0. 27 ± 0.11 NU; p=0.02] and during the sleep (0.28 ± 0.13 NU; 0.18 ± 0.10 NU; p=0.02). The HF component during sleep was lower in non-dippers (0.34 ± 0.11 NU) than in dippers (0.48 ± 0.19 NU; p=0.03). In conclusion, a blunted fall in nocturnal DBP is associated with a higher GFR and sympathetic activity in normoalbuminuric normotensive IDDM patients.

2084

INCREASED URINARY ALBUMIN EXCRETION IN PATIENTS WITH IMPAIRED GLUCOSE TOLERANCE. JM. Lu, CY. Pan, H. Tian, et al. Department of Endocrinology, Chinese PLA General Hospital, Beijing, 100853, China

In order to observe the urinary albumin excretion (UAE) of impaired glucose tolerance (IGT) and its related factors. The UAE was measured in 772 cases of IGT and 787 normal controls. The results showed that the UAE (6. 84+6. 30ug/min) and the incidence of microalbuminuria (10. 5%) were significantly higher than that of normal subjects (5. 20+4. 28ug/min and 4. 32%, respectively). In addition, BMI, blood pressure, serum levels of Ch, Tg, Cr were remarkedly higher, while serum HDL-ch level was lower than that of normals. The UAE (8. 32+8. 84ug/min) in IGT patients with hypertension was higher than that of IGT patients with normal blood pressure (6. 29+5. 16ug/min)

Among the IGT patients, the UAE (7. 07+6. 45ug/min) in obesity patients was higher than non-obesity (5. 77+4. 53 ug/min). Multiple analysis showed that UAE in IGT and normal subjects had significantly positive correlation with BMI, MBP, 2h blood glucose, and significantly negative correlation with serum HDL-ch. It was suggested that the UAE was elevated in IGT, and they had more risk factors of cardiovascular diseases.

Key words Impaired glucose tolerance Urinary albumin excretion

2085

RENAL MORPHOLOGICAL CHANGES PREDICT URINARY ALBUMIN EXCRETION RATE 6 YEARS LATER IN PATIENTS WITH IDDM AND MICROALBUMINURIA.

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We investigated in a prospective 6 years study the relationship between morphological changes at start, GFR at start, hyperglycemia and blood pressure during the study and urinary albumin excretion rate (AER) at the end of the study in 18 patients with IDDM and microalbuminuria. Age *(median and range)*: 19 (18-29) yrs, duration of diabetes 12 (7-18) yrs, HbA1$_c$ 10.3 (7.9-12.6) % mean AER 31 (15-194) ug/min. Renal biopsies were taken at 0 and 2,5 years, HbA1$_c$ and AER were measured 3-4 times a year. Glomerular structures were assessed by stereological methods. AER *(median + 95 CI)* decreased (NS) during the study, from 31 (18-40) to 18 (7-90) μg/min. Only 4 patients increased their AER and 7 patients were no longer microalbuminuric at the end of the study. HbA1$_c$ (mean + 95 CI) was reduced from 10.1 (9.2-11.1) to 8.4 (7.8-9.1)% (p=0.01). Mean arterial blood pressure (MAP) remained unchanged, 96 (92-100) to 95 (91-98) mm Hg. AER at 6 years (AER$_6$) showed a significant positiv linear correlation with the morphological changes at the start of the study [basement membrane thickness (BMT), r=0.71, mesangial volume fraction (r=0.46) and matrix volume fraction (r=0.42)], but not with mean 6-yrs HbA1c (r=0.26). In a stepwise regression analysis with AER$_6$ as the dependent variable, BMT (p=10^{-5}), mean 6-years HbA1c (p=10^{-3}) systolic BP (p=0.005) at 6 years and GFR (p=0.04) at the start of the study contributed significantly to the explained variation (R^2 = 0.86). With change in AER during the study as the dependent variabel, BMT (p=0.006) at start of the study and systolic BP (p=0.01) at 6 years contributed significantly (R^2=0.53). We conclude that the degree of morphological glomerulopathy after 10 years duration of diabetes is highly predictive of kidney funcion measured by AER six years later and that the development in AER is influenced by the level of hyperglycemia and possibly by systolic blood pressure.

2086

GLUCOSE-INSULIN AXIS AND MICROALBUMINURIA AMONG FIRST DEGREE RELATIVES OF NEPHROPATHS

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Diabetic nephropathy (DN) is common among Polynesians. We investigated whether the risk for DN was independent of the risk for NIDDM. A 2x2 factorial design was used: Polynesians ± a first degree relative with NIDDM (FHDM±) ± a first degree relative with nephropathy (FHN±). FHN were recruited through patients with end stage renal failure. Other subjects were recruited through randomly selected households. Subjects attended for a 75g oral glucose tolerance test and provided timed 2 hour urine samples for urinary albumin:creatinine ratio (UACR) on three separate days. Subjects ± FHN (n=173 and 174 respectively) were well matched for age, body mass index and blood pressure. FHN+ (vs FHN-) had a higher fasting glucose (FBG:5.1±0.7 vs 4.6±0.8 mmol/l, p<0.001), a similar 2 hour glucose (2HBG: 5.1±1.4 vs 5.0±1 mmol/l), a lower fasting and 2 hour geometric mean insulin (FINS:17.8 vs 22.9, 2HINS: 43.7 vs 70.8 mU/l, p<0.01) and a similar geometric mean UACR (1.26 mg/mmol creatinine). Subjects ± FHDM were well matched for age (n=181 and 166 respectively). FHDM+ (vs FHDM-) were more obese (33.3±7.4 vs 31.3±6.7 kg/m^2, p<0.05), had a higher systolic blood pressure (113±14 vs 110±16 mm Hg, p<0.05), a higher FBG (5.0±0.8 vs 4.7±0.8, p<0.001) but comparable FINS and 2HINS (20.4 vs 20.4; 55.0 vs 57.5 mU/l) and UACR (1.14 vs 1.24). Overall 12% of subjects had microalbuminuria with no differences between family history groups. FHN+ are relatively hyperglycaemic and hypoinsulinaemic, while FHDM+ are hyperglycaemic with a "normal" insulin (ie insulin resistant). Presence of microalbuminuria was independent of family history.

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GLOMERULAR FILTRATION RATE AND MICROALBUMINURIA IN NEWLY DIAGNOSED IDDM PATIENTS M. Georgescu, T. Popa, C. Serafinceanu, I. Ferariu, T. Mogos, R. Strachinaru, C. Ionescu-Tirgoviste, Clinic of Diabetes, Institute "N.C. Paulescu", Romania

Both glomerular hyperfiltration and microalbuminuria were found to be markers for early stages of diabetic nephropathy. Hyperfiltration was often associated with renal hypertrophy and increased intraglomerular pressure leading to enhanced transglomerular passage of plasma proteins which might predispose to the accumulation within glomeruli of extracellular matrix and to the expansion of the mesangium. This is the baseline analysis of a prospective study regarding renal function in IDDM patients. The glomerular filtration rate (GFR) was determined by 24h clearance of endogenous creatinine and microalbuminuria determined by imunoturbidimetry. Hyperfiltration was defined by a GFR value > 115 ml/min/1.75m^2 in females and 130ml/min/1.75 m^2 in males. Microalbuminuria was defined as albumin excretion between 30-300mg/24h (20-200μg/min) and macroalbuminuria > 300mg/24h (>200μg/min). This study included 50 newly diagnosed IDDM patients, 31M/19F, aged 34.28 yrs +/- 16.95 (38.9 yrs +/- 17.78 for males and 28.95 yrs +/- 17.71 for females) of which < 20yrs, 15 patients (19.4% M and 48% F); 21-40 yrs, 15 patients (29% M and 32% F) and > 40 yrs, 20 patients (51.6% M and 22% F). Of the 31 males, 15 (51%) had hyperfiltration more often in the young (<20yrs.) or older (>40yrs.) age groups. Of 19 females, hyperfiltration was encountered in 12 cases (63%) more often in age groups over 20 yrs. Microalbuminuria was found in 7 cases in males (22.58% M) and in 4 cases in females (20.5% F). In addition we found AER with borderline values 20-30 mg/24h in 9 cases, 7 males (23% M) and 2 females (11% F), which represent a possible risk group for further renal disease. It is important to follow this group up prospectively in its evolution to microalbuminuria. The correlation coefficient between GFR and AER is very low (0.11) and shows a lack of correlation between these variables at the onset of disease. This correlation could become tight as the disease advances.

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CELL REPLICATION AND Na/H ACTIVITY IN INSULIN-DEPENDENT DIABETIC PATIENTS WITH PROTEINURIA.

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Higher Na/H exchange (NHE) activity and thymidine incorporation may characterise human skin fibroblasts (HSF) from patients with IDDM and overt nephropathy (DN). Whether this may be paralleled by enhanced cell replication is unclear. Primary HSF cultures were obtained from 16 patients with IDDM and overt nephropathy, and from 17 age-matched patients with long-term IDDM but normoalbuminuria. Glycated haemoglobin levels was comparably distributed in both groups (HbAIc, 8.7±0.3 vs 9.3±0.3 %, p=NS). HSF were studied at the 6th passage. Cell proliferation rates were higher in the patients with DN, as measured by counting cells on day 2, 5 and 7 after seeding 10,000 cells/well (0.179±0.016 vs 0.127±0.013 10^5cells·day^{-1}, p=0.015). Enhanced proliferation was paralleled by increased activity rates of NHE among the patients with DN, both at pH_i=6.2 (Vmax, 12.6±1.3 vs 6.6±0.9 mmol/l/min, p=0.0006) and at pH_i=6.5 (2.2±0.3 vs 1.3±0.3 mmol/l/min, p=0.023). These findings were not associated with differences in resting pH_i (6.87±0.02 vs 6.84±0.02, p=NS), nor with older patient age or longer duration of diabetes. Cytofluorimetric analyses in subsets of syncronised cell cultures suggested that cells in phase S were present in similar proportions among these groups of patients (17.8±4.2 vs 25.6±3.1 %, p=NS), G0-G1 (62.9±3.7 vs 56.9±2.4 %, p=NS) and G2-M (19.3±1.9 vs 17.5±2.6%, p=NS). These findings support the idea that faster *in vitro* replication rates may characterise HSF in diabetic nephropathy and further corroborate the association of proteinuria with higher activity of NHE. The genetic mechanisms that may underlie these abnormalities remain to be clarified.

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THE CHANGE IN RENAL BLOOD FLOW IN THE EARLY DIABETIC NEPHROPATHY.

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Diabetic nephropathy (DN) is a progressive renal disease. Although persistent proteinuria is a characteristic of DN, this sign represents a serious dysfunction of kidney in late DN. Thus, it is important to evaluate the change of renal function in early DN. The present study examined the relationship between the change of renal blood flow (RBF) and the impairment of renal function in NIDDM. 68 NIDDM (mean age 46 years old, male 37, female 31) and 40 normal control (mean age 42 years old, male 16 and female 24) were included in this study. According to the period of NIDDM, patients were subdivided into two groups (Group I < 5 years, n=30 and Group II > 5 years, n=38). The RBF was measured using duplex ultrasound. The resistive index (RI), calculated from the duplex doppler waveform, was compared with clinical and laboratory finding. In present study, RI > 0.70 and urea> 25 ug/ml were defined as renal dysfunction. It was found that abnormal rate of RI was 17% in group I and 74% in group, and increase of serum urea level was 40% in group I and 89% in group II. RI and serum urea level were significantly higher in group II than that in group I and normal control (RI: 0.71±0.05 vs 0.65±0.08 and 0.62±0.04, p< 0.01; serum urea level (ug/ml): 86±10 vs 42±5 and 19±5, p< 0.01). Both abnormal rates of RI and urea were 13 % in group I and 63% in group II. It concluded that the decrease of RBF is closely related to the period of illness and the impairment degree of renal function in DN.

2090

PREVALENCE OF GAD_{65} AND IA-2 AUTOANTIBODIES IN IDDM PATIENTS WITH NEPHROPATHY.

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Diabetic nephropathy develops in about one third of patients affected by insulin-dependent diabetes mellitus (IDDM). The reason only this subset of IDDM patients develops nephropathy is unknown. Circulating autoantibodies to islet antigens are found in the majority of patients at IDDM onset, and in a minority of patients antibodies persist throughout life. Whether islet antibody persistence underlies a susceptibility to develop diabetic nephropathy is presently controversial. We studied the prevalence of GAD_{65} and IA-2 antibodies in a total 100 IDDM patients either characterised by normoalbuminuria, as defined by an albumin excretion rate lower than 20 µg/min (n=52) or by presence of established diabetic nephropathy, as defined by an albumin excretion rate higher than 200 µg/min in two out of three consecutive overnight urine collections (n=48). The two groups of patients had similar age (42.3±1.6 vs 42.9±1.6) and duration of diabetes (25.5±1.1 vs 24.5±1.2). No patients were on hemodialysis. GAD_{65} and IA-2 antibodies were measured by radiobinding assays with [^{35}S]-methionine labelled in vitro translated recombinant antigens. There were no differences in the prevalence (30.8% vs 39.6%) of GAD_{65}-antibodies between patients with normo- and macroalbuminuria. IA-2 antibody prevalence was low, suggesting that their prevalence declines after disease onset, and did not differ between groups (9.6% vs 12.5%). These data suggest that persistence of islet autoantibodies does not appear to be directly related to the presence, and perhaps development, of diabetic nephropathy in IDDM patients.

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HYPERFILTRATION IN NON-COMPLICATED IDDM IS UNRELATED TO METABOLIC CONTROL, BLOOD PRESSURE OR PLASMA VOLUME.

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Intra-glomerular hypertension and hyperfiltration are dominant features in the pathogenesis of diabetic nephropathy in IDDM. The exact pathogenesis of early hyperfiltration remains elusive. In a prospective study we will assess the role of hyperfiltration in the genesis of diabetic renal disease. Therefore we have studied at entry 54 non-complicated IDDM patients (DP) and 50 healthy control subjects (C) in whom renal function, blood pressure (BP), sodium-lithium countertransport (Na-Li-CT), plasma volume and transcapillary escape of albumin (TER_{alb}), basal albumin excretion ratio (AER) and metabolic control were measured. Renal clearances of inulin and PAH were used as markers of GFR and ERPF respectively. Glucose concentrations were controlled in IDDM patients with levels between 5.0 and 8.0 mmol/l during the study. Blood pressure was measured intra-arterially during cannulation of the brachial artery. Plasma volume and TER_{alb} were measured by ^{125}I-albumin injection. The disappearance rate of ^{125}I-albumin was measured and extrapolation to time zero was used for calculation of the plasma volume. HbA1c was used as a measure of metabolic control. Results are expressed as means±SE. Data were analyzed by *t*-test for Gaussian distribution and Mann-Whitney-U test for non-normally distributed data. Glomerular filtration rate (GFR) was significantly increased in DP (GFR 120±2 in DP and 106±2 ml/min/1.73m^2 in C, $P<0.01$). GFR of 130 ml/min/1.73m^2 was the upper limit in C. DP were divided into quartiles. Hyperfiltration (GFR > 130ml/min/1.73m2) was found in the highest quartile (n=13). Those were compared to the lowest quartile of DP (n=13, GFR 100±2 ml/min/1.73m^2). There were no significant differences between non-hyperfiltrating and hyperfiltrating DP with respect to blood pressure (MAP 80.5±1.8 versus 82.5±2.0 mmHg), Na-Li-CT (V_{max} and K_m 733±70 and 55±4 and 826±81 and 70±4 respectively), plasma volume (2900±143 versus 3114±156 ml), TER_{alb} (5.1±0.6 versus 4.9±0.4 %/hr), basal AER (6.4±1.3 and 9.1±1.3 µg/min) and HbA1c (8.4±0.4 versus 8.4±0.4%). There were no differences in age (26.5±1.8 and 27.8±1.9 yr) or diabetes duration (8.4±0.6 and 8.2±0.8 yr). It is therefore concluded that hyperfiltration in non-complicated diabetic patients is not related to BP, plasma volume, capillary and glomerular leakage of albumin, plasma volume or metabolic control.

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PROTEINURIA IS STILL USEFUL FOR THE DIAGNOSIS OF OVERT DIABETIC NEPHROPATHY.

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The aim of this study was to assess the performance of urinary total protein measurements in timed 24h urine collection (24hUP) and in a diurnal random urine specimen (RUS) for the diagnosis of overt diabetic nephropathy. One-hundred-sixty seven diabetic patients (20 IDDM, 147 NIDDM; 78 females; aged 20 - 84 years) collected 217 timed 24h urine specimens. Albumin was measured by immunoturbidimetry, total protein by the sulfosalicylic acid technique and creatinine by Jaffé's method. According to 24h urinary albumin excretion rate (UAER), samples were divided into Normoalbuminuric (Normo;UAER<20 µg/min; n=84), Microalbuminuric (Micro; UAER 20-200 µg/min; n=78) and Macroalbuminuric (Macro; UAER ≥ 200 µg/min; n=55). Eighty-six patients also collected 106 RUS (Normo,n=47; Micro, n=37; Macro, n=22) after completing timed 24h urine collection and urinary protein concentration (UPC) and protein:creatinine ratio (UPCR) were measured. The relationship between 24h UAER vs 24hUP and RUS (UPC and UPCR) were calculated by Spearman's correlation coefficients (rS). The receiver operating characteristics (ROC) curve approach was used to analyze the performance of the diagnostic tests. The rS of 24h UAER vs 24hUP was 0.95 ($p < 0.001$) and of 24h UAER vs UPC and UPCR were 0.76 and 0.73, respectively ($p < 0.001$). The calculated areas (± SE) under the ROC curve for the diagnosis of overt diabetic nephropathy for 24hUP was 0.9987 ± 0.001, for UPC 0.9805 ± 0.014 and for UPCR 0.9708 ± 0.015. On the ROC curves the first points with 100% sensitivity were: for 24hUP 541mg (specificity 95 7%), for UPC 185 mg/L (specificity 72.6%) and for UPCR 0.2 (specificity 77.4%). In conclusion, 24hUP presented a better performance than UPC and UPCR for the diagnosis of overt diabetic nephropathy. The 24h proteinuria presented an almost perfect accuracy for the diagnosis of clinical diabetic nephropathy, is simpler and less expensive than UAER and yet can be used in the evaluation of clinical nephropathy.

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IMPAIRED AUTOREGULATION OF GLOMERULAR FILTRATION RATE IN NON INSULIN DEPENDENT DIABETIC PATIENTS WITH DIABETIC NEPHROPATHY

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Afferent arteriolar hyalinosis frequently present in diabetic glomerulosclerosis, might impair the normal myogenic responses to pressure changes leading to impaired autoregulation of glomerular filtration rate (GFR). To evaluate this hypothesis we investigated the effect of acute lowering of arterial blood pressure (BP) upon kidney function in 14 non insulin dependent diabetic (NIDDM) patients with diabetic nephropathy and 12 NIDDM patients with normoalbuminuria. The study was performed twice within 2 weeks with the subjects receiving an intravenous injection of either clonidine (150-225 µg) or saline (0.154 mmol/l) in random order. We assessed GFR (single bolus ^{51}Cr-EDTA plasma clearance technique), albuminuria (ELISA), and BP (TAKEDA TM-2420). The two groups were well matched with respect to demographic data and baseline GFR (ml/min/1.73 m^2) 90 (SEM 6) vs 93 (4) and baseline BP (mm Hg) 164/85 (5/2) vs 167/92 (5/3) in patients with and without nephropathy, respectively. The clonidine injection induced similar reductions in mean arterial blood pressure 19 and 21 mm Hg, respectively. While GFR and urinary albumin excretion rate remained nearly unchanged in the control group after clonidine, GFR diminished from 90 (6) to 81 (7) ml/min/1.73 m^2 ($p = 0.006$) and albuminuria declined from 708 (antilog SE 1.3) to 532(1.2) µg/min ($p=0.06$) in nephropathic patients. A significant correlation between relative reductions (%) in MABP and in GFR ($r = 0.78$, $p<0.001$) was demonstrated in albuminuric NIDDM patients. A complete pressure passive vasculature was found in 4 out of 14 patients with nephropathy (Δ GFR% = Δ MABP%). Our study suggests that NIDDM patients with diabetic nephropathy frequently are suffering from impaired/abolished autoregulation of GFR.

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PHYSICIAN'S OFFICE EVALUATION OF A NEW 7- MINUTE QUANTITATIVE ASSAY FOR URINARY ALBUMIN AND CREATININE.

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Increased levels of albumin excretion are an early indicator of diabetic nephropathy. Early recognition may lead to more timely medical intervention and decreased need for kidney dialysis and/or transplants. Most quantitative albumin estimations do not provide rapid results to the physician. A new method has been developed by Bayer, using a dry cartridge format in the DCA 2000® Analyzer. The cartridge contains reagents for both albumin and creatinine assays. The concentration of albumin is measured by an immunoturbidimetric method, and the creatinine by a modified Benedict-Behre reaction. Results of the two analytes, as well as an albumin/creatinine ratio, are available in 7 minutes. The objective of the clinical evaluations was to assess the performance of the DCA 2000 methods in the hands of typical end-users. Overnight timed and random specimens were tested (58 - Site 1, 49 - Site 2). Results for the two specimen types were equivalent. DCA albumin results were compared to immunoturbidimetric methods and creatinine results to kinetic Jaffe methods. DCA results correlated well with comparative methods (Albumin: y = 0.1 + 0.857x, r = 0.99, (Site 1); y = 0.6+1.06x, r = 0.99 (Site 2). Creatinine correlations: y = (-)2.3 + 1.02x, r = 1.00 (Site 1); y = (-)3.0+1.12x, r = 0.99 (Site 2). Albumin precision on two levels of controls was 3.4 - 6.6% CV. Creatinine precision was 2.6 - 3.6%CV. It is concluded that the DCA 2000 method is sensitive, reliable and provides albumin, creatinine and albumin/creatinine ratio results to the clinician during the patient's office visit. The method correlates well with immunoturbidimetric methods. The simplicity of the test allows physician's office personnel to do the test routinely. This will permit detection of earlier stages of diabetic nephropathy and effective counseling while the patient is still in the office.

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EXERCISE-INDUCED VARIABILITY OF GLOMERULAR FILTRATION RATE (GFR) IN DIABETIC SUBJECTS.

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Introduction. Plasma clearance rate of intravenously given ^{51}Cr-EDTA (CrB) bolus (radioactive edetic acid), calculated by computer analysis (TS–method, Sambataro-Thomaseth, JANS, 1996), provides accurate description of GFR without need of steady-state condition and resting body position during the test. Moreover, GFR changes due to physiological cardiovascular stimulation have never been investigated. Aim. (i) to verify the accuracy of GFR measurement using the TS-method after CrB, in different physiological operative conditions; (ii) to evaluate changes of GFR in NIDDM after acute maximal muscular exercise. Subjects and Methods. An intravenous bolus of 1 μCi/kg of ^{51}Cr-EDTA was performed in 13 NIDDM patients (age 53-68 yr; BMI 30±1 kg/m^2; AER<150 mg/24h). The plasma clearance was calculated by multiexponential model using a weighted least squares estimation routine. Basal GFR was assessed in all subjects. After 6 months, 5 randomly selected patients (D1) underwent a maximal muscular stress by Tapis Roulant (5 km/h for 5 min with 12% slope) immediately before a CrB test (mGFR); the other 8 patients (D2) were instead reinvestigated at rest (rGFR). Results. Basal GFR (bGFR) was 102±6(SE) ml/min/1.73m^2 in all subjects, but was different in the two subgroups: 122±7 in D1 and 91±6 in D2 (p<0.007). D1 after muscular exercise had similar renal function of D2 at rest (mGFR=90±7, rGFR=86±8, p=0.711), with lower GFR in the two operative conditions (D1: bGFR–mGFR = 31±7; D2: bGFR–rGFR = 5.0±4.6) (p<0.007). Linear regressions between repeated GFR yielded r^2=0.726, slope=1.2, p=0.007 for D2 (bGFR vs. rGFR), and r^2=0.27, slope=0.5, p=0.37 for D1 (bGFR vs. mGFR). Conclusions. (i) Redistribution of vascular blood flow by maximal muscular exercise is a physiological variability factor of renal filtration rate; (ii) the hyperfiltering glomerular high risk condition in diabetes could be a functional and not an anatomical factor; (iii) modelling analysis of tracer plasma disappearance rate well quantifies the effects of rapid physiological stimuli on renal function; (iv) the TS-method gives accurate descriptions of physiological variations of GFR.

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A NEW RELIABLE AND RAPID METHOD FOR ESTIMATION OF URINARY ALBUMIN CONCENTRATION

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A new method has been developed by Bayer using a dry cartridge format in the DCA2000® analyzer. The method, which gives a urinary albumin and creatinine results in seven minutes, uses an immunoturbidimetric reaction for albumin and a modified Benedict-Behre reaction for creatinine. Results of the two analytes are combined for an albumin/creatinine ratio. Overnight, 24-hour, and spot urine samples were analyzed from 105 subjects with Type 1 diabetes mellitus. Comparisons were made using the DCA2000® methods against a RIA method for albumin and a kinetic Jaffe method for creatinine. DCA methods correlated well with RIA results, y = (-) 1.0 + 0.899x, r = 0.99 and Jaffe results y = (-) 1.4 + 1.07x, r = 0.99. The correlation was equivalent with the three types of collections (overnight, spot or 24 hrs.). Diagnostic performance expressed as Sensitivity, Specificity, Positive and Negative Predictive Values was greater than 97%. Total precision (CV) using two levels of controls was 5.8% and 2.7% (32 and 201 mg/L albumin); 2.8% and 2.2% (100 and 396 mg/dL creatinine). Finally, 75 urine samples were tested in duplicate using DCA2000® and a Beckman Array® immunoturbidimetric method. The correlation was excellent (y=0.6+0.992x, r=1.00). The albumin precision was 3-4% CV. It is concluded that the DCA2000® cartridge assay is sensitive, reliable, and rapidly provides the albumin/creatinine ratio as well as individual test results to the clinician. The new method correlates well with both RIA and immunoturbidimetric methods. The rapid availability of results will allow physicians' office personnel to do the test routinely and will allow patients to receive effective counseling while still in the doctor's office.

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DETERMINANTS OF GLOMERULAR HYPERFILTRATION IN IDDM PATIENTS

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The increase of glomerular filtration rate (GFR) is considered characteristic of early diabetic nephropathy in patients with IDDM. Actually it is not know the real predicting value of hyperfiltration for development of incipient and overt nephropathy. In a cross-sectional study of 171 normotensive, normoalbuminuric IDDM patients (mean age 32,4±9,2 yrs, duration of disease 14±7 yrs) and 20 controls subjects (mean age 33±10 yrs) we have evaluated GFR and renal plasma flow (ERPF) by single injection of Cr^{51}EDTA and I^{125}Hippuran and we studied the relationship between GFR and ERPF, age, sex, IDDM duration, HbA1c, BMI. Mean value of GFR was 113±20 ml/min/1.73m^2 in controls and 141±25 ml/min/1.73m^2 in IDDM (p<0.0001); ERPF was 474±99 ml/min/1.73m^2 and 633±140 ml/min/1.73m^2 respectively (p<0.002), while FF (GFR/ERPF) was similar in controls and patients (0.23±0.03 vs 0.23±0.02 p=ns). Patients with GFR more than 95° percentile of controls (135 ml/min/1.73m^2) are defined hyperfiltering: they represent the 53% of our population study. An inverse correlation between age and GFR is well known. In a simple regression analysis we found the same correlation between GFR and age (p<0,01 but in a multiple regression analysis including ERPF, age, sex, IDDM duration, HbA1c, BMI, ERPF was the only independent determinat of GFR (p<0.0001). In our cross-sectional study ERPF seems the only determinat of glomerular hyperfiltration in IDDM.This is probably due to vasodilation of afferent arterioles caused by hyperglycaemia per se.

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DIABETIC NEPHROPATHY SCREENING WITH MICRAL TEST
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Back grouhd: Micral test is semikuantitatif protein urine test,especially to detect microalbumin uria on incipient Diabetic Nephropathy.This screening test is very usefull and practicable primary faster diagnostic for out patient and study epidemiology ;Normal result micral test negatif (0-15mg/ml),threshold (15-30mg/ml),+(20-80mg/ml),++(80-100mg/ml). Aim and method: We pre formed study incidence of incipient diabetic Nephropathy from 200 NIDDM was suffered from diabetic more than one year and we collect urine sample after excercise from diabetic club and 50 normal control group. Result: Up to the final study 195 NIDDM was ixamined by micral test consist of 62 male and 133 female, all group got sulfonylurea and dieet normal renal function and liver function,Micral test result 37% male was normal, 21 % threshold 23% + and 19% ++, in the male group + and ++ result mostly come from 56-65 age group and female group from 46 - 55 age group .
Comparing with control group significant different positif-result ($P < 0{,}001$).
Conclussion : Micral test is a simple test to detect incipicient Diabetic Nepropathy.

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RENAL RESERVE CAPACITY IN TYPE 1 DIABETIC SUBJECTS AFTER ORAL PROTEIN LOAD

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AIM: Glomerular hyperfiltration is a known feature in insulin dependent diabetes mellitus (IDDM). In this study we defined glomerular filtration rate (GFR) and renal reserve capacity in IDDM patients without any clinical sign of diabetic nephropathy.
METHODS: A single shot sinistrin and p-aminohippurate (PAH) clearance was used to measure GFR and renal plasma flow (RPF) before and after ingestion of a protein load (1g/kg body weight). The kinetic marker data were evaluated by a two compartment modelling. During the whole experiment blood glucose in the diabetic subjects was kept between 120 and 199 mg/dl by variable i.v. insulin infusion.
RESULTS: GFR and RPF were measured in 10 well controlled IDDM patients ([mean±SD] age: 33±7 years, diabetes duration 12±8 years, BMI: 24±3, HbA1c: 7.6±0.6) and 10 controls (age: 29±5, BMI: 22±2). All subjects were normotensive (RR<130/85) and had normal albumin excretion (4±3 and 3±1 mg/24h). Basal GFR in the diabetic subjects was elevated (139±17 ml/min/1.73 [normal range 80-130] compared to the control group (116±16, $p<0.02$). After protein load GFR decreased in the diabetic group to 127±12 ($p<0.01$), whereas the healthy subjects showed an increase to 128±8 ($p<0.05$) reflecting the renal reserve capacity. Furthermore, in the diabetic patients we found a significant correlation between the baseline GFR and the respective decreases after protein load($r=0.73$, $p<0.05$). RPF and total renal resistance did not change in both groups.
CONCLUSION: GFR in IDDM subjects without nephropathy is elevated and decreases after oral protein load. This lack of renal reserve capacity may be explained by intrarenal haemodynamic changes.

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EFFECTIVE SCREENING FOR DIABETIC NEPHROPATHY BY SELF-TEST FOR MICROALBUMINURIA

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The accuracy of two consecutive versions of a qualitative self-test for microalbuminuria (Micral-Test ® and Micral-Test ® S) was assessed in comparison to quantitative analysis by immunoturbidimetry in diabetic patients. For evaluation of the Micral-Test ® 339 diabetics from hospitals independent of age, sex, duration and type or therapy of diabetes were included in a screening program for microalbuminuria unless one of eight defined exclusion criteria was present. The patients performed a self-test for microalbuminuria on three days within one week in the first morning urine. All urine samples were retested by a quantitative analysis of urinary albumin concentration using immunoturbidimetry. 24 patients could not be analyzed due to incorrect number of urine samples. In the second part of the study the new version of the self-test, Micral-Test S, was evaluated in 112 consecutive diabetic inpatients following the same methods. Four patients had to be excluded due to insufficient number of urin samples. Statistical analysis included frequency distribution of test results and evaluation of sensitivity, specifity, positive and negative predictive values. If at least two positive out of three self-tests during one week are necessary for assessment of an elevated urinary albumin concentration, the Micral-Test shows a sensitivity of 0.70, specificity of 0.81, positive predictive value of 0.63 and a negative predictive value of 0.85. The new version Micral-Test S showed a better accuracy with a sensitivity of 0.81, specificity of 0.92, positive predictive value of 0.71 and negative predictive value of 0.95. When the assessment of microalbuminuria, however, was based on at least one positive out of three self-tests using the Micral-Test S, sensitivity increased to 0.90 at the cost of a decrease of specificity to 0.77. The improved sensitivity and specificity of the the new version, Micral-Test S, reflects a better practicability. Due to these data and the fact that only in few cases a regular microalbuminuria screening in primary care is performed, the self-test Micral-Test S should be integrated into widespread screening programs for incipient diabetic nephropathy using the capabilities of most diabetics for self-monitoring like blood and urinary glucose, ketonuria and blood pressure.

2101

Large kidneys as marker for subsequent nephropathy in IDDM.

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Approximately 30-40% of IDDM patients develop diabetic nephropathy. Previous examinations on the significance of the kidney size prior to the manifestation of nephropathy produced varying results. A longitudinal study, therefore, was designed to assess the correlation between sonographically determined kidney size and kidney function over eight years, and to evaluate a potential risk pattern. Data could be collected from 71 (59%, m38/f33) of initially 120 IDDM patients whose sonographically determined kidney volume (cm^3=Lcm x W cm x D cm x pi/6) and kidney function (creatinine, albuminuria) had been examined eight years ago, and who had a diabetes duration of 1 month to 25 years at that time. KV of more than 170 cm^3 was defined as large kidney on the basis of 44 age matched healthy controls. During the observation period, eight (6,7%) diabetic patients had died (7 in terminal renal insufficiency), 4 (3,3%) presently require dialysis and 1 (0,8%) has received a kidney transplant. All deceased patients due to renal insufficiency had normal or slightly increased creatinine values eight years ago, yet enlarged kidneys at the time (> 170 cm^3). Of the IDDM patients who had large kidneys, 42% developed pathological creatinine values (>106µmol/l) versus 20% in the group of patients with a kidney volume of under 170 cm^3 ($p<0,05$). In additon, all eight diabetic patients had a kidney volume of over 170 cm^3 whose creatinine values rose more than 50% during the observation period. The examined endpoints (serum creatinine > 106µmol/l, creatinine increase of more than 50% in the past eight years, death in terminal renal insufficiency, receiving dialysis and/or renal transplantation) were reached from 77% of patients with initially normal creatinine, but a large kidney volume, in contrast to only 20% of patients with normal kidney volume ($p<0,0001$). These results indicate that large kidneys might be a morphological marker for subsequent diabetic nephropathy and as a consequence renal insufficiency.

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PERFORMANCE CHARACTERISTICS OF MICRAL TEST AND SPOT URINE ALBUMIN CONCENTRATION FOR DETECTION OF MICROALBUMINURIA IN DIABETIC PATIENTS

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Microalbuminuria is both an independent predictor of progressive renal disease and a clinical marker of atherosclerotic heart disease and premature cardiovascular death in diabetic individuals. The definition of microalbuminuria in IDDM and NIDDM is generally accepted as a urinary albumin excretion rate of 20-200 ug/min (30-300 mg/day). Because of the difficulties involved in estimating urinary albumin excretion rates, many physicians use simple dipstick methods to detect microalbuminuria. One of these methods, the Micral test (Boehringer-Mannheim, Germany) has been shown to be a clinically useful test, and readings between 20 to 200 mg/l are regarded as indicative of microalbuminuria. However, being semi-quantitative, and affected by fluctuations in urine output, the Micral test has many limitations. Our study sought to define the performance of the Micral test and the spot urine albumin concentration (UAC-RIA) as measured with a double antibody radioimmunoassay method (sensitivity 0.3 mg/l) against the spot urine albumin-creatinine ratio (ACR). We studied 211 patients (175 NIDDM, 36 IDDM) attending the Toa Payoh Hospital Diabetes Center. Early morning spot urine specimens were first tested using the Micral test and the Albustix test (Ames, USA). The same urine specimens were then immediately sent for radioimmunossay for urine albumin concentration, as well as estimation of urine creatinine concentration. We found a linear relationship between ACR and UAC-RIA (ACR=1.316 X UAC-RIA, r=0.888, p<0.0005). Using a reference ACR level of <30 mg/g as a cutoff to delineate normoalbuminuria from microalbuminuria, and subjecting the UAC-RIA data to receiver operating characteristics analysis (ROCLAB program) we found that the UAC-RIA value that yielded optimum sensitivity and specificity as a cutoff for microalbuminuria was 53 mg/l (sensitivity 91%, specificity 95%). Micral test (semi-quantitatively rated as 10, 20, 50 or 100 mg/l) demonstrated a clear correlation with both UAC-RIA and ACR values. As the Micral cutoff that most closely approximated a UAC-RIA of 53 mg/l was 50 mg/l, we feel the latter would be a more useful cutoff for microalbuminuria than the traditionally suggested value of 20 mg/l. We conclude that both the ACR and UAC-RIA are useful screening tests for microalbuminuria, and that a Micral test of >50 mg/l is a useful office screening method for microalbuminuria in diabetic patients.

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INCREASED FREQUENCY OF MICRO- AND MACROALBUMINURIA IN SINGLE-KIDNEY NIDDM PATIENTS.

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The role of glomerular hyperfiltration as a risk factor for future development of diabetic nephropathy is still unsettled. The presence of a single kidney represents a state of pronounced hyperfiltration. We have previously demonstrated that single-kidney NIDDM patients have a higher frequency of renal disease when compared to NIDDM patients with both kidneys. We are now comparing the prevalence of renal disease between single-kidney individuals with and without NIDDM. The aim of this study was to analyse the prevalence of micro- and macroalbuminuria in 20 single-kidney NIDDM patients, 13W/7M, aged 63.7 ± 9.9 (50-84) yrs with diabetes duration of 10.9 ± 9.8 (1-40) yrs. Seventeen non-diabetic individuals with one kidney, 15W/2M, aged 56.8 ± 13.2 (35-75) yrs formed the control group which was age-, sex- and body mass index-matched with the NIDDM group. The period of time with one kidney was similar in the diabetic patients [23.4 ± 17.2 (5-63) yrs] and in the control group [20.7 ± 17.6 (5-73) yrs]. Albumin excretion rate (AER) was measured in 24-h urine samples, by immunoturbidimetry. Glomerular filtration rate (GFR) was measured by ^{51}Cr-EDTA single injection method. The prevalences of micro- (AER 20-200 µg/min) and macroalbuminuria (AER >200µg/min) were significantly higher in the diabetic group (45% and 25%, respectively) in comparison with the non-diabetic group (18% and 6%; p<0.05). There were no differences between the groups regarding GFR (67.2 ± 22 vs. 73.5 ± 20 ml/min/1.73m^2), mean blood pressure levels (106 ± 17 vs. 106 ± 21 mmHg), serum cholesterol (5.66 ± 0.98 vs. 5.39 ± 1.2 mmol/L) and HDL (1.3 ± 0.4 vs. 1.2 ± 0.3 mmol/L), respectively, in non-diabetic individuals and NIDDM patients. Serum triglycerides were significantly higher in diabetic patients (1.4 vs. 2.3 mmol/L) In conclusion, hyperfiltration related to the single-kidney status is associated with an increased frequency of renal disease only in the presence of diabetes.

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Utility of the urine glycated albumin/serum glycated albumin ratio as a marker for diabetic nephropathy

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Measuring albumin in urine is essential to diagnosing initial nephropathic lesions at an early stage. An albumin excretion value of less than 30 mg/g. Creat.,a value from 30 mg/g Creat. to 300 mg/g Creat.,or a value of 300mg/g. Creat. of above are defined as normoalbuminuria, microalbuminuria, or macroalbuminuria,respectively. We studied the ratio of glycated albumin in urine to that in serum (GA ratio) as a possible marker for the onset of diabetic nephropathy. Compared to an GA ratio of 2.099 ±0.300 (means±SD) for controls consisting of 11 healthy adult subjects (61.5 ± 5.4 years), the GA ratio was 2.097 ± 0.577 for a normoalbuminuria group of 14 subjects (60.4 ± 3.6 years) , 1.271 ± 0.394 for a microalbuminuria group of 15 subjects (59.1 ± 9.7 years) , and 0.950 ± 0.075 for a macroalbuminuria group of 13 subjects (60.5 ± 11.8 years). In the progression from normoalbuminuria to the onset of nephropathy, GA ratios gradually declined from 2.000, clustering at a value of 1.000. This indicates that a GA ratio of about 1.000 represents the region of irreversible injury. Further, the point of intersection between the line of about this ratio of 1.000 and the regression curve determind polynomially for the GA ratio in the microalbuminuria group corresponded to a value of about 150 mg/g.Creat.. The above findings thus suggest that a GA ratio of about 1.000 marks the onset of diabetic nephropathy, and the threshold value for the onset of nephropathy in the progression from microalbuminuria is that of about 150 mg/g Creat..

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LOW BIRTH WEIGHT - A PROGRESSION PROMOTER IN DIABETIC NEPHROPATHY?

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Intrauterine growth retardation, defined as birth weight below the 10 th centile, gives rise to a reduction in nephron number. Oligonephropathy has been suggested to increase the risk for systemic and glomerular hypertension in adult life as well as enhance risk for expression of renal disease following exposure to injurious renal stimuli e.g diabetes mellitus. The aim of this study was to determine if low birth weight is a risk factor for progression of established diabetic nephropathy. We investigated 114 (66 men) IDDM patients with diabetic nephropathy (persistent albuminuria > 300 mg / 24 hours) (mean age (SD) 41 (9) years, mean duration of diabetes 32 (8) years). All patients had been followed for at least 3 years, median (range) 9 (3-17) with at least 3 measurements (9 (3-28)) of GFR (51Cr-EDTA). 79 % of the patients were treated with one or several antihypertensive agents. Information about weight at birth was obtained from the midwifes original registrations. The mean (SD) rate of decline in GFR was 3.4 (3.7) ml/min/year. There was no correlation in univariate analysis between birth weight and rate of decline in GFR, neither in men nor in women. A backwards stepwise multiple regression analysis with rate of decline in GFR as dependent variable and mean values of albuminuria, haemoglobin A1c, arterial blood pressure, and s-cholesterol during the whole observation period in addition to sex and birth weight as independent variables revealed a significant association only with albuminuria and haemoglobin A1c. Furthermore, the 10 patients with intrauterine growth retardation had a rate of decline in GFR similar to the 104 patients with birth weight above the 10 th centile, mean (SE): 2.0 (0.6) vs 3.2 (0.4) ml/min/year, respectively (ns). In conclusion, our study does not suggest that low birth weight is associated with accelerated progression of diabetic nephropathy in IDDM patients.

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URINARY ALBUMIN TO CREATININE RATIOS 10 AND 16 YEARS AFTER DIAGNOSIS OF NIDDM.

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Renal deterioration in the early years after diagnosis of diabetes has been associated with hyperglycaemic degree: does this relationship hold later? The urinary albumin:creatinine (A/C) ratio was measured radio-immunologically in 77 patients less than 82 years old 16 years after their diagnosis as NIDDM. The mean value was 11.2±SD 29.7mg/mmol: 42 had values below 2.0 and 18 values above 6.0mg/mmol. The values were unrelated to age, gender, mode of hypoglycaemic therapy or fasting glycaemia either then ($p<0.15$), at diagnosis or as a mean of the levels 1,3,5 and 10 years later, but were correlated non-parametrically directly with systolic blood pressure ($p<0.002$) and sub-scapular skin-fold thickness ($p<0.006$) and inversely with 10-50 min. post-glucose growth hormone radio-immunoassay levels ($p<0.005$). The A/C values of 52 of these patients had also been measured 10 years after diagnosis (along then with 46 other such patients). The results correlated with those at 16 years ($p<0.001$) but had a smaller mean at 2.8±3.9 mg/mmol ($p<O.O2$). All 98 10-year values were correlated with past glycaemia, either at diagnosis or averaged over several years (both $p<0.004$) as well as systolic blood pressure ($p<0.04$). These results typically were also related to mortality in the next 6 years ($p<0.02$). The changes from 10-16 years were correlated with 10-year plasma triglyceride ($p<0.001$) and BMI ($p<0.01$) values but not with glycaemia or blood pressure. The results, while confirming glycaemia and blood pressure as associates of albuminuria in diabetic patients, also suggest its evolution to be at least a 2-stage process.

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IMPAIRED NOCTURNAL DECLINE IN ARTERIAL BLOOD PRESSURE IN NORMO- AND MICROALBUMINURIC NIDDM PATIENTS.

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Lack of the normal nocturnal decline in arterial blood pressure is associated with increased cardiovascular complications in diabetic and non-diabetic subjects. The insertion(I) / deletion(D) polymorphism in the angiotensin converting enzyme gene (ACE/ID) is associated with ischaemic heart disease which is prevalent in NIDDM patients with elevated urinary albumin excretion rate (UAE). The aim of our study was to describe the diurnal rhythm of arterial blood pressure (A&D TM2420) and to evaluate the impact of the ACE/ID polymorphism in 135 microalbuminuric NIDDM patients (UAE 30-300 mg/24h, 55 (SD, 7) years, 103 men, group 1). As control groups we used 41 normoalbuminuric NIDDM patients (UAE < 30 mg/24h, 58 (6) years, 32 men, group 2) and 22 non-diabetic subjects (58 (8) years, 15 men, group 3) matched for age and sex. Patients in group 1 were studied on their usual antihypertensive treatment (AHT) (50%) whereas patients in group 2 had their previous AHT (30%) withdrawn. None of the patients in group 3 had received any AHT. The nocturnal blood pressure reduction (daytime-nighttime)/daytime (mean (SE)) was impaired in group 1, 11.6 (0.8) % and in group 2, 10.0 (1.5) % as compared with group 3, 17.6 (1.7) % ($p < 0.01$, ANOVA). In group 1 the nocturnal reduction in blood pressure was similar in patients with or without AHT 12.2 (1.1) % vs 10.8 (1.2) % (NS). In group 1 patients without AHT no significant difference in the nocturnal blood pressure reduction was observed comparing patients with II genotype (n = 15) 14.9 (1.7) %, ID genotype (n = 29) 11.1 (1.8) % and DD genotype (n = 24) 11.8 (2.0) % ($p = 0.47$, ANOVA). In conclusion: the nocturnal blood pressure reduction is equally impaired in normo- and microalbuminuric NIDDM patients as compared with non-diabetic control subjects. The ACE/ID polymorphism does not have a major impact on the diurnal blood pressure rhythm in microalbuminuric NIDDM patients.

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URINARY PROTEINS AND DIABETIC COMPLICATIONS IN NIDDM

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The aim of this study is to clarify the clinical significance of urinary proteins in NIDDM. Urinary albumin index (Alb), N-acetyl-beta-D-glucosaminidase index (NAG), alpha 1-microglobulin (AMG), beta 2-microglobulin (BMG), immunoglobulin-G (Ig-G), and transferrin (Tf) in random spot urine samples were determined 60 NIDDM patients aged 39 to 86. Pateints were divided four groups; normoalbuminuria, microalbuminuria (≧20mg/g·Cr), proteinuria (Albustix positive) and overt nephropathy (Cr>2mg/dl). Multiple regression analysis was used to investigate the relationship between Alb index, NAG index, AMG, BMG, Tf, or Ig-G value (dependent variable) and independent variables (age, fasting plasma glucose(FPG), lipoprotein(a), systolic blood pressure, HDL-cholesterol(HDL-C), LDL cholesterol, srum thrombomodulin(Tm), pulse wave velocity of aorta, common carotid artery blood velocity and ankle pressure index(API)). Mean Alb index, AMG, BMG, Tf, and Ig-G values significantly tended to increase with the degree of diabetic nephropathy. Mean NAG index was different among 4 groups, but not different between normoalbuminuric group and microalbuminuric group. On multiple regression analysis, the independent predictive factors for NAG index was FPG, for Alb index or Tf was Tm, for AMG or Ig-G were HDL-C and Tm, and for BMG were age, HDL-C and API.

We concluded that these parameters without NAG index in random spot urine samples may serve as early functional indicators of diabetic nephropathy ,and also as indicators of macroangiopathy.

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24-H BLOOD PRESSURE AND AUTONOMIC FUNCTION IS RELATED TO ALBUMIN EXCRETION IN NORMOALBUMINURIC IDDM PATIENTS.

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Background: Significant changes in both blood pressure, autonomic function and kidney ultrastructure are observed in IDDM patients with microalbuminuria. Intervention strategies are evaluated at even earlier stages of disease. Identification of patients at risk of developing microalbuminuria must be based on thorough knowledge of the relations between key pathophysiological parameters in normoalbuminuric patients. *Aim*: To characterize interactions of urinary albumin excretion (UAE), 24-h ambulatory blood pressure (AMBP), and sympathovagal balance in a large group of normoalbuminuric IDDM patients. *Methods*: In 117 normoalbuminuric (UAE <20 µg/min) patients we performed 24-h AMBP (Spacelabs 90207), with assessment of diurnal blood pressure and heart rate (HR) variation, and short-term (three times 5 min.) power spectral analysis of RR interval oscillations, as well as conventional cardiovascular reflex tests. *Results:* Patients with UAE above the median (4.2 µg/min) had significantly higher 24-h systolic and diastolic AMBP (125±10.1 / 76±7.2 mmHg) compared to the low normoal-buminuric group (120±8.4 / 74±5.1 mmHg), $p<0.01$ and 0.02, respectively. Patients with UAE above the median had significantly reduced short-term RR interval variability including both the high frequency component (5.47 ±1.36 versus 6.10±1.43 ln ms^2), and low frequency component (5.48±1.18 compared to 5.80±1.41ln ms^2), $p<0.02$ and $p=0.04$. In addition, patients with high-normal UAE had reduced mean RR level (faster heart rates) 916ms ±108 compared to 963ms ±140, $p<0.04$. These differences were not explained by age, duration of diabetes, gender, level of physical activity, or cigarette smoking. HbA_{1c} was significantly higher (8.6±1.2 versus 8.2%±1.0, $p=0.03$) in the group with high normal UAE. *Conclusions:* Comparing normoalbuminuric IDDM patients with UAE above and below the median value, we found significantly higher AMBP in combination with significant differences in sympathovagal balance (attenuation in vagal tone and concomitantly increased sympathic activity) and significantly poorer glycemic control in the group with high-normal albumin excretion. Our data demonstrate interactions between albumin excretion, blood pressure, autonomic function, and glycemic status, already present in the normoalbuminuric range and may describe a syndrome indicative of later complications.

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INCIDENCE OF UREMIC NEPHROPATHY IN IDDM IN FINLAND

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Finland has the highest incidence of IDDM in the world. We have investigated the incidence of uremic DNP related to period of and age at debut, gender and area of living in order to detect possible variations in epidemiology. We have extracted data from two large national registries; one registering all Finnish IDDM patients with disease debut before 30 years of age from 1964 onwards, or living in 1967, and the other, The Finnish Registry of Kidney Diseases (FRKD), which has registered all patients that have entered chronic renal replacement therapy (RRT), 572 patients between 1972 and 1995. They comprise renal death out of 14 775 patients with diabetes debut between 1960-1989. When calculating renal death in IDDM, pre-dialysis death associated with uremia or far advanced DNP should be added. We therefor investigated causes of death of patients who had died over 10 years after debut, but who had not entered RRT. Fifteen % of these deaths were associated with far advanced DNP. Adding these numbers, we calculate that 25% of Finnish IDDM patients, who have survived 10 years, will have end stage diabetic renal disease at death. So far, there has not been any detectable decrease in renal death, although median renal survival time from diabetes debut to RRT has increased from 21.8 y. in the '70:s to 26.8 y. in the '90:s (P<0.001). Patients with IDDM debut during puberty, 12-15.9 years, have a higher relative risk (1.25, CI 1.01-1.55, P=0.042) than other IDDM patients to develop DNP. Renal survival is similar in males and females. The relative risk for DNP is higher in the Northern and Eastern (where prevalence of hypertension and cardiovascular morbidity are higher) compared with the Southern and Western regions of the country (1.31, CI 1.11-1.45, P=0.005). Our results indicate the need to identify risk patients and to intensify early therapeutic measures, and for increasing RRT resources.

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RENAL SODIUM AND DOPAMINE HANDLING IN DIABETIC CHILDREN WITH FAMILY HISTORY OF ESSENTIAL HYPERTENSION

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Diabetic nephropathy (DN) develops only in a subset of patients with type 1 diabetes mellitus (DM). It has been suggested, that diabetic patients with a genetic trait for essential hypertension (EH) are susceptible for DN. Since sodium handling is altered both in DM and EH , and dopamine (DA) is the main natriuretic hormone, the **aim** of our study was to assess the difference in sodium and DA excretion after sodium challenge in diabetic patients with and without a genetic trait for essential hypertension. **Patients and Methods:** Eight diabetic children with (FH+) and eight patients without (FH-) a family history of hypertension have been studied. Age, duration of diabetes, metabolic control (HbA1c) and body mass index were comparable in the two groups. All patients underwent a 3 day sodium challenge by ingesting 8g NaCl per day beside a sodium and carbohydrate fixed diet. Blood pressure was recorded by ambulatory blood pressure monitoring, and urinary DA excretion was measured by HPLC before and after the sodium load. **Results:** Systolic and diastolic blood pressure were unaltered and comparable in the two groups before and after sodium challenge. After sodium load urinary sodium excretion was significantly higher ($p< 0.03$) in FH- group (212± 47 mmol/day) compared to FH+ group (146±51 mmol/day). Natriuresis was accompanied by significantly ($p< 0.005$) higher urinary DA excretion in the FH- group (2.48±0.7 µmol/day) compared to the FH- children (1.48±0.3 µmol/day). **Conclusion:** Diabetic children with a family history of EH fail to increase urinary sodium and DA excretion following high salt diet. The importance of these phenomena in the development of DN is unknown.

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OUTCOME OF UREMIC NEPHROPATHY IN IDDM IN FINLAND

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We have investigated the outcome of renal replacement therapy (RRT) of 909 patients with end stage diabetic nephropathy (DNP) of IDDM with debut < 30 years of age, who have entered RRT between 1972 and 1995. Patient characteristics and results of therapy are presented according to period of initial RRT, and as median or per cent.

	'71-80	'81-85	'86-90	'91-95
No. of pats, M/F	104/56	135/73	164/93	165/119
Age at debut, M/F, y	13.6/9.5	12.9/11.3	13.7/10.9	12.1/11.2
Interval debut-RRT, M/F,y	21.8/21.5	22.9/23.0	24.3/23.6	26.8/26.2
% transplanted (Tx)	58	63	62	....
Survival in RRT, M/F, y, Tx+	3.0/2.4	8.9/12.9	>10/>10	....
Tx-	0.4/0.9	1.3/1.5	2.0/1.5	1.9/1.5
CV deaths, %, Tx+/Tx-	80/68	78/75	70/70	80/74

After 1993 the yearly number of new IDDM patients entering RRT has not increased. Females with DNP have constantly had their IDDM debut 1-3 years younger of age than males. Time from debut until start of RRT (length of renal survival in patients entering RRT) has successively increased by about 5 years within the follow-up period (P<0.001). Life table analyses indicate that the 5-year survival of transplanted diabetics has improved from 45% to >90%, whereas patients, who have not been considered suitable for Tx, have a poor prognosis. The proportion of cardiovascular (CV) deaths remains unchanged, in spite of the drastic survival improvement in time. Thus improvement of therapy has occurred, but outcome with regard to renal death and causes of patient death are basically unchanged.

2113

THE EFFECT OF THE DIETARY SODIUM INTAKE ON URINARY ALBUMIN EXCRETION IN THE PATIENTS WITH IDDM AND INCIPIENT DIABETIC NEPHROPATHY.

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It has generally been accepted that hypertension in patients with insulin-dependent diabetes mellitus (IDDM) is salt-sensitive, i.e. it depends on a dietary sodium intake. The aim of the study was to evaluate an influence of the dietary sodium intake on the urinary albumin excretion (UAE) in patients with IDDM. We investigated 30 patients with IDDM and incipient diabetic nephropathy (UAE 20-200µg/min) with mean duration of diabetes 16.7±7.2 years and average age 40.7±8.3 years. The control group consisted of 20 patients with IDDM and normoalbuminuria (UAE < 20µg/min). Microalbuminuria was estimated twice: on normal sodium diet (mean urinary sodium excretion 83,1 mmol/24h) and on high sodium diet (mean urinary sodium 302,6 mmol/24h), using RIA method. We found significantly lower ($p<0,001$) UAE in the patients with incipient nephropathy on the normal sodium diet in comparison with the patients on the high sodium diet (47.48±18.7 µg/min vs 76.6±29.1 µg/min). Also in the control group we found significantly lower UAE in the patients on the normal sodium diet compared with those on the high sodium diet. In the group of patients with incipient nephropathy an increase in microalbuminuria on the high sodium diet was accompanied by a significant ($p<0.01$) increase of the mean arterial pressure (MAP) (93.8±11.2 vs 98.9±9.1 mm Hg), which was not observed in the control group of patients with normoalbuminuria (89.6±7.8 vs 90.4±8.6 mm Hg). We conclude that dietary sodium intake influences markedly UAE and MAP in incipient diabetic nephropathy; therefore an excessive sodium chloride intake should be avoided in these patients.

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HUGE INCREASE OF END STAGE RENAL DISEASE (ESRD) IN DIABETIC PATIENTS: THE FRENCH SURVEY .

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In all developped countries diabetes represents the first cause of ESRD in the USA prevalence rate 35% of dialysed patients. For France we conducted the first national survey on this topic: in1989 (mainland) and in1992 (overseas territories = OT).Results were: a very low prevalence for mainland (6.9% : 884 on 12903)) the lowest of developped countries asking for specific explanations : nutrition, genetic (another french paradox?) with a North-South gradient (higher in the North) . In OT a highest prevalence: (22%) nutrition and population different. Because of the huge increase of ESRD due to diabetes worldwide we decided to reexamine the situation in 1995 (after 6 and 4 years respectively). All the french dialysis centers (n=244 among them 12 in OT) were asked by a questionaire: total number of dialysed patients, total number of diabetics, new cases in 1995 (incidence), type of diabetes, history of hypertension and of the kidney disease, result of kidney biopsy when avalaible, type of dialysis, kidney transplantation planed, other diabetic complications. Geographic origin of the parents of the patient and where the patient spent the most part of his life, habits, to try to distinguish ethnic from environemental factors.Results: 79 %of the centers answered, total prevalence 14.3%,2313 of 16212, mainland 13,09% vs 6,9 %, 6 years ago. In overseas territory 26% vs 22% 4 years ago and 35.6% incidence for1995. Details of the data will be discussed. However the dramatic increase of medical cost of diabetic's treatment and hypertension in diabetic patients during the 10th last years is accompanied by an increase of ESRD. This must be analyzed.

2115

ALTERATIONS OF RENAL RESISTIVE INDEX (RI) DURING 3 YEARS IN NIDDM PATIENTS WITH NEPHROPATHY

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Since the resistive index (RI) obtained by intrarenal duplex Doppler ultrasonography is reflecting the status of renal vascular resistance, we reported the increased RI in NIDDM patients with nephropathy, and noted that this method is available non-invasive test for evaluating the progression of diabetic renal disease. Now, we examined the alterations of renal function and RIs during 3 years follow-up study in 96 NIDDM subjects with nephropathy (46 males and 50 females, age 60±1y) in order to evaluate the RI as a surrogate marker of diabetic nephroparthy. Mean duration of diabetes in these subjects was 14±1y. Forty subjects were treated with insulin, 45 with an oral anti-diabetic drug and 11 with diet alone. The RI was calculated from Vmax-Vmin/Vmax. Mean levels of serum creatinine, urinary albumin excretion (UAE) or RI in these subjects was 62.8±1.8 μmol/L, 233±55mg/d or 0.67±0.01 in the starting year, and then increased significantly to 73.4±5.3 μmol/L, 511.6±108.6mg/d or 0.69±0.01, respectively, after 3 years, but a mean HbA_{1c} was 9.0±0.3% and did not change.
There was increased UAE or RI in 33% or 67% of these subjects, and 7 cases who had inreased RI (>0.80) were introduced to hemodialysis during 3 years. The increments or decrements of the RI was positively correlated with those of UAE or serum creatinine in these subjects.
Thus, it suggests that the intrarenal RI may be reflecting the status of renal progression in NIDDM patients with nephropathy.

2116

CHRONIC RENAL FAILURE DURING UP TO 25 YEARS OF IDDM IN THE ERFURT DISTRICT

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The aim of the study was to find out the incidence of chronic renal failure and associated factors in IDDM within a geographically defined population. The centralized diabetes care system in the former GDR had been the basis for the almost complete registration of all diabetic patients. Development of chronic renal failure (creatinine >150 μmol/l) was evaluated by checking the original charts up to 1990 of all the 1132 patients with IDDM onset until 40 years of age in the Erfurt district between 1966 and 1988. Cumulative risk of chronic renal failure after 25 years of IDDM was 8.87 + 0.02 %, that of end-stage renal failure amounted to 3.45 + 0.02 %. Chronic renal failure developed after 14 + 5 years of IDDM at an age of 40 + 10 years. In comparison ($p<0.05$) to patients with similar age and duration of IDDM at the end of follow up (n=373) patients with chronic renal failure (n=28) showed a higher BMI (25.0 vs 22.7 kg/m²), were more often obese (21 vs 9 %) and had higher systolic blood pressure (134 vs 127 mm Hg) at IDDM onset as well as higher systolic and diastolic blood pressure during the course of IDDM. They also developed more frequently hypertension (71 vs 31 %) and proliferative retinopathy (25 vs 4 %), suffered more often from myocardial infarction (11 vs 3 %), stroke (7 vs 2 %) as well as major foot amputation (11 vs 1 %) and died with higher frequency (39 vs 8 %). In conclusion in the Erfurt district the development of chronic renal failure in IDDM followed increased body weight and blood pressure at diabetes onset and clustered with higher risk of hypertension, myocardial infarction, stroke, foot amputation, severe retinopathy and death.

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RENAL TUBULAR ENZYMURIA IN NON-INSULIN-DEPENDENT DIABETES (NIDDM): RELATIONSHIP TO RETINOPATHY

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20 years after diagnosis a third of NIDDM patients have proteinuria; but some 30% of these will have non-diabetic renal pathology. Diabetic nephropathy is correlated with the presence of retinopathy. N-acetyl glucosamine (NAG) and retinol binding protein (RBP), markers of renal tubular dysfunction, or transferrin (TF) which reflects glomerular damage, may be more specific for diagnosing diabetic renal disease than microalbuminuria (MA). 89 randomly selected NIDDM patients (mean age 60.9 [range 38 to 77] years, 32 female) collected 3 timed overnight urine samples for albumin excretion rate (UAER), and one 24 hour urine sample for NAG, RBP and TF. 49 patients [13 retinopathy] were normoalbuminuric (NA); 20 [9 retinop.] MA and 20 [10 retinop.] macroalbuminuric. Patients with retinopathy compared to those without had similar UAERs (median [range]), 58.3 [<5 to 3890] vs 10 [<5 to 3740]μg.min^{-1}; TF in MA retinopathic patients was increased to 6.4 [0.8-112] vs 0.7 [0.3-17.2]mg/mmol creatinine, $p<0.03$; NAG in macroalbuminurics with retinopathy was decreased from 2.14[1.3-5.1] to 2.42[0.9-3.0] IU/mmol creatinine, $p<0.001$, as was RBP 13.7[3.4-128] compared to 29.1[1.4-74]mg/mmol creatinine, $p<0.001$. NIDDM patients with retinopathy and MA have raised TF, while those with macroalbuminuria have lower levels of the tubular enzymes, NAG and RBP. Patients with retinopathy are more likely to have a diabetic cause of nephropathy, therefore, this would confirm that glomerular rather than tubular dysfunction is of importance in its early development. Those with macroalbuminuria and no retinopathy may have other renal pathology resulting in tubular dysfunction. The pattern of tubular enzymuria may help in defining the cause of the nephropathy.

2118

IS RENAL BIOPSY NECESSARY IN PATIENTS WITH NIDDM?

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Non-diabetic renal disease (NDRD) in NIDDM apts can alter the management and prognosis of renal disease. We retrospectively reviewed renal biopsies (RB) performed for suspected NDRD in 40 NIDDM pts, 29 males and 11 females, mean age 59,7 years, mean duration of NIDDM 5,9 years. Indications for RB were: rapidly progressive renal failure, unexplained renal failure, macrohematuria, unexpected nephrotic syndrome and acute nepritic syndrome in pts with normal or enlarged kidneys. Eight pts (20%) had only diabetic nephropathy (DN). Thirty-two pts (80%) had NDRD alone (14 pts) or in combination with DN (18 pts). Glomerulonephritis (GN) was found in 27 pts (mesangio-proliferative IgA GN and Henoch-Schonlein 11, focal necrotising and extracapillary GN 4, membranous GN 4, endocapillary postinfectious GN 2, minimal change GN 2, focal segmental glomerulosclerosis 1, membranous and mesangial GN 1, membrano-proliferative GN 1, and anti GBM GN 1 pt) Acute interstitial nephritis (AIN) had 3 pts (drug-induced 2 pts, bacterial 1 pt). One pt had fibrillary glomerulopathy and 1 pt cholesterol microembolization. Sixteen pts out of 40 were treated. Four pts with focal necrotising and extracapillary ANCA positive GN (2 pts with Wegener disease and 2 pts with microscopic polyangiitis) and pt with anti GBM GN received steroids and cytoxane, 3 pts with postinfectious GN and bacterial AIN antibiotics, 2 pts with minimal change GN steroids. In 4 pts with membranous GN-secondary, drug induced, the suspected drug was excluded and pts were cured. Two pts with drug induced AIN and acute renal failure recovered after treatment with steroids and exclusion of suspected drug Four of 6 dialysis dependent pts regained sufficient renal function. Conclusions: renal biopsy is indicated and useful in a group of NIDDM pts with an unusual clinical course. Renal biopsy findings indicated different therapy in a half of our NIDDM pts.

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IGF-1 AND ERYTHROCYTE SODIUM-LITHIUM COUNTERTRANSPORT IN TYPE 1 DIABETES

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It is well known that patients with systemic hypetension and diabetic children and adolescents with persistent microalbuminuria had a sodium-lithium countertransport (Na^+/Li^+ CT) activity significantly higher than healthy controls. Moreover, sometimes, raised serum IGF-1 can be concomitant of elevated Na^+/Li^+ CT in essential hypertension. In order to understand if the relationship between elevated Na^+/Li^+ CT and IGF-1 serum levels is present also in diabetic patients with persistent microalbuminuria, we studied a group of 31 (14 male and 17 female) children and adolescents with persistent microalbuminuria (defined as an albumin excretion rate >20 mcg/min) and high level Na^+/Li^+ CT activity: their age ranged from 10.4 to 19.9 years, their duration of disease ranged from 4.5 to 9.5 years; their mean HbA1c was 8.4±2.6%. The Na^+/Li^+ CT activity was higher that a control group of healthy children (0.47 mmol Li^+/L RC/h vs 0.35 mmol Li^+/L RC/h); the mean value of IGF-1 in diabetic patients was 321.1±37.9 mcg/L while in healthy controls was 329.9±53.9 mcg/L (p=ns). We found no significant correlation between Na^+/Li^+ CT activity and IGF-1 serum levels. In conclusion, in diabetic patients with persistent microalbuminuria we found high levels of Na^+/Li^+ CT activity but normal values of IGF-1. We suggest that Na^+/Li^+ CT activity is not influenced by serum IFG-1 levels in diabetic children with persistent microalbuminuria.

2120

ANALYSIS OF FACTORS WHICH POSSIBILITY RESPONSIBLE FOR THE PROGRESSION OF DIABETIC RENAL FAILURE

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In 52 NIDDM patients with renal failure, the rate of deterioration of renal function in each case was examined by linear regression analysis using the reciprocal of serum creatinine concentration versus time. It was found that the slope of the regression line differed for each case and had high coefficients of correlation(mean ± SD:r=0.973 ± 0.018). We investigated that the slope and its 10% reduction times(10% RT) as to relation to fifteen clinical characteristics such as sex, age at onset, duration of diabetes, type of treatment and several labolatory data which might be responsible for those individual change in the course of renal failure. The presense of hypoproteinemia and anemia were found the factors significantly related to the slope of the regression line. The mean slope in cases with hypoproteinemia was -2.21±0.99 and in cases with normoproteinemia was -1.16±0.63 at the start of trace($p<0.001$). In the mean 10% RT, the former was 1.8 times shorter than the latter. Similarly the mean slope in cases with anemia and in cases without anemia were -2.00 ± 1.13 and -1.20 ± 0.59($p<0.05$), respectively. In the mean 10% RT, the former was 1.4 times shorter than the latter. No other clinical data were found to be contributory. However, hypoproteinemia and anemia in the late stages of renal failure were not showed any relation to the reduction of the renal function. These suggest that hypoproteinemia and anemia may be one of the risk factors of the advancement to renal failure in the early stages of diabetic nephropaty, but it cannot be prove to have any effects by itself on the later progression of diabetic renal failure. So we need to turn our attention to the treatment.

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Plasma Concentrations of Immunoreactive Vascular Endothelial Growth Factor (IR-VEGF) in Diabetic Patients with Retinopathy (RP) and Nephropathy (NP)

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VEGF, a secretory protein expressed in various tissues, has been suggested to be involved in the pathogenesis of proliferative RP and proteinuria in diabetic subjects. We measured plasma concentrations of VEGF using a highly sensitive enzyme immunoassay in 110 diabetic patients. Half of these patients showed measurable levels of VEGF(>15.6pg/ml). The prevalence of detectability increases in urinary albumin excretion rate (35.1% in normo-, 54.8% in micro-, 61.3% in macroalbuminuria), but not with severity of RP. Smoking caused an acute increase in plasma IR-VEGF in only 22.6% (12/53) of subjects studied. Smoking-induced elevation was more frequent in patients with macroalbuminuria compared with those with normo-or microalbuminuria (33.3% vs 16.7% or 17.6%, respectively). The magnitude of response also tended to be greater in the former than in the latter. There was no difference among groups with different degrees of RP. These findings suggest that circulating IR-VEGF is related to the progression of NP, and that smoking may contribute to this process by causing tissue hypoxia in susceptible diabetic subjects.

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FASTING SERUM C-PEPTIDE IMMUNOREACTIVITY IN DIABETIC PATIENTS ON HEMODIALYSIS

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Indicators for blood glucose control and fasting serum C-peptide immunoreactivity level (SCPR) were investigated by the cross-sectional method in diabetic patients on hemodialysis who were classified according to the type of diabetic therapy. In addition, the relationship between the changes in SCPR and in blood glucose was assessed. The subjects consisted of 133 patients with diabetes who had undergone hemodialysis. Among them, 83 undergoing dietary therapy alone were classified as group D, while 50 receiving dietary therapy plus insulin were classified as group I. SCPR, fasting plasma glucose levels (G), HbA_{1c}, and serum fructosamine levels (F) were measured in both groups. In addition, SCPR and HbA_{1c} were, as a rule, measured every 6 months. Changes in these parameters were followed for up to 4 years in 34 patients. SCPR was significantly lower, while G, HbA_{1c}, and F were significantly higher in group I than in group D. In group I, SCPR inversely correlated with the daily insulin dosage (R=-0.60). Changes in SCPR and HbA_{1c} followed pattern P (SCPR increased when HbA_{1c} increased) or pattern PS (SCPR tended to increase when HbA_{1c} increased, but the change was small or indistinct because of the short observation period) in 22 patients. SCPR can be used as an index for the initiation and cessation of insulin therapy as well as adjustment of insulin dosage. However, since SCPR often increased during poor blood glucose control, it may be preferrable to evaluate the blood glucose control at the time of measurement.

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HEPATOCYTE GROWTH FACTOR AND DIABETIC NEPHROPATHY

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Hepatocyte Growth factor (HGF) possesses mitogenic and morphogenic activities for renal epithelial cells. It was shown in rats that HGF acts as a renotropic factor for renal regeneration through not only paracrine but also endocrine mechanism. Recently it was also shown that HGF has a potent mitogenic activity to endothelial cell of vessel. We measured serum HGF levels in 298 diabetic patients by the ELISA kit (Otsuka, Japan) using antihuman HGF monoclonalantibodies as the solid phase and antihuman HGF rabbit polyclonal antibodies as the liquid phase. The serum HGF levels of diabetic patients were significantly higher than normal subjects (0.38 ± 0.11 ng/ml vs. 0.19 ± 0.05 ng/ml; $p<0.001$). In patients with diabetic nephropathy elevated urinary albumin or N-acetyl-d-glucosaminidase, serum HGF levels were significantly increased. We also recognized a significant relationship between HGF production and hypertension but not diabetic retionopathy or glycemic control. According to these results, HGF appears to play a role in the development of diabetic complication, including diabetic nephropathy.

2124

KIDNEY TRANSPLANTATION ON PATIENTS HAVING DIABETES MELLITUS TYPE I (IDDM) AND DIABETES MELLITUS TYPE II (NIDDM) WITH END-STAGE RENAL DISEASE (ESRD)

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After Saint-Vincent Declaration, a multidisciplinary team was created for patients with diabetes mellitus and ESRD. **Aim:** to evaluate the results of kidney transplantation on IDDM and NIDDM patients with ESRD on dialysis. **Materials and methods:** A total of 618 patients with ESDR were transplanted (1980-Sep/1996). 28 of them were diabetics. Those were divided in two groups: Group I - 17 IDDM *(2.75%); 11M, 6F; mean age: 33.2; mean years of diabetes: 21.8; proliferative retinopathy: 100%; macroangiopathy: 63.6% M, 33.3% F; neuropathy: 100%; mean years of dialysis: 2.6. Group II - 11 NIDDM (1.78%); 9M, 2F; mean age: 53.0; mean years of diabetes: 14.1; proliferative retinopathy: 100%; macroangiopathy: 100%; neuropathy: 100%; mean years of dialysis: 2.7. They received a cadaver kidney and triple (AZA+P+CsA) immunosuppression in 92.9% cases and a sequential quadruple (AZA+P+CsA+ATG or OKT3) in 7.1%. **Results:** Group I- mean days of hospitalisation: 20.9; mean survival graft: 31.7 months; patients with functioning graft: 15/17; HbA1c: 8.3%; most frequent post-transplant complications: acute rejection; infections, vascular, tumours..., mortality 1/17. Group II- mean days of hospitalisation: 28.8 mean survival graft: 31.1 months; patients with functioning graft: 8/11; HbA1c: 8.2%; most frequent post-transplant complications: acute rejection; infections, vascular, tumours..., mortality 3/11. Actuarial patient survival (1995): a) non-diabetic: 1st year- 95.55%; 5th year- 90.76% and 10th year- 81.54%; b) IDDM: 1st year- 92.86%; 5th year- 92.86%; c) NIDDM: 1st year- 60%; 5th year- 60%. Actuarial graft survival (1995): a) non-diabetic: 1st year- 91.82%; 5th year- 79.58% and 10th year- 67.65%; b) IDDM: 1st year- 86.2%; 5th year- 86.2%; c) NIDDM: 1st year- 60%; 5th year- 60%. **Conclusions:** After Saint-Vincent Declaration and start of our multidisciplinary team, more diabetic patients were transplanted. Diabetics have a lower actuarial patient and graft survival than the non-diabetics ones. The NIDDM had the worst results. In the last years there was an improvement of results, so for IDDM kidney transplant is our best option. In NIDDM the decision to transplant or to keep on dialysis should be taken on a per person basis.

*3 patients received a combined pancreas and kidney transplant

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THE PROGRESSION OF DIABETIC NEPHROPATHY AND PHAGOCYTOSIS OF MONONUCLEAR CELLS IN NIDDM

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The aim of this study is to examine connection between different stages of diabetic nephropathy (DN) and phagocyte function of mononuclear peripheral blood cells (MN) of patients with NIDDM. In order to evaluate this connection 45 patients with NIDDM were divided into three groups:A-normal creatinine (Cr) and no macroproteinuria; B-normal Cr with macroproteinuria; C- high Cr with macroproteinuria. The groups were homogenous concerning parameters of glycoregulation, liporegulation, antidiabetic therapy, sex and age. The control group consisted of 15 healthy volunteers. All subjects were examined by method of ingestion of particles of inactivated yeast labeled with neutral-red; after these all system is labeled by acrydil-orange and analyzed by fluorescent microscopy. Percent of phagocytosis (PP), index of phagocytosis (IP) and absolute index of phagocytosis (AIP) are determined. PP are 20.48 +/- 5.42%; 22.07 +/- 7.11%; 30.64 +/- 6.90% and 24.12 +/- 9.15% in groups A, B, C and control respectively (statistically significant difference exists between groups: C vs A, p=0.0008; C vs B, p=0.006 and C vs control, p=0.0026). IP are 3.06 +/- 0.65, 3.17 +/- 0.87, 3.55 +/- 0.84 and 3.13 +/- 0.82 in groups A, B, C and control respectively ($p>0.05$). AIP are 61.10 +/- 17.91, 66.15 +/- 5.87, 107.84 +/- 41.05 and 74.17 +/- 29.41 in groups A, B, C and control respectively (A vs C, $p<0.05$). In conclusion, advanced stages of DN are connected with increasing of phagocyte activity of MN cells.

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ASYMPTOMATIC BACTERIURIA IN PATIENTS WITH NIDDM. HW Lee, KC Won, IH Cho, CK Lee, Hy-W Lee and IK Lee*. Yeungnam University, Keimyung University*, Taegu, Korea.

The urinary tract is a frequent site of infection in patients with diabetic mellitus. The majority of investigators have reported that the prevalence of asymptomatic bacteriuria is higher in women with diabetic mellitus than nondiabetic women. Several studies, however, report little or no difference between diabetic and nondiabetic group. Several promoters of bacteriuria have been suggested in the diabetic host, such as glycosuria, immune defence defect or autonomic neuropathy affecting bladder and urethral function.

The prevalence of asymptomatic bacteriuria was studied in 279 diabetic patients and 182 nondiabetic controls. The possible role of host factors such as glycosylated hemoglobin, duration of diabetic mellitus, cardiovascular autonomic reflex, diabetic microangiopathies was also assessed in diabetic patients with and without bacteriuria. The prevalence of asymptomatic bacteriuria was significantly higher in diabetic women than in nondiabetic controls(15.8 vs 1.5%, $p<0.001$), but not in the diabetic men. In this survey of 133 diabetic women, asymptomatic bacteriuria was more common in the older patients. E. coli was found in 70.3% of urine culture with significant growth from diabetic patients. In nondiabetic controls, E.coli was found in 2/2 (100%) cultures. Risk of bacteriuria was not related to level of glycosylated hemoglobin(HbA_1), mean duration of diabetes mellitus, diabetic nephropathy and retinopathy. Abnormalities of cardiovascular autonomic function and diabetic neuropathy were common in diabetic patients with bacteriuria than in those without bacteriuria. This results suggest that diabetic patients with abnormal cardiovascular reflex test and autonomic neuropathy appear to be at increased risk of developing bacteriuria. Further study is necessary to determined relationship between cardiovascular test of autonomic function and autonomic abnormalities in other system, especially bladder innervation.

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THE RELEVANCE OF KIDNEY ATHEROSCLEROSIS IN THE EVOLUTION OF KIDNEY FUNCTION IN NIDDM.
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Hystomorphological aspects of renal atherosclerosis are commonly found but, in the absence of reliable and non-invasive techniques, the relevance of macroangiopathy has still to be defined. The Duplex Doppler Ultrasuonography provides data about kidney morphology and characteristics of the intrarenal blood flow up to the level of interlobar arteries. Aim of this study is to evaluate the role of the atherosclerosis of the reno-parenchymal vasculature in the kidney involvement in NIDDM. 44 NIDDM and 10 control subjects entered the study. Patients with acute kidney failure and chronic urinary tract infections were excluded. Clinical and metabolic characteristics of subjects and vascular sequelae of the disease were evaluated. (Microangiopathy: fundus oculi or fluorangiography; albumin/creatinine; video-capillaroscopy. Macroangiopathy: Doppler ultrasuonography (carotid, posterior tibial and pedidia arteries). Duplex Doppler Ultrasuonography (DDU) of the kidney: Resistive Index (R.I.) and renal volume were calculated. No correlations were found between kidney R.I. and fasting blood glucose, HbA1 and total cholesterol; HDL-cholesterol correlates negatively ($p<0.05$), Tryglycerides and uric acid correlate positively ($p<0.05$). The R.I. correlates positively with serum creatinine ($2p<0.05$) and negatively with kidney volume($2p<0.01$). An increased R.I. is significantly associated with the macroangiopathy of the lower body ($2p<0.05$), while it does not seem to be influenced by macroangiopathy of the upper body or by microangiopathy. It suggests that renal macroangiopathy is mainly responsible for the modifications of DDU and for the evolution of kidney failure in NIDDM.

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EFFECT OF CALCIUM - ANTAGONISTS ON BLOOD PRESSURE AND MICROALBUMINURIA IN TYPE 2 DIABETICS WITH HYPERTENSION
The Lacidipine Multi-Center Study, Diabetes Center, Hippokration Hospital, Athens University, Athens, Greece.

In order to investigate the hypotensive effect of calcium-antagonists, in Type 2 Diabetics with hypertension as well as any effect on the development of albumin excretion, we studied 136 patients, 68 males, 119 females, in 9 Diabetes Centers, with mean age 60,6± .6, diabetes duration 8,2± .6 and hypertension duration 5,1± .6 years. Thirty-five percent of the patients were treated for the first time with antihypertensive drugs. After a 2 week run-in period without antihypertensive treatment, 4mg/d of the calcium antagonist Lacidipine were given for 12 weeks. Serum lipids, HbA1c, safety tests and urinary albumin were measured at the beginning and the end of the study. Blood glucose and blood pressure were measured at each visit, i.e. at weeks 0, 1, 2, 4, 8 and 12. Both Systolic (SBP) and Diastolic (DBP) blood pressure showed a significant decrease after one week of treatment, from 167.1 to 154.5 and from 97.6 to 89.0 mmHg respectively, p<.001. Both SBP and DBP decreased progressively till the 8th week, when they were stabilized, SBP 167.1, 154.5, 151.3, 148.9, 146.1, 145.7 and DBP 97.6, 89.0, 85.6, 84.5, 82.7, 82.0 mmHg,p<.001. At the 12th week the final decrease was 12.6% for SBP and 15.9% for DBP. There were 87 subjects without microalbuminuria, Albumin ≤ 20mg/L and 14 subjects with microalbunminuria, Albumin >20 and ≤200 mg/L, while subjects with macroalbuminuria, Albumin >200 mg/L were excluded from this analysis. At the end of the 12 week treatment period with Lacidipine 18.4% of the normoalbuminurics progressed to microalbuminuria, while a greater percentage, 42.9%, of the microalbuminurics became normoalbuminurcs. The mean urinary albumin in the normoalbuminuric group increased significantly from 3.3 ±.5 to 12.8 ± 2.6 mg/L, p<.001, but remained in the normal range, while in microalbuminurics it decreased significantly from 39.7 ± 4.9 to 25.3 ± 4.8 mg/L, p<.05. **Conclusions:** Treatment of hypertensive Type 2 diabetics with the Calcium antagonist Lacidipine for 12 weeks results in: **a)** A fast, significant and sustained decrease of both Systolic and Diastolic Blood Pressure **b)** A significant decrease of the albuminuria in those with microalbuminuria **c)** Reversal to normoalbuminuria of a significant percentage of the microalbuminurics.

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DECREASED ACTIVITY OF CALCIUM PUMP IN SKIN FIBROBLASTS OF IDDM PATIENTS WITH NEPHROPATHY.
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Human essential hypertension is characterized by an abnormal cellular calcium metabolism, and a decreased activity of the calcium pump has been observed in platelets of hypertensive patients. A predisposition to essential hypertension has been suggested to underlie the development of proteinuria in IDDM patients, but whether Ca^{2+} handling may be abnormal in these patients is presently unknown. We have therefore addressed Ca^{2+} transport pathways in cultured human skin fibroblasts (HSF) from insulin-dependent patients with diabetic renal disease. Forearm skin biopsies were taken and HSF grown from 13 patients with proteinuria and from 11 age-matched normoalbuminuric patients with a comparably long duration of diabetes (26±6 vs 29±2 years, p=NS). Exchangeable cell Ca^{2+} pool, Ca^{2+} influx and Ca^{2+} efflux were measured by established tracer (^{45}Ca) techniques in subconfluent cultures of actively proliferating cells after seven passages. The exchangeable Ca^{2+} pool was comparably distributed in the above groups of patients (21.4±3 vs 22.4±3 $nmol \cdot mg_{protein}^{-1}$, p=NS). Ca^{2+} influx was lower among the patients with nephropathy, though failing to reach significance (0.68±0.07 vs 0.95±0.1 $nmol \cdot mg_{protein}^{-1} \cdot min^{-1}$, p=0.1). Finally, Ca^{2+} efflux was significantly lower in patients with proteinuria (0.09±0.01 vs 0.14±0.02 $nmol \cdot mg_{protein}^{-1} \cdot min^{-1}$, p=0.03). None of these variables was quantitatively related with HbAIc, age or duration of diabetes. These results suggest that reductions in the activity of Ca^{2+} pump may represent one more ion transport abnormality shared by essential hypertension and diabetic nephropathy. A normal exchangeable Ca^{2+} pool may reflect a parallel reduction in Ca^{2+} influx, but the mechanisms of these abnormalities remain to be seen.

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RENAL BIOPSY & IMMUNOSUPPRESSIVE THERAPY IN PROTEINURIC TYPE-II DIABETIC PATIENTS.

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In proteinuric type-II diabetic subjects, absence of diabetic retinopathy (DR) or presence of erythrocyturia is usually suspicious for non-diabetic glomerulopathy. Renal biopsy in such cases is important for diagnosis and planning appropriate therapy. Aim of the present study was to evaluate the justification of renal biopsy and the role of individualized immunosuppressive therapy in clinically diagnosed cases of so-called diabetic nephropathy. Studied subjects comprise 29 (m=14, f=15) proteinuric type-II diabetic patients having erythrocyturia or absence of DR of mean age 55.07 (SD 6.36) yr. with 8.9 (SD 6.67) yr. of known diabetic duration. Renal biopsy revealed diffuse & nodular glomerulosclerosis (suggestive of diabetic nephropathy - DN) in only 8 (m=6, f=2) cases all of which also had interstitial reaction, rest 21 (m= 8, f=13) had different forms of glomerulonephritis (GN). All were treated with prednisolone with or without cyclophosphamide (including pulse doses). Intensity & duration of the therapeutic regimen were individualized according to histology, severity of disease and clinical response. Patients were followed-up for 5.4 (SD4.11) months. Proteinuria decreased from 6.69± 2.15 to 2.98±1.34 gm/24 hr ($p<0.001$) in DN group and from 5.44±3.09 to 2.18±1.69 ($p<0.001$) in GN group; serum creatinine decreased from 2.42±0.73 to 1.94±0.44 mg% ($p<0.001$) in DN group and from 2.22±1.16 to 1.83±1.00 mg% ($p<0.001$) in GN group. However proteinuria and serum creatinine at last follow-up correlated significantly with baseline proteinuria ($p=0.0031$) and serum creatinine ($p<0.001$) even in multiple regression analysis where they were adjusted for each other and other factors as well. Response to therapy was better in female compared to their male counterpart which may be related to their less severe initial presentation. We conclude that renal biopsy in selective proteinuric type-II diabetic patients is justified and individualized pathogenetic treatment may be helpful in non-diabetic nephropathy in type-II DM and early intervention should be recommended Further follow-up is going on to confirm our findings.

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THE EFFECT OF INTENSIVE INSULIN THERAPY ON THE PROGRESS OF EARLY DIABETIC NEPHROPATHY AND RETINOPATHY

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Evidence in animals and humans has suggested that the developement of diabetic chronic complications(diabetic nephropathy, retinopathy, neuropathy, ect.) is related to metabolic abnormality associated with hyperglycemia, In the clinical study, we observed the effect of the intensive insulin therapy on the progress of early diabetic complications. 16 patients with early diabetic nephropathy and 14 patients with minimal background retinopathy were randomly divided into a the intensive insulin therapy group and a placebo— controlled group. The results showed that 1. the daily administration 0. 2～1. 0 U/kg. d of biosynthetic human insulin(Actrapid, Novo Nordisk) to normalize the level of blood glucose(fasting<7. 2, 2 hours <10. 0 mmol/ L) throughout 12 week course of the experiment sharply decreased the glomerular filtration rate (clearance of ^{99}Tc) comparing with placeo group(treated group: from 140±20 to 116±11 ml/min. 1. 73 m^2, $P<0.05$; placeo group: from 138±22 to 136±16 ml/min. 1. 73 m^2, $P>0.05$); 2. Urinary albumin excretion(radioimmunoassy) was apparently decreased in the treated patients, whereas no changes was seen after placeo(treated: from 86±21 to 43±15 ug/min, $P<0.05$; placeo: from 90±11 to 83±25 ug/min, $P>0.05$)); 2. Neither the intensive insulin therapy nor placeo— controlled patients were found the statistical significant reduce in the microaneurysm counts(treated group: from 3. 6±1. 9 to 4. 1±3. 0; placeo group: from 3. 8± 2. 5 to 4. 4± 1. 8 , determined by flurorescence retino— angiography). In conclusion, intervention with the intensive insulin therapy can reduce the progress of glomerular filtration rate and urinary albumin excretion, but at least in short time, has no significant effect on the course of minimal retinopathy.

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CLINICAL EFFECTS OF LONG-TERM ERYTHROPOIETIN (EPO) TRAETMENT IN JAPANESE NIDDM WITH NEPHROPATHY

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EPO (recombinant human erythropoietin] has been used for the treatment of anemia associated with end stage diabetic nephropathy. However, there were only a few clinical data about the effects of EPO administration on amenia and renal function at more earlier stage of diabetic nephropathy before hemodialysis. In this prospective study, we have tried to investigate the long term clinical effects of EPO in NIDDM patients with anemia due to diabetic nephropathy at more early stage.

[Subjects and methods] Four Japanese NIDDM patients with anemia due to diabetic nephropathy were investigated (serum creatinine (sCr.): 2.0 ±0.4 mg/dl, erythrocyte (RBC): 285±10 x10^4/mm^3, hemoglobin: 8.6±0.7 g/dl]. EPO was administered at the dosage of 6000 units every two weeks for 96 weeks. Fasting blood sample was taken periodically and investigated the clinical effects of EPO.

[Results] After EPO administration, anemia was significantly improved [24 weeks ; RBC:328±17 x10^4/mm^3 ($p<0.01$), 96 weeks ; 343±34 x10^4/mm^3 ($p<0.05$)]. Although serum creatinine concentrations were increased in these patients, the changes were not statistically significant [24 weeks: 2.3±0.4, 96 weeks: 2.7±0.5 mg/dl, n.s.]. Mean changing rates of serum creatinine was significantly reduced than control data [- 0.0016 ±0.0010 (1/mg · dl/week) , $p<0.01$]. During this prospective study, there were no significant changes in blood pressure and blood glucose control levels.

[Conclusion] It was found that in NIDDM patients with anemia due to nephropathy, long term EPO treatment was clinically effective to improve amenia and also to attenuate the speed of deterioration in renal function.

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DECREASE OF URINARY ALBUMIN EXCRETION AFTER GLYCEMIC CONTROL IN DIABETES.

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Raised urinary albumin excretion (UAE) is a marker for incipient nephropathy when comprised between 30 and 300 mg/24h. The aim of this work was to assess the influence of high blood glucose level on the rate of UAE and its evolution after glycemic control. Subjects and methods: 32 diabetic patients were included (22 M, 11 F; 13 IDDM, 19 NIDDM; mean age 51 ± 18 years, diabetes duration 8.1 ± 18 years). All patients had a poor glycemic control (fasting blood glucose level > 250 mg/dl, HbA1c > 9%) requiring short term intensive insulin therapy. The 24hour UAE was measured daily during an 11 days period. Poor glycemic control at admission was demonstrated by patients' parameters (glycemia 342 ± 102 mg/dl, HbA_1c 11.3 ± 2.3%, fructosamine 0.44 ± 0.09 mmol/l). Results : Daily measurement of UAE showed a continuous decrease until a stable level which was reached around the 8th day. A significant difference ($p<0.001$) for mean UAE rate was found between the first day (35.6 ± 61 mg/ 24h) and the last day (14.2 ± 10 mg/24h). A positive correlation was noted between the initial HbA_1c level and the magnitude of UAE's decrease ($r = 0.395$, $p< 0.02$). Comments : A long time of high blood glucose level induces an increase of urinary albumin excretion and the restoration of glycemic control allowed a decrease of UAE which becomes stable after 8 days. Thus, in diabetic patients, urinary albumin excretion rate should be interpreted with consideration of glycemic control.

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LOW PROTEIN DIET AND KIDNEY FUNCTION IN INSULIN DEPENDENT DIABETIC PATIENTS WITH DIABETIC NEPHROPATHY

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Initiation of low-protein diet (LPD) in patients with varies nephropathies induces a faster initial and slower subsequent decline in glomerular filtration rate (GFR). Whether this initial phenomenon is reversible or irreversible remains to be elucidated. We performed an eight weeks prospective, randomized, controlled study comparing the effect of LPD (0,6 g/kg/24 h) with normal protein diet (NPD, 1,0 g/kg/24 h) in 29 insulin dependent diabetic (IDDM) patients with diabetic nephropathy. At baseline the patients were randomized to either NPD (n=15) or LPD (n=14) for 4 weeks. Between week 4 and 8 all patients received NPD. The following variables were measured at baseline and after 4 and 8 weeks follow-up: Dietary protein intake (DPI, estimated by the 24 h urinary nitrogen excretion, g/kg/24 h), GFR (^{51}Cr-EDTA, ml/min/1,73 m²), albuminuria (ELISA, mg/24 h) and blood pressure (BP, Hawksley random zero sphygmomanometer, mm Hg). During the investigation 14 patients in the NPD group and 12 patients in the LPD group received their usual antihypertensive treatment. Baseline data, the NPD group vs the LPD group; DPI (mean, SD) 0,9 (0,3) vs. 1,2 (0,3)($p < 0.05$), GFR (mean, SD) 92 (21) vs. 94 (21)(NS), albuminuria (geometric mean, antilog SEM) 550 (1,3) vs. 269 (1,5)(NS), and BP (mean, SD) 140/79 (17/10) vs. 135/76 (12/7)(NS). Changes in DPI (ΔDPI), GFR (ΔGFR), albuminuria (ΔUAE) and BP (ΔBP), from baseline to 4 weeks, and from 4 to 8 weeks, were compared between the LPD group and the NPD group:

	mean difference (95% CI) between LPD group and NPD group			
	0 to 4 weeks	p	4 to 8 weeks	p
ΔDPI (g/kg b. w./24 h)	-0,4 (-0,6 to -0,3)	<0,0001	0,3 (0,2 to 0,5)	<0,0001
ΔGFR (ml/min/1,73 m²)	-6,0 (-12,6 to 0,5)	=0,07	8,8 (2,9 to 14,7)	<0,005
ΔUAE (%)	-28,1 (-46,5 to -5,6)	<0,05	21,5 (-8,0 to 60,3)	=0,16
Δsystolic BP (mm Hg)	0,0 (-8,8 to 8,9)	NS	-2,5 (-12,2 to 7,2)	NS
Δdiastolic BP (mm Hg)	-3,1 (-8,3 to 2,1)	NS	-0,7 (-7,1 to 5,8)	NS

In conclusion, short-term LPD induces a reversible (hemodynamic?) reduction in GFR and albuminuria in IDDM patients with diabetic nephropathy.

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SECONDARY FAILURE IN NON-INSULIN-DEPENDENT DIABETES MELLITUS (NIDDM): EFFECT OF INSULIN ON RENAL FUNCTION

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Some 50% of NIDDM patients fail to achieve adequate glycaemic control after 10 years on oral hypoglycaemic agents (OHA), thus necessitating insulin therapy. OHA failure patients were defined as those having an HbA_{1c}>7.2% (ref <6.2%), on maximum OHA for >1 year with C-Peptide concentrations >0.6 nM 6 mins after I mg iv glucagon. HbA_{1c}, fasting C-peptide and insulin, blood pressure (BP), morning urine for albumin/creatinine ratio (ACR) and 3 timed overnight urine collections for albumin excretion rate (UAER) [n=28], with glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) [plasma isotopic clearance after single combined intravenous injection of Cr^{51}-EDTA and Tc^{99}-MAG3, corrected to 1.73m² surface area] [n=17], assessed immediately before and repeated after 3 months on insulin. HbA_{1c} improved significantly from 10.6 [0.3] mean [SEM] to 8.5[0.3]%, p<0.01. Fasting C-peptide decreased from 965 [100] vs 686 [70]pmol.l^{-1}, p<0.001; insulin increased form 12.16 [1.3] to 25.41 [9.6]mU.l^{-1}, p< 0.01. Body mass index increased from 27.3 [0.9] to 28.3 [1.0] kg.m², p<0.001. Systolic BP increased 133 [3.3] vs 138 [3.7]mmHg, p< 0.04, with no change in diastolic BP 75 [2.0] vs 77 [2.1]mmHg. ACR (median [range]) 3.2 [<5 to 390] vs 2.5 [<5 to 182]mg.mmol^{-1} and UAER at 8.7 [<5 to 3110] initially vs 6.9 [<5 to 1028]μg.min^{-1} were not significantly lower on insulin. GFR, ERPF and filtration fraction remained stable at 90.1 [6.6] vs 88.2 [7.4] ml.min^{-1}; 330 [29] vs 320 [30] ml.min^{-1} and 0.28 [0.01] vs 0.28 [0.02]% respectively. Insulin therapy produces the expected increase in BMI, serum insulin concentrations and improvement in glycaemic control. There was also the less expected increase in systolic BP. Inspite of these changes there is no alteration in renal haemodynamics or urinary albumin excretion.

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METABOLIC EFFECTS OF A MILD DIETARY PROTEIN RESTRICTION IN NIDDM PATIENTS WITH NEPHROPATHY

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Aim of this study was to assess the effects of a short term (about 2 weeks) mild dietary protein restriction (0.8g/kg) on the renal and metabolic state in hospitalized NIDDM patients with diabetic nephropathy. **Patients & Methods:** 22 NIDDM patients (14 M/ 8 F. age 56.2±14.5y, diabetes duration 17.3±7.7y, S-creatinine 2.2±1.6 mg/dl, M±SD) were studied. 10 NIDDM patients without nephropathy (7M/3F ,age 52.2±13.7 y, diabetes duration 9±0.2y,Scr 0.9±0.2) treated without dietary protein restriction (1.45g/kg) served as controls . On admission and before discharge , 24 -hour urine samples were collected and analyzed for protein, nitrogen excretion, creatinine clearance (CCr) and nitrogen balance (NB). Serum total protein (TP),albumin(ALB), lipids (TC, TG), glycated albumin (GA)were also checked. Those data were compared between on admission and before discharge within the two groups. **Results:** Estimated protein consumption (g/kg/iw) of patients at admission and discharge were 0.93±0.25 vs 0.77±0.13,p=0.0026, but that in controls did not differ. CCr., S-cr.,U-protein did not differ in either group. TP and ALB in patients were decreased significantly at discharge,but not in controls. NB(g/day) at discharge in patients was 0.28 ±1.12 and in controls was 2.88±2.44,p=0.004, significantly lower in patients . Lipids in patients were decreased significantly. GA(%) was decreased significantly at discharge in controls, but not in patients. **Conclusion:** A protein restricted diet showed some demerits, even though the restriction was a mild and the duration was short, nutritional status should be monitored closely during a protein modified diet.

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PROGRESSION OF LEFT VENTRICULAR MASS DURING ANTIHYPERTENSIVE TREATMENT IN DIABETIC NEPHROPATHY

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Increased left ventricular mass (LVM) may contribute to the increased relative mortality from cardiovascular disease in insulin dependent diabetic (IDDM) patients with diabetic nephropathy. The effect of antihypertensive treatment on LVM and systolic function was evaluated in a one year, double blind, randomised, parallel study with a long-acting calcium antagonist (nisoldipine, 20-40 mg/day) or an angiotensin converting enzyme inhibitor (lisinopril, 10-20 mg/day). Fifty hypertensive IDDM patients with diabetic nephropathy were enrolled. Three patients dropped out and 7 patients were not evaluable due to technical difficulties. M-mode echocardiography was performed and the results analyzed blinded and independently by two observers before and after treatment with nisoldipine (n=20) or lisinopril (n=20). Twenty four hours ambulatory blood pressure was measured with the A&D TM2420 device every 3 months. During the study, 24h mean arterial blood pressure was reduced from (mean(SE)) 108 (3) at baseline to 101 (2) in average during treatment in the lisinopril group and from 105 (2) to 103 (2) in the nisoldipine group (p=0.06 comparing changes in the two groups). LVM, corrected for body surface (LVMI) was comparable between groups at baseline and increased from (mean(SE)) 96 (5) to 107 (6) g/m² (p=0.007) in the nisoldipine group and from 95 (4) to 103 (5) g/m² (p=0.03) in the lisinopril group. The mean difference between the change in LVMI in the two groups were 2,9 (95% CI: -6,8 to 12,7) g/m². The prevalence of left ventricular hypertrophy rose from 18 % to 30 % during the study period. A multiple linear regression analysis revealed, that LVMI increased with higher systolic blood pressure level and declining GFR, while sex, age, metabolic control, diastolic blood pressure, urinary sodium excretion and treatment group did not contribute to explain the variation in LVMI, R^2 = 0.25. Systolic function, assessed by fractional shortening, were within normal range and similar at baseline and during follow up 42 (1) vs 41 (1) %, nisoldipine vs lisinopril, respectively. Conclusion: Antihypertensive treatment to a blood pressure level within the normal range, does not hinder a progressive rise in LVM in IDDM patients with diabetic nephropathy. The impact of more aggressive treatment should be evaluated.

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LOW PROTEIN DIET INCREASES INSULIN-STIMULATED GLUCOSE DISPOSAL AND ENERGY PRODUCTION RATE IN UREMIC PATIENTS.

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Low protein diets (LPD) increase insulin-mediated glucose disposal in chronic renal failure (CRF), but the fate of the better utilized glucose and the effect on energy production rate are unknown. Using a two-step (1 and 5 $mU.kg^{-1}.min^{-1}$) euglycemic hyperinsulinemic clamp combined with indirect calorimetry, we studied the effects of a LPD (0.3 $g.kg^{-1}.d^{-1}$, supplemented with essential aminoacids and ketoanalogues) in 6 non dialized patients with chronic renal failure (Glomerular Filtration Rate: 13.6±3.1 $ml.min^{-1}$). After 3 months of diet, no significant change was observed concerning GFR, body weight, arterial pH. At the postabsorptive state, plasma glucose and insulin were significantly lower (Glucose: 4.7±0.1mmol.l^{-1} vs 5.0±0.1 before LPD, insulin 8.1±1.5 $\mu U.ml^{-1}$ vs 14.0±3.7 before LPD; both $p<0.05$) and energy production rate higher (17.16±0.67 $Cal.kg^{-1}.min^{-1}$ vs 15.72±0.48 before LPD; $p<0.05$). Insulin-stimulated glucose oxidation (3.37±0.35 $mg.kg^{-1}.min^{-1}$ vs 2.36±0.29 before LPD; $p<0.05$ at first clamp step) and non oxidative disposal (2.58±0.68 $mg.kg^{-1}.min^{-1}$ vs 1.29±0.51 before LPD at first clamp step; 5.31±0.41 vs 3.74±0.74 at 2nd step; $p<0.05$ at both steps) increased after LPD. This confirms that LPD ameliorates insulin sensitivity in CRF, even for low plasma insulin concentrations. Since energy production rate is increased by LPD, the caloric intake should be increased when protein intake is restricted.

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ACE-INHIBITION DURING TWO YEARS DID NOT IMPROVE U-AER IN NORMOTENSIVE MICROALBUMINURIC IDDM PATIENTS.

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The aim was to investigate if a low dose of ramipril(1.25mg daily) is as effective as 5mg daily to reduct urinary albumin exretion rate (U-AER) in normotensive (diastolic blood pressure ≤ 90 mmHg) microalbuminuric (U-AER 30-300mg/24h) IDDM patients. The study was performed as a multicenter randomized placebo controlled double blind parallell group study during two years. U-AER was measured in 2 24h urinary collections every 3 month, GFR and 24h ABPM at time 0, 1 and 2 years.

Group of patients	Placebo	1.25mg Ramipril	5 mg Ramipril
Male/female (n)	14/4	13/6	14/4
Duration of diabetes	21.1	27.7	22.5
HbA1c (%)	7.4	7.6	7.2
U-AER* 0y (mg/24h)	103	109	69
U-AER* 2y (mg/24h)	95	94	81

*values is median, no differences are significant

There was no significant changes in 24 h ABPM or GFR in any group.

Conclusion: Normotensive IDDM patients with microalbuminuria and good glycaemic control had no difference in UAER progression when treated with placebo or ramipril 1.25mg or 5mg daily during 2 years. Probably, this group of microalbuminuric patients does not need Ace-inhibition.

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n - 3 FATTY ACID THERAPY IN DIABETIC PROTEINURIA - A CLINICAL STUDY.

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Twenty four hour urine proteins were estimated when the urine culture was sterile in 36 patients ***(Group 1)*** (diabetes mellitus of 5-10 years duration) with age *50 ± 12 years (M:F = 14:12)* before and six months after n-3 fatty acid therapy (EICOSAPENTAENOIC ACID 1080 mg/day & DOCOSAHEXAENOIC ACID 720 mg/day in three divided doses. Commercial name MAXEPA). Glycemic control was achieved with insulin alone (n = 10), oral antidiabetics (n = 16) and a combination of both (n = 10). Nine were treated with ACE inhibitors, and three with calcium blockers. The mean ± SD biochemical parameters fasting and post prandial plasma glucose (FPG and PPPG - mg%), serum creatinine (SC - mg%), 24 hour urine total volume (24 TV - ml/day), 24 hour urine proteins (24 UP - mg/day), 24 hour urine creatinine (24 UC - mg/day) were *213 ± 94; 300 ± 112; 0.9 ± 0.34; 2650 ± 1045; 530 ± 600; 928 ± 447 respectively before initiation of therapy and 135 ± 68; 180 ± 88; 0.98 ± 0.5; 2394 ± 1002; 436 ± 515 respectively six months after therapy.* Forty seven percent ***(Group 2)*** (n = 17) showed a significant decline in proteinuria ($P < 0.01$) when compared to ***(Group 3)*** (n = 19) who showed a rise in proteinuria. The *mean ± SD of FPG, PPPG, SC, 24 UTV, 24 UP, 24 UC in group 2 at the time of initiation of therapy were 167 ± 57; 264 ± 94; 0.99 ± 0.33; 2740 ± 1081; 766 ± 769; 921 ± 334 and six months after therapy 146 ± 68; 191 ± 82; 1.02 ± 0.45; 2295 ± 1218; 357 ± 374; 779 ± 507 respectively.* The corresponding *values in group 3 were 254 ± 102; 333 ± 119; 0.9 ± 0.35; 2568 ± 1033; 318 ± 271; 933 ± 538 at initiation and 127 ± 69; 170 ± 93; 0.95 ± 0.56; 2483 ± 785; 506 ± 617; 1015 ± 304 respectively after six months of therapy.* There was a significant difference between Group 2 and 3 among FPG ($P < 0.01$), PPPG ($P < 0.1$) at initiation of therapy FPG and PPPG ($P < 0.01$) six months after therapy, and the fall in 24 UP ($P < 0.01$). There was a negative correlation ($P < 0.05$) between the degree of fall in 24 UP and 24 UP before initiation of therapy in group 2 only. There was no correlation between serum creatinine and creatinine clearance before and after therapy in both groups. The reduction of proteinuria by n-3 fatty acid therapy in diabetic proteinuria is complementary to glycemic control and significant when the proteinuria is mild or moderate.

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ACE INHIBITORS IN TREATMENT OF ADOLESCENT PATIENTS WITH IDDM AND DIABETIC NEPHROPATHY.

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The purpose of the study was to assess the effect of ACE inhibitor Enalapril maleate in patients with IDDM on early stages of diabetic nephropathy and normal level of BP. 42 IDDM patients (13 male, 29 female, aged 11.5 - 20.9 ys) were included into the study. 35 of them had microalbumin - uria and 7 had proteinuria. All patients received 5-10 mg of Enalaprili daily during 3-6-9 months. The effect of treatment was evaluated by dynamics of microalbuminuria ("Beringer Mangheim"), HbAl blood level and BP. After 3 months of treatment with enalapril showed a decrease of urine albumin excretion and in 21 of them the urine albumin contents became normal and was normal in 75% of patients after 3 months and in 72,2% after 9 months completion of treatment. Patients who showed no changes of microalbumin-uria level after 3 months of treatment continued to receive the same dose of enalapril for 6 to 9 months. After the period of prolonged treatment improvement was achieved only in patients with decreased level of HbA1% (11.94± 0.6 vs 10.1 ± 0.4 p. > 0.05). In the group of proteinuria 6 of 7 patients showed the reduction of protinuria and 1 of them - the total absence of proteinuria after 3 months of treatment with enalapril (daily dose 5 mg.) Treatment with increased dose (10 mg. daily) o f enalalpril was continued in patients with remaining proteinuria. After the next 3 months of treatment the half of them showed the following reduction of proteinuria, the later correlated with improvement of blood HbA1% (13.2 ± 1.2 vs 10.6 ± 0.78, p > 0.05) level. BP was stable in all patients during the whole period of study. Conclusion: Enalapril is effective for treatment of patients with diabetic nephropathy with normal BP on the stages of microalbuminuria and proteinuria. Effect of treatment is better in patients with compensated carbohydrate metabolism.

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IS VEGETARIAN PROTEIN DIET (VPD) RENOPROTECTIVE ?

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Dietary protein load is recognized to influence the course and progression of chronic renal diseases including diabetic nephropathy (DN). Earlier observations by ourselves and others had suggested that in addition to quantity, the quality (vegetable vs animal origin) of the dietary protein may also influence the pathogenesis and progression of DN. Urinary albumin excretion (UAE) rates (mg/24h) were measured by immunoturbidometry in 227 consecutive NIDDM subjects. The results were correlated with clinical and demographic data. Compared to vegetarians, non-vegetarians had higher UAE (X:166 vs. 205), specifically in the ODN group (858 vs. 1402). Advanced stages of DN were associated with increased diabetes duration, systolic blood pressure (SBP), and glycated hemoglobin, and tendency towards dyslipidemia (increased LDLC and TG, decreased HDLC); age at diagnosis and OHA failure had no significant associations. Conclusions: Despite VPD being potentially renoprotective and the relative predominance of vegetarianism in India, other negative influences (social deficiencies in comprehensive diabetes care - glycemic control, B.P.Control possible increased genetic susceptibility) are likely overwhelming, resulting in overall higher prevalence of advanced stages of DN in Asian Indians.

Group	NAE	IDN-1	IDN-2	ODN	P
UAE	<31	31-150	150-300	>300	
Prevalence%	22	58	10	11	
GHb %	7.8	8.3	8.1	9.1	
SBP mm Hg	137	138	143	156	0.0009
DM duration	6.1	8.0	10.2	11.0	0.012

NAE = Normal Albumin Excretion
IDN: Incipient DN
ODN: Overt DN

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LISINOPRIL REDUCES EXERCISE-INDUCED MICROALBUMINURIA IN NORMOTENSIVE NORMOALBUMINURIC IDDM PATIENTS

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In order to study the effect of lisinopril on exercise-induced urinary albumin excretion twentysix IDDM patients with normoalbuminuria were randomized into two groups having either placebo (n=13, age 36±3 yrs, BMI 24.5±1.1 kg/m^2) or lisinopril (15 mg) (n=13, age 34±2, BMI 24.4±0.9 kg/m^2) as their treatment. Overnight and exercise-induced urinary albumin excretion rate were measured at baseline and after 1 and 2 yr treatment. Two patients from the placebo group and none from the lisinopril group developed microalbuminuria. The exercise-induced urinary albumin excretion rate diminished 46% after the first (p=0.059) and 66% (p<0.01) after the second year in the lisinopril group, but remained unchanged in the control group. Systolic and diastolic blood pressure were similar in the baseline and after 1 year, but at 2 years systolic blood pressure was 13 mmHg (p=0.03) and diastolic blood pressure 9 mmHg lower (p=0.052) in the lisinopril group as compared to the control group. Diastolic blood pressure decreased significantly at 1 and 2 years in the lisinopril group, while there was no significant change in the systolic blood pressure. At baseline, overnight albumin excretion rate correlated with HbA_{1c} (r=0.50, p<0.01) and the duration of diabetes (r=0.39, p<0.05), and systolic blood pressure correlated with both overnight (r=0.42, p<0.05) and exercise-induced (r=0.48, p<0.05) albumin excretion rate in the whole group.

In conclusion, glycaemic control and blood pressure are directly related to overnight albumin excretion rate already in normotensive, normoalbuminuric IDDM patients. Lisinopril treatment reduces exercise-induced urinary albumin excretion rate in such patients. These data suggest a protective effect of lisinopril for the development of microalbuminuria.

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BASAL AND AFTER ORAL METHIONINE ADMINISTRATION HOMOCYSTEINE LEVELS IN NIDDM PATIENTS. RELATIONSHIP TO MICROALBUMINURIA.

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Recent studies conferred an atherogenetic role to Homocysteine.Homocysteine has been suggested as a new independent risk factor for atherosclerosis. Aim of the study was to evaluate the relationship between Homocysteine and Microalbuminuria in NIDDM patients.In this study 33 NIDDM patients (16 males and 17 females) and 16 healthy controls (7 males and 9 females) were enrolled. We determined Homocysteine levels before and after oral administration of L Methyonine (100 mgs/kg) and microalbuminuria, HbAlc, total and HDL cholesterol, tryglicerides. All the patients were treated by hypoglicemic agents or by diet. Homocysteine plasmatic levels in fastin state (8.12 +/- 3.17 micromol/l vs 7.19 +/- 2.4 micromol/l p=ns) and after a methionine load (26.51 +/- 11.5 micromol/l vs 25.06 +/-10.76 micromol/l) did not differ in NIDDM patients versus controls. There was a significant correlation between Homocysteine levels and microalbuminuria (p< 0.005). In NIDDM patients with vascular complications presented higer homocystein levels than patients without any complications (p< 0.0001).
Homocystein could be a relevant prognostic factor warsening the progognosis in well controlled in NIDDM patients.

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EFFECTS OF LISINOPPRIL AND NIFEDIPINE ON THE PROGRESSION TO OVERT PROTEINURIA IN NORMOTENSIVE INSULIN DEPENDENT DIABETIC PATIENTS WITH INCIPIENT NEPHROPATHY

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Microalbuminuria (MA) is observed in as high as 15-25% of IDDM patients in Europe, even in absence of arterial hypertension. It is not clear whether MA does predict overt nephropathy to the same extent when associated or not to arterial hypertension. Intervention trials on renal function in MA IDDM patients should adopt as outcome measurement the rate of decline of glomerular filtration rate (GFR). However normotensive MA IDDM patients show no change in GFR over a follow-up period of 4-10 yrs irrespective of antihypertensive teatment. In the present study we used either 1) the cumulative incidence of progression to proteinuria (albumin excretion rate-AER>250 µg/min) or 2) the rate of yearly increase in AER 50% above baseline, to study the course of renal function in 137 normotensive IDDM patients during double-blind, double dummy treatment either with lisinopril or with slow release nifedipine in comparison with placebo. 103 patients completed the 3 yrs follow-up period. Postural hypotension and hyperkaliemia did not occur in any patient. Time to event analysis indicated a reduction in the risk of progression to proteinuria of 58.1% (95% C.I.: 27.8-68.4%) in the lisinopril (p<0.02 and of 62.5% (95% C.I.: 32.5-73.4%) in the nifedipine (p<0.02) groups respectively, after adjustment for mean blood pressure, glycated hemoglobin and baseline AER. Baseline AER was 71 µg/min (range 21-230) in progressors and 73 µg/min (range 20-233) in nonprogressors (NS). The percentage of patients who showed an yearly increase of AER>50% above base line values was significantly lower in the lisinopril (14 of 34, 41%, p<0.02) but not in the nifedipine (17 of 27, 63%) than in the placebo (31 of 42, 74%) groups. Lisinopril group had significantly lower systolic values during the follow-up than nifedipine (p<0.002) and placebo (p<0.03) groups. In conclusion our data show that both lisinopril and nifedipine are effective in delaying the occurrence of overt proteinuria in normotensive IDDM patients with MA. As overt proteinuria strongly predicts end stage renal failure both treatments appear capable to prevent such complication in normotensive IDDM with MA. Lisinopril appears to improve the patterns of AER also in the range of microalbuminuria. This latter result needs to be emphasized as the patterns of AER have been suggested not be an indicator of but to contribute themselves to the progression of renal damage.

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LONG-TERM PICOTAMIDE, A DUAL THROMBOXANE INHIBITOR, DECREASES URINARY ALBUMIN EXCRETION RATE IN TYPE 2 DIABETIC PATIENTS WITH MICROALBUMINURIA AT REST

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Picotamide inhibits thromboxane-synthetase and acts as a thromboxane antagonist at the receptor level. We investigated in a randomized, double-blind, placebo-controlled trial the long-term effect of picotamide on urinary albumin excretion (UAE) at rest and induced by exercise in 30 non insulin dependent (type 2) diabetic patients normotensive (supine BP $<140/90$ mmHg) and with microalbuminuria (24-h UAE between 20 and 200 μg/min) while at rest. The subjects of our study had mean age 52.5 ± 1.6 yrs and mean BMI 28.5 ± 0.7 Kg/m^2, diabetes duration of 9.1 ± 1.8 yrs and were in fairly good glycometabolic control (HbA1c: 7.0 ± 0.8%). The patients were randomly allocated to receive for 1 year either picotamide, 300 mg, 3 tabs/day or placebo, 3 tabs/day. The patients were seen after 1, 3, 6, 9 and 12 months of treatment. At all times, BP, UAE at rest (mean of three 24-h urine samples), bood glucose, serum creatinine, serum picotamide and creatinine clearance were measured; at baseline and after 6 and 12 months all patients underwent a submaximal physical exercise for evaluation of exercise-induced microalbuminuria. After 6 months of picotamide treatment, at rest (30 ± 24 μg/min) and exercise-induced (80 ± 35 μg/min) microalbuminuria were significantly decreased as compared to the baseline (at rest: 60 ± 40 μg/min; after exercise: 250 ± 150 μg/min) and placebo level without further drops at month 12 of picotamide treatment. On placebo treatment, UAE at rest (80 ± 40 μg/min) and after exercise (170 ± 35 μg/min) was slightly, even if not significantly, increased as compared to baseline values. The effects of picotamide occurred without either significant side effects or changes in either blood pressure levels or glycometabolic control. Our study is the first, long-term, intervention trial in type 2 diabetes showing that an anti-thromboxane agent is able to dramatically decrease microalbuminuria, which in this disease is a dual marker of macro- and micro-angiopathy.

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COMPARISON OF THE EFFECTS OF PERINDOPRIL AND METOPROLOL ON KIDNEY FUNCTION AND ALBUMINURIA IN EARLY DIABETIC NEPHROPATHY IN IDDM PATIENTS

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The action of angiotensin converting enzyme inhibitors (ACEI) and of beta receptors blockers (beta RB) on the pathophysiological events in early diabetic nephropathy is a subject of controversy. The main question is: do the ACEI posses any specific influence on kidney function in diabetics, which is not exerted by beta RB? To explain the problem the double blind, randomised study was designed. In the study the effects of 2-8 mg of perindopril or 50-100 mg of metoprolol daily in IDDM subjects with albuminuria less than 1g/24h were measured. 30 IDDM subjects were randomised into 2 subgroups: 14 cases for perindopril and 16 cases for metoprolol and subjected to 1 year study. At the baseline and 3 times every 3 months the following observations were recorded: clinical examination, HbA1c, blood pressure (BP), daily albuminuria, glomelular filtration rate (GFR), effective renal plasma flow (ERPF), filtration fraction (FF). All IDDM subjects showed the HbA1c level <7%, did not have any additional morbidity and had free access to observes. The obtained: the mean systolic BP dropped down in the perindopril group from 151±15 to 134±10 mm Hg, in metoprolol group from 158±12 to 137±8 mm Hg, the mean diastolic BP decreased respectively from 94±6 to 78±5 mm Hg and from 93±4 to 84±6 mm Hg. The differences observed in BP decrease in both groups were not significant. Contrasting results were observed in the intensity of albuminuria. The mean daily albuminuria decreased in the perindopril group from 376±92 to 103±57 mg/24h. It did not change in the metoprolol group. Differences between both groups are statistically significant. Such functional parameters like ERPF, GFR and FF did not change significantly in the perindopril group, while they showed the tendency to deterioration in the metoprolol group. Conclusion: Both types of therapy equally reduced BP, however ACEI therapy decreased the albuminuria and contributed to the preservation of kidney function in a more visible manner than metoprolol. This outcomes are in favour of the specific ACEI significance.

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LONG-TERM PICOTAMIDE, A DUAL THROMBOXANE INHIBITOR, DECREASES BOTH THE URYNARY EXCRETION OF THROMBOXANE B_2 (TXB_2) AND ALBUMIN IN PATIENTS WITH TYPE 2 DIABETES

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It has been suggested that platelet hyperreactivity in patients with diabetes mellitus is associated with increased platelet production of thromboxane as compared to normal subjects. It has been hypothesized that this platelet activation could contribute to the pathogenesis of microangiophatic diabetic complications. In fact, recents studies have shown that in type 2 diabetes (NIDDM) the urinary excretion of TXB2 (stable metabolite of TX) is elevated with respect to normal subjects. Aim of our study was to evaluate the urinary excretion of TXB2 and albumin in 10 NIDDM normotensive patients (8M/2F) with microalbuminuria at rest treated with picotamide (300 mg tid; a new antiplatelet drug acting as antagonist of both TX-receptors and TX-synthetase) or placebo (3 tabs/day) for 12 months. At the end of the 12 month-period all the patients underwent to: 1) microalbuminuria assay at rest and after cycloergometric submaximal exercise; 2) urinary TXB2 assay (RIA; extracted from 20 ml aliquots of each urine sample on SEP-PAK C18 cartridge and eluted with ethil-acetate); 3) serum picotamide assay (HPLC). All the patients treated with picotamide showed urinary TXB2, microalbuminuria at rest and after exercise values significantly lower than the patients in the placebo group. In the whole group of patients we found: a significant inverse correlation ($r= -0.93$; $p=0.000$) between serum picotamide and urinary TXB2; a significant direct correlation between microalbuminuria after exercise and urinary TXB2 ($r=0.735$; $p=0.015$) and between microalbuminuria at rest and urinary TXB2 ($r= 0.5$; $p< 0.05$). On the basis of our data we can hypothesize that: 1) TX could be involved in the pathogenesis of microalbuminuria in diabetes; 2) the long-term efficacy and the clinical potential use of picotamide in diabetic patients with microalbuminuria at rest is due to its effective TX synthetase inhibition.

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ACE INHIBITORS TREATMENT REDUCES MICROALBUMINURIA LEVELS IN NIDDM.

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Nephropathy is a relevant cause of death in Diabetic Mellitus. Many studies have shown that the treatment with Ace Inhibitors reduces incidence of the kidney impairment throught the decrease of microalbuminuria (earliest marker of nephropathy). Aim of the study was to compare microalbuminuria levels in NIDDM patients before and after six months treatment with Ramipril (orally administrated at the dose of 1,25 mgs/day).Were evaluated 55 NIDDM patients (36 males and 19 females), 45-75 years aged, 16 of them affected by arterial hypertension, in treatment with other drugs in good control of hypertension; nobody was affected either by nephropathy or vascular diseases. Microalbuminuria was determined before and after 8 weeks and 24 weeks of treatment with Ramipril. After six months treatment was found a significant decrease in microalbuminuria (64,46 mg/dl at T0 VS 45,54 mg/dl at T6 p=0.04), this reduction being more relevant in females (64,17 vs 30,37 p=0.002) than in males (75,53 mg/dl vs 54,,78 mg/dl p=0.043). Our data would suggest that Diabetic patients with microalbuminuria request a prolonged treatment with Ace Inhibitors.

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GLYCOSAMINOGLYCANS - A NEW THERAPEUTIC APPROACH FOR DIABETIC NEPHROPATHY (DN)

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The Steno hypothesis suggests that the key event in the development of DN is the loss of glycosaminoglycan (GAG) content of glomerular basement membrane, including the loss of negatively-charged heparan sulphate that impairs membrane charge-selectivity and lead to the onset of albuminuria. Experimental studies demonstrated amelioration of DN under the treatment with GAG (Gambaro,1994). The aim of our study was to evaluate the antiproteinuric effect of GAG administration in type I diabetic patients. Eighteen p-ts with good metabolic control (mean HBA1c 8.1%) were included in an open controlled GCP study: gr.1 (n=9) - with microalbuminuria 30-100 mcg/min and gr.2 (n=9) - with proteinuria 500-1500 mcg/min. We used low-molecular heparin Sulodexide (Alfa Wasserman, Italy) for 3 weeks (600 U/day i.m.). In gr.1 there was a signif. decrease in albuminuria after 2 wk of therapy (up to normal level) and it was stable after 6 wk of withdrawal. In gr.2 the signif. decrease in proteinuria was reached after 3 wk of therapy, but it quickly increased 6 wk after withdrawal. Sulodexide therapy also showed a lipid-lowering effect in gr.2 : plasma cholesterol reduced from 7.3 to 5.6 mmol/l. So GAG therapy has an antiproteinuric and hypolipidemic effects , both of which induce nephroprotection in DN. It is suggested that GAG can improve the glomerular sulfate incorporation and restore the glomerular charge-selectivity.

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Long and Short-acting ACE inhibitors in the management of microalbuminuric diabetics.

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Background : Microalbuminuria is a predictor of proteinuria, and a prognostic indicator of early mortality. The micoalbuminuric patients mostly have blood pressures above that of age, sex and duration matched IDDM with normoalbuminuria. Reducation of elevated blood pressure will reduce proteinuria. ACE (Angiotensin converting enzyme) inhibitors selectively normalize intrarenal blood pressure,and reduce AER. This class include short-acting and long-acting drugs. I conducted a short-term study on 15 diabetic- hypertensive patients with microalbumnuria, proteinuria. Three groups, each of 5 Patients, with same duration of diabetes and hypertension, were randomized and given either captopril, ramipril or benazepril. Glycaemic control and adequate blood pressure were maintained through the study and proteinuria was monitored at regular intervals of 3 months. The two groups on long-acting ACE inhibitors showed statistically signigicant improvement in proteinuria than the group on short acting ACE. They were superior to short-acting ACE in absence of side-effects ($P<0.001$). Long-acting ACE inhibitors do improve proteinuria better than short-acting ACE inhibitors, with minimal side effects.

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A PILOT STUDY OF GLYCOSAMINOGLYCANS (GAG'S) ACTIVITY ON MICROALBUMINURIA OF TYPE I DIABETIC PATIENTS

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Nephropathy in subjects with diabetes mellitus is a major complication that leads to renal failure and death. Diabetic nephropathy ultimately develops in 30% of patients suffering from in diabetes mellitus and is initially characterized by microalbuminuria. Sulodexide is a highly purified preparation containing an endogenous -like fast moving heparin fraction (Iduronyl-Glycosaminoglycan sulphate) -60% and a dermatan fraction-40%. Fifteen out-patients with type I diabetes mellitus and microalbuminuria (mean±SEM 95.4±13.9mcg/min), were administered the glycosaminoglycan sulodexide, with the aim of investigated its influence on the rate of albumin excretion. Sulodexide (Vessel Due F-Alfa Wassermann-Italy) was given by intramuscular route with the dose of 600 Liporoteinolipase Releasing Units/day by for 21 days. At the control visits a recent anamnesis was recorded, a complete physical examination was performed, body weigh and blood pressure were measured. Two times during experiment (before and after treatment) renal function tests (creatinine clearance), hematological and coagulative parameters (blood count, APTT, fibrinogen), hematochemical parameters (creatininemia, uricemia, proteinemia, glycemia, cholesterol, triglycerides) were examined. Urinary albumin excretion was estimated six times (before, after each week of treatment, three and six weeks after end of the treatment) by immunoprecipitationes assay. All the patients completed the study. Sulodexide yielded a clear-cut and statistically significant lowering of the albumin excretion already after the first week of treatment (53,6±11.1mcg/min; $p<0.005$), the decrease was still more evident at treatment completion (26.5±6.05mcg/min; $p<0.007$) and was maintained during the follow-up period, at the end of which the mean value of albumin excretion rate was still significantly lower than at baseline (36.3±6.7mcg/min; $p<0.004$). Sulodexide short-term administration did not influance the routine hematological, hematochemical and coagulative tests contemporarily performed. Patients compliance with treatment was very good and no adverse events were ever reported. In conclusion, our study suggests that sulodexide (Vessel Due F) plays an important role in reducing urinary albumin excretion in diabetic patients with nephropathy

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EFFECT OF ANGIOTENSIN CONVERTING ENZYME INHIBITOR (IMIDAPRIL) ON MICROPROTEINURIA IN NON-INSULIN-DEPENDENT DIABETIC PATIENTS

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In diabetic patients, microproteinuria [urinary-albumin (ALB), transferrin (TF), β_2-microgloburin (BMG) and N-acetyl-β-D-glucosaminidase (NAG)] are markers for early renal dysfunction. We evaluated the effect of imidapril hydorochloride in blood pressure and progression of diabetic nephropathy using these urinary-proteins. In this study, 30 patients with NIDDM (non-insulin-dependent diabetes mellitus) complicated by hypertension, without severe clinical nephropathy (serum creatinine $\leq$1.2mg/dl), but with microalbuminuria, received 5~10mg orally of Imidapril from 24 to 52 weeks. They were required to revisit the outpatient clinic every 4 weeks to have their blood pressure checked, and the first urine specimen in the early morning was used to test for ALB, TF, BMG and NAG. A creatinine correction was made for microproteinuria and parametric statistics were performed using a paired t-test. Imidapril reduced both systolic and diastolic blood pressure significantly after 4 weeks of treatment, and the effect continued until the 32nd week. Also, albuminuria and transferrinuria were reduced significantly with treatment of Imidapril after 4 weeks and the effect continued until the 52nd week. The value of ALB and TF correlated well ($R=0.934$), in all of the urinary samples. No significant changes occurred in the urinary BMG and NAG levels. We also performed the renography using ^{99m}Tc-mercaptoacetyltriglycine (MAG_3) to eliminate the other renal diseases, and found that it is useful for the diagnosis of incipient diabetic nephropathy and the effective renal plasma flow (ERPF) slightly increased among early stage patients. Our study indicated that Imidapril had a proteinuria (ALB, TF)-reducing effect and might be useful for the therapy of incipient diabetic nephropathy with hypertension.

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BASELINE DATA FROM THE PIMAGEDINE ACTION TRIALS

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Baseline demographic and clinical data of two large cohorts of well defined type I and II diabetic patients with overt diabetic nephropathy and retinopathy, enrolled in the phase III pimagedine trials in the USA and Canada, are presented. Pimagedine (aminoguanidine HCl) is a potent inhibitor of non-enzymatic glycosylation and a selective inhibitor of the inducible isoform of Nitric Oxide Synthase (iNOS). Two studies, ACTION I and II, were designed to test the efficacy and safety of pimagedine in slowing the progression of diabetic nephropathy and retinopathy, and lowering morbidity and/or mortality. The ACTION I trial (**A** Clinical **T**rial **I**n **O**vert **N**ephropathy) enrolled type I diabetics between 22 and 50 years of age with overt diabetic nephropathy (estimated creatinine clearance of 0.67 to 1.50 ml/sec, based on the Cockcroft and Gault [C&G] equation, ≥ 500 mg/24 hour proteinuria). The ACTION II study recruited type II diabetics between 30 and 70 years of age with overt diabetic nephropathy of approximately identical magnitude as the type I diabetics in ACTION I (estimated creatinine clearance of ≥ 0.67 ml/sec by C&G, serum creatinine of ≥ 106.1 umol/L [males], or ≥ 88.4 umol/L [females], ≥ 500 mg/24 hour proteinuria). Study patients in both trials were required to have retinopathy by exam or history. The stage of retinopathy was determined with standardized stereoscopic fundus photography. Enrollment for ACTION I was completed on August 31, 1996 with 690 randomized patients. Enrollment for the ACTION II study was closed at 597 patients on December 2, 1996. The two populations demonstrated statistically significant differences for the following baseline parameters ($p < .05$: Mann-Whitney Wilcoxon or Fisher's exact test, two-sided): gender, ethnicity, duration of diabetes, smoking and cardiovascular history, prevalence of allergies, weight, BMI, heart rate, systolic BP, EKG findings, glycemic control, mode of diabetes therapy, renal function (GFR, C&G, and 24 hour urine creatinine clearance, corrected for body surface), proteinuria, c-peptide, ANA titers, triglyceride and HDL cholesterol levels, stage of retinopathy, and history of photocoagulation. Both cohorts exhibited surprisingly low 24 hour urinary calcium levels (mean±SD ACTION I 1.2±1.3, ACTION II 1.2±1.4 mmol/24hr) with normal serum calcium levels. These data are a unique contribution to the understanding of the epidemiology of type I and II diabetes with advanced diabetic complications.

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IMPACT OF CONVERTING ENZYME INHIBITION ON MICROALBUMINURIA IN NIDDM

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Overnight urinary albumin excretion rate(AER) was measured in 45 NIDDM patients having no evidence of overt proteinuria or urinary tract infection. Diagnosis of microalbuminuria was confirmed by estimation of three urine samples at one month intervals. Eight patients had microalbuminuria at presentation. There was no difference in mean age between patients with microalbuminuria and those with normal AER(46.25±11.65 years vs. 49.5±10.04 years; $p>0.05$). Compared to patients with normal AER serving as controls, microalbuminuric patients had significantly higher duration of the disease, mean arterial pressure(MAP) and glycated haemoglobin levels(17.21±2.1 years vs. 9.40±4.73 years; 123.72±5.8 mmHg vs. 101.35±14.49 mmHg and 9.33±0.23% vs. 7.67±1.37%, $p<0.001$ in all cases). 12 months' therapy with enalapril, 2.5-15 mg/day, normalized AER in two patients while microalbuminuria persisted in the remaining six; none progressed to overt proteinuria. Although significant reduction in MAP or glycated haemoglobulin occurred in one year, there was no difference in MAP or glycated haemoglobin levels between those patients in whom AER normalized and those in whom it did not; the mean duration of disease also did not differ between the two groups.
Conclusion: Enalapril therapy may delay the progression to overt proteinuria or even normalize AER in microalbuminuric NIDDM patients irrespective of age, duration of the disease, MAP or glycated haemoglobin levels.

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METABOLIC EFFECTS OF ISRADIPIN IN NORMOTENSIVE NIDDM PATIENTS WITH MICROALBUMINURIA

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Several studies have suggested that ACE-inhibitors may be effective in preventing the onset of nephropathy and treatment of it in both insulin-dependent diabetic subjects and non-insulin dependent diabetic subjects. In contrast, these effects of calcium antagonists are controversial. Therefore, we aimed to evaluate the effects of isradipin, a new antihipertansive dihydropyridine calcium antagonist, on microalbuminüria, fasting plasma glucose, plasma lipids, plasma creatinin, uric acid, C-peptid, insulin, Hb A1, fructosamine, systolic and diastolic blood pressure and heart rate in normotensive patients with NIDDM. 18 subjects received sustained-release (SRO) formulation of isradipin at dosages of 5 mg once daily for 3 monrhs. After 3 months of isradipine treatment urinary albumin excretion rate (UAER) fell from 72.5 ± 40.2 to 52.9+39.5 mg/24 h ($p<0.01$). Diastolic blood pressure decreased from 85.8+4.9 to 81.9+3.0 mmHg ($p<0.05$). Other parameters were not significantly influenced by isradipin treatment. No serious clinical or metabolic side effects were observed. We therefore conclude that isradipin may be useful in regression of nephropathy in normotensive patients with NIDDM.

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INSULIN-LIKE GROWTH FACTOR I PREVENTS PROGRAMMED CELL DEATH IN NEURONS.

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We have shown that cultured neurons undergo programmed cell death (PCD) when exposed to high glucose. Insulin-like growth factor-I (IGF-I) can prevent these changes. We are now examining the consequences of IGF-I treatment on the threshold regulators of PCD. The cysteine kinases Yama (CPP 32β) and ICE/LAP3, and the *bcl* family member *bax* propagate PCD, while *bcl*-2 and *bcl*-X_L inhibit PCD. Employing SH-SY5Y human neuroblastoma cells, we find that IGF-I rescues cells from apoptosis induced by hyperglycemia. Neuroblastoma cells were cultured in control medium (DMEM) containing 25 mM glucose, or DMEM and excess glucose, or DMEM/high glucose ± 10 nM IGF-I. At selected timepoints, cells were harvested and Western immunoblotting performed for *Yama, bax, bcl*-2, and *bcl*-X_L. Between 6 and 24 h after exposure of neuroblastoma cells to high glucose (20-150 mM excess glucose), *bcl*-2 and *bcl*-X_L protein levels are decreased, and there is increased activation of Yama and ICE/LAP3. No change was observed in the absolute level of *bax*. In the presence of hyperglycemia, IGF-I treatment sustains *bcl*-2 and *bcl*-X_L levels and suppresses the conversion of inactive cysteine kinases to their active forms, thereby blocking PCD. These results help elucidate an important pathway by which IGF-I suppresses the neurotoxic effect of neuronal stressors, and suggest dual IGF-I regulation of *bcl* family members and cysteine kinase propagation. Furthermore, these data suggest that IGF-I may have an important role in the treatment of diabetic neuropathy. These studies were supported by NIH grants K08 NS01938 and R29 NS32843.

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INSULIN-LIKE GROWTH FACTOR-I SIGNALING PATHWAYS IN NEURITE OUTGROWTH.

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We are interested in the potential role of insulin-like growth factor-I (IGF-I) in the treatment of diabetic neuropathy. We have previously shown that IGF-I can enhance lamellipodia formation and neurite initiation. We are now investigating its role in neurite outgrowth. As part of these studies, we are characterizing the signaling pathway that mediates IGF-I's effects on neurite elongation. In our studies, we employ a cell culture model of neuronal growth, the SH-SY5Y human neuroblastoma cells. When SH-SY5Y cells were treated with IGF-I, there was a dose-dependent increase in the tyrosine phosphorylation of the type I IGF receptor (IGF-IR), and 2 MAP kinases: ERK1 and ERK2. While IGF-IR phosphorylation was immediate, maximal phosphorylation of ERK1 and ERK was not reached for 30 min and maintained for up to 4 hr. IGF-IR, ERK1 and ERK2 phosphorylation were inhibited by the IGF-IR blocking antibody, α-IR_3, while ERK1 and ERK2 phosphorylation were inhibited by a compound which specifically blocks MEK activity (PD98059). In parallel, PD98059 blocked neurite outgrowth and decreased the expression of growth cone associated protein 43 (GAP-43). Collectively these studies show that IGF-I induces a unique pattern of MAP kinase phosphorylation in neurons which results in neurite outgrowth, supporting the use of IGF-I in the treatment of diabetic neuropathy. Sponsored by R29 NS32843 and grants from the American Diabetes Association and the Juvenile Diabetes Association.

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INCREASED OXIDATIVE STRESS IN EXPERIMENTAL DIABETIC NEUROPATHY

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Recent reports that anti-oxidants restore nerve function in diabetic rats have implicated increased oxidative stress in the etiology of complications. Evidence for increased oxidative stress and/or reduced endogenous protection at the tissue sites of diabetic lesions, although suggestive, have not been consistently observed. Our aim was to validate a method for quantitating tissue levels of total (Tglut) and reduced glutathione (GSH), to characterize the effects of diabetes on these parameters and to identify possible mechanisms linking changes in glutathione levels to diabetic hyperglycemia. Tglut and GSH levels were quantitated with an enzymatic recycling assay that couples glutathione reduction to formation of the chromophore 2-nitro-5-mercapto-benzoic acid. Sciatic nerve Tglut and GSH levels were not different between 6 and 24-week old male Sprague Dawley rats (11.2±1.5 and 10.0±1.6 vs 9.9±1.2 and 8.9±1.3 nmol/mg protein), indicating that these parameters are unaffected by maturation. Six weeks of untreated diabetes induced in 19-week old rats, however, significantly reduced nerve GSH levels by 42±12% ($p<0.001$). To determine if depletion of GSH may be a consequence of increased competition for NADPH between aldose reductase (AR) and glutathione reductase, nerve glutathione levels were measured in 19-week old non-diabetic rats fed a 50% galactose diet for 6 weeks. In these animals GSH was significantly reduced by 50±17% ($p<0.001$) but was normalized within 4 days with AR inhibitor treatment (ARI-509, 10 mg/kg/day). These data link increased oxidative stress and AR activity to impairment of the glutathione redox cycle which may contributes to the etiology of nerve dysfunction in experimental models of diabetes.

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CYCLOOXYGENASE-DEPENDENT EFFECT OF PENTOXIFYLLINE ON NEUROVASCULAR FUNCTION IN DIABETIC RATS.

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Pentoxifylline can improve blood rheology and tissue perfusion. The aim was to ascertain whether 2 weeks pentoxifylline (40 mg/kg) treatment could correct nerve conduction velocity (NCV) and blood flow deficits in 6 week streptozotocin-diabetic rats. Pentoxifylline is a phosphodiesterase inhibitor and could, therefore, act to potentiate the actions of vasodilator prostanoids that use cAMP as a second messenger: a further aim was to examine whether the effects were blocked by co-treatment with the cyclooxygenase inhibitor flurbiprofen (5 mg/kg). Diabetic deficits of 20.6 ± 0.9% (± SEM; $p < 0.001$) for sciatic motor and 12.1 ± 1.9% ($p < 0.001$) for saphenous sensory NCV were corrected by 56.5 ± 7.6% ($p < 0.001$) and 69.8 ± 9.8% ($p < 0.01$) respectively with pentoxifylline treatment. For motor NCV, the value remained reduced compared to that for a nondiabetic control group ($p < 0.001$). Flurbiprofen co-treatment completely abolished the improvement in saphenous NCV with pentoxifylline and markedly attenuated (52.9 ± 12.6%; $p < 0.05$) the effect on motor NCV. Sciatic nutritive endoneurial blood flow was 48.4 ± 2.2% ($p < 0.001$) reduced by diabetes. This was 50.4 ± 9.1% ($p < 0.001$) attenuated by pentoxifylline, although a 24.0 ± 4.4% ($p < 0.001$) flow deficit remained compared to nondiabetic rats. Flurbiprofen co-treatment largely (62.2 ± 11.2%; $p < 0.01$) abolished the effects of pentoxifylline on blood flow. Thus, pentoxifylline has beneficial vascular effects in experimental diabetic neuropathy which depend at least in part on products of cyclooxygenase-mediated metabolism.

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NA+,K+-ATPASE AND PROTEIN KINASE C ARE ALTERED IN SCIATIC NERVE FROM DIABETIC RATS.

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Experimental diabetic neuropathy, like the human form, is characterised by a decrease in Na^+,K^+-ATPase activity. Treatment of diabetic rats with gangliosides prevents or restores reduced Na^+,K^+-ATPase activity and corrects altered polyphosphoinositide turnover in the sciatic nerve. Phosphoinositide metabolism and Na^+,K^+-ATPase may be linked through a protein kinase C (PKC)-mediated step. We evaluated the effects of *in vivo* GM1 ganglioside (GM1) administration on PKC and Na^+,K^+-ATPase activities in sciatic nerves from control and streptozotocin (STZ)-induced diabetic rats. We also measured GM1 and PKC activators' effects *in vitro* on nerve Na^+,K^+-ATPase. Groups of diabetic and age-matched controls rats were injected daily with either saline or GM1 (10 mg/kg i.p.) for one month, starting two or five months after STZ. GM1 almost completely restored the drop in Na^+,K^+-ATPase in the diabetic sciatic nerves. PKC activity was slightly increased in the membranous fraction from diabetic nerves, while the cytosol fraction was unchanged. GM1 treatment resulted in a further increase in membrane-associated PKC activity, amounting to 20 and 17% respectively at three and six months. When nerve segments from untreated diabetic rats were incubated with PKC activators, homogenized and assayed for Na^+,K^+-ATPase, phorbol esters and 1,2- but not 1,3-dioctanoylglycerol restored the 30% Na^+,K^+-ATPase deficit to normal; GM1 also corrected it in a dose-dependent manner. Our data showed that diabetic nerves have reduced Na^+,K^+-ATPase activity and elevated membrane-associated PKC. GM1, which restores Na^+,K^+-ATPase, further increases PKC in *vivo*. These findings agree with *in vitro* observations that PKC activators and GM1 restored Na^+,K^+-ATPase.

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ISLET TRANSPLANT EFFECT ON DIABETIC NEUROLOGICAL DAMAGE: PRIMARY VERSUS SECONDARY INTERVENTION.

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Neuroelectrophysiological alterations in diabetes indicate progressive nervous function failure. This study was performed to establish whether restoration of euglycemia following islet transplantation immediately after diabetes onset or after an extended period of disease can prevent or reverse neurophysiological abnormalities. Pancreatic islets (1200-1500) were injected into the portal vein of inbred Lewis rats 15 days (group A) or 8 months (group B) after induction of diabetes by streptozotocin. The rats were then followed for a total period of 12 months from streptozotocin injection. Groups of control and diabetic rats were also studied in parallel. Auditory (BAEPs) and somatosensory (SEPs) evoked potentials and metabolic parameters were measured at the beginning and at the end of the study. The metabolic parameters (blood glucose, HbA1 and body weight) in both groups of transplanted animals did not differ from those of the control group but differed significantly from those of diabetic animals ($p<0.001$). BAEP latencies (wave I, II and III) of group A were similar to those of the control rats whereas group B showed significantly altered wave I and II values ($p<0.005$ and $p<0.01$ vs control values respectively). Group A and control rat SEP conduction velocities did not vary, whereas conduction velocities of group B showed only a partial improvement since values were lower than those of control rats but higher than those of diabetic rats (significant at T5-cortex level: $p<0.05$). These results show that precocious normalisation of metabolic control by islet transplantation prevents central and peripheral neurological alterations. However, after an extended period of hyperglycemia, the beneficial effect is only partial and observable mainly at peripheral nervous system level.

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INHIBITION OF PHOSPHOINOSITIDE SYNTHESIS BY GLUCOSE IN RETINAL PIGMENT EPITHELIAL CELLS TRANSFECTED WITH ALDOSE REDUCTASE

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Constitutive expression of high aldose reductase (AR) in retinal pigment epithelial (RPE) cells leads to glucose (Glu)-induced inhibition of sodium-dependent myo-inositol (MI) transport (SMIT) activity, MI-content and phosphoinositide (PI) synthesis. The *aim of the study* was to examine the effect of Glu on these parameters in low-AR expressing RPE-47, and in RPE-75 cells derived by stable transfection of the RPE-47 with AR-gene. SMIT activity was determined by measurement of [2-^{3}H] MI (1 μM)-uptake in Krebs buffer (5 mM Glu), and PI-synthesis by quantifying the PI-synthase substrate CDP-diglyceride (CDP-DG) by labeling with ^{3}H-cytidine followed by thin layer chromatography, and by liquid scintillation spectrometry. MI-content was determined by gas-liquid-chromatography. In RPE-47, Glu (30 mM) modestly inhibited SMIT activity in a time-dependent fashion, with maximum inhibition (90.2±2.7% control) reached at 12-16 h, and persisted for 5 d. The Glu inhibition was significantly higher in RPE-75 (73.6±0.8%* control at 48 h) (*$p<0.05$ vs. RPE-47). Increasing the osmolality of the MI-uptake buffer by sucrose increased SMIT activity in a dose-dependent fashion, and reversed the inhibition by Glu. In RPE-75 incubated with 20 mM Glu for 1, 2, 4 and 7 d, the average sorbitol and MI contents were respectively 1129.8±210.5% and 72.3±8.7% of that present in RPE-47. During a 72 h incubation with 15, 20, 30, and 50 mM Glu, the CDP-DG contents in RPE-47 were respectively 110.07±5.33%, 101.10±4.27%, 114.98±3.03% and 110.13±6.84% control, and in RPE-75 cells, 108.26±4.17%, 137.98±4.18%*, 135.47±3.35%* and 148.36±2.92%* control. These studies support the notion that Glu-induced MI-depletion and inhibition of PI-synthesis previously identified in certain RPE cells is consequent to their high constitutive expression of AR. The RPE-75 and RPE-47 cells may serve as suitable models for exploring the AR-related and -unrelated components of glucose-induced pathways leading to diabetic complication.

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THE EFFECTS OF SULINDAC ON PERIPHERAL NERVE MORPHOLOGY IN THE CHEMICALLY INDUCED DIABETIC DOG.

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Diabetes was induced in male beagle dogs at the age of 4 months with a mixture of Alloxan and Streptozotocin. Thereafter moderate diabetes control (blood sugar 13-20mmol/l) was achieved by once daily Monotard insulin injections. 3 groups of 3 animals were studied: Control (C), Diabetic (D), Diabetic on Sulindac (prostaglandin synthesis inhibitor and in vitro Aldose Reductase Inhibitor) (DS) for 4 years. HbA1c (%) prior to biopsy was C-3.0 ± 0.6, D- 7.4 ± 0.9, DS- 7.3 ± 0.9. The Sciatic nerve was biopsied from each animal and prepared for detailed light and electron microscopy. Myelinated fibre density (no/mm^2) was not significantly different in D(4746(4414-7107)) compared with C(6354 (5951-6607)) nor did it change with treatment in DS(5760(5650-7265)). Regenerative cluster density was however marginally significantly increased ($p<0.08$) in DS (95(75-102)) v D (19 (11-50)) and C (63 (35-67)). Paradoxically, myelinated fibre and axon areas (47(42.9-66.3), 17(12-22.9)) were increased in D ($P<0.08$) compared to C (39.1 (34.2-42.5), 13.7(11.3-15.8)) without any effect of treatment in DS (47(42.9-66.3), 12.8 (9-15.2)). Unmyelinated fibre density (no.mm^{2}x10^3) failed to differ between C (148(121-155)), D (143(122-157)) and DS (139(115-153)). Axon diameter (μm) also failed to differ between C (0.65(0.58-0.65)), D (0.79 (0.69-0.82)) and DS (0.68 (0.62-0.86)). Endoneurial capillary parameters did not differ between any group for: Capillary density (no.mm^2)(C-52 (47-56), D- 58(50-81), DS- 53 (43-56)), Basement membrane area (μm^2)(C-13.8 (12.2-16.2), D-14.7 (13.6-21.9), DS- 17.2 (15.5-20.5)), Luminal area (μm^2)(C-9.5(8.7-11.6), D- 9.1 (8.6-14.5), 6.0(5.8-10)) and Endothelial cell profile no. (C-3.9(3.3-4.1), D-3.8(3.4-4.4), DS (3.8 (3.7-4.3)). The diabetic dog demonstrates only minor structural abnormalities in the peripheral nerve consistent with findings in other fully mature animals models with moderately controlled diabetes. Sulindac fails to ameliorate any of these abnormalities.

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ASSOCIATION OF ATP1A1 GENE POLYMORPHISM AND Na/K ATPase ACTIVITY, ISOFORMS EXPRESSION AND DIABETIC NEUROPATHY.

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The aim of this study was to characterize subunits expression and red blood cell activity of Na/K ATPase, according to ATP1A1 gene polymorphism which encodes for α1 isoform. In IDDM patients, the red blood cell Na/K ATPase activity was decreased compared to controls and more in patients with neuropathy. There was a positive correlation between red blood cell Na/K ATPase activity and nerve conduction velocity in diabetic rats and humans. A RFLP with Bgl II enzyme was described in first intron of ATP1A1 gene and was associated with diabetic neuropathy (relative risk 7.7%). In a serie of 100 IDDM patients with or without neuropathy and 100 controls, the restricted allele frequency was 0.10 and 0.09, respectively. Red blood cell Na/K ATPase activity was significantly decreased in IDDM patients with restricted allele compared to patients with unrestricted allele (237±11 vs 313±9) whereas there was no change in controls (401±12 vs 395±9). To understand the correlation between ATP1A1 gene polymorphism and red blood cell Na/K ATPase activity, we studied isoform expression in this tissue. Only α1 isoform, predominant in nervous tissue, is expressed on red cell membrane. There was a positive correlation between α1 expression and Na/K ATPase activity in IDDM patients and controls. No significant difference of α1 expression was observed between IDDM patients with or without restricted allele for a same activity. In all subjects with restricted allele, we found α1 ß1 dimers on red blood cell membrane but also ß1 subunit alone compared to subjects with unrestricted allele. These results showed an abnormality of α1 isoform in diabetic patients with restricted allele which might be due either to protein expression or degradation or binding to membrane.

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BENEFICIAL EFFECTS OF ACETYLSALICYLIC ACID TREATMENT ON CENTRAL AND PERIPHERAL NEURAL RESPONSES IN DIABETIC RATS

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The effects of acetylsalicylic acid (ASA) on nerve conduction velocity (NCV), somatosensorial evoked potentials (SEPs) and neural levels of malondialdehyde (MDA), a product of lipid peroxidation, were studied in streptozotocin-diabetic rats. ASA (100 mg/kg, in normal rat chow) was given to diabetic rats after the induction of diabetes for 8 weeks. NCV was measured from caudal nerve and SEPs were measured by stimulating via caudal nerve and recording via cortex at the 4th and 8th week during the treatment. Diabetes caused 20-30% deficits in NCV and SEPs slightly but not significantly at the 4th week. However, ASA treatment had a significant restoring effect at NCV and SEPs at the 8th week ($p<0.05$ vs.untreated diabetic rats, respectively). Diabetes caused elevation in neural MDA levels ($p<0.05$ vs. non-diabetic group), which was prevented by ASA ($p<0.05$ vs. untreated diabetic rats). Weight and the glucose levels were not influenced by ASA treatment. Our results suggest that the beneficial effects of ASA on diabetic neuropathy are not associated with the regulation of glycemia, but these effects may be related with prevention of lipid peroxidation.

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ARI EFFECT ON INTERLEUKIN 6 EXPRESSION AND NERVE FIBER REGENERATION.

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At the scene of nerve fiber damage, acute action of citokines, an inflammatory mediator, may have an important role to elicit the nerve fiber regeneration . To test this hypothesis, IL-6 synthesis was quantitated in axotomized sciatic nerve using bio-assay technique in streptozocin (STZ) induced diabetic rats. Furthermore, we performed RT-PCR to evaluate mRNA-IL-6 levels , confirming IL-6 synthesis in axotomized nerves. The IL-6 levels elevated from right after axotomy , was maximum at 3 days after in all three groups (normo-glycemic, hyper-glycemic and hyper-glycemic with ARI(WP-921, Wakamoto and Toyama Chemical Co. Ltd., Japan) treatment, which were 10.4+0.9 pg/mg/ww, 3.9+0.9 and 7.7+1.8 respectively. RT-PCR demonstrated IL- 6 mRNA expression in sciatic nerve tissue in all conditions but pronounced significantly less in hyperglycemia without ARI treatment. Immunocytochemicaly, ED2 positive cells (macropharges) were appeared at axotomized stump. These cells also showed positive staining with IL-6 antibody. The fequency of appearance of macropharges seemed less in hyper-glycemic condition. Myelinated fiber regeneration was quantitated using light-microscopic morphometriy. At 35 days after axotomy, regenerated fiber density was significantly reduced in hyper-glycemic condition to 32% normo-glycemia. However, fiber density was preserved to 78% normal with ARI treatment. We concluded that ARI may exert its beneficial effect on nerve fiber regeneration via activation of the IL-6 synthesis consequent on impaired recruitment of macropharges.

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EFFECT OF HYPERGLYCEMIA ON MITOCHONDRIAL MEMBRANE POTENTIAL OF NEURONS: LASER CONFOCAL STUDY

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Hyperglycemia and decline in contents of some neuropeptides in primary afferent neurons have been reported to be associated with the development of diabetic neuropathy. To elucidate the implications of hyperglycemia for nerve dysfunction and the associated changes in energy metabolism, we measured the mitochondria potential using laser confocal cytometric technique (ACAS 570, Meridian, USA) in single nerve cell body freshly isolated from newborn rat dorsal root ganglion (DRG) and loaded with rhodamine 123. Totally 40 small (average of 17.15±0.89μm in diameter) DRG neurons were observed in the study. The fluorescence distributed mainly around the edges of the cell , in particular in the two opposite poles of the cells.However, fluorescence intensities in the nucleus area was minimal. In some of the tested cells, the fluorescence declined gradually, in particular, when frequently shined by laser beam. In average, the fluorescence declined by the rate of 10% every 400 sec. When 30 mmol/L glucose was added into the medium of the cells, the fluorescence of the mitochondria was rapidly decreased in the majority of the cells (8/9) by 21.2±5.44-22.8±4.72 during 100-400 seconds after the addition, which returned towards the control line then, but seldom returned to the control value (the value measured before the administration of glucose). When the artificial cerebral spinal fluid (aCSF) (in volume of 20μl , same as the glucose solution) was added to the medium of the cells, no changes in the fluorescence was observed (n=7). The changes in the fluorescence was significantly difference ($P<0.05$, ANOVA) between that measured after addition of glucose and aCSF. The finding indicate that the energy metabolism in the small sensory neuron might be impaired by acute hyperglycemia.

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A NOVEL, HIGH SELECTIVE ALDOSE REDUCTASE (AR) INHIBITOR, NZ-314, AND ITS THERAPEUTIC MEANING.

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To clalify the beneficial role of aldose reductase inhibitors (ARIs) in prevention of diabetic complications, we investigated the *in vitro* and *in vivo* effects of a new and highly potent ARI, 3-(3-nitrobenzyl)-2,4,5-trioxoimidazolidine-1-acetic acid (NZ-314). NZ-314 was found to possess uncompetitive and reversible inhibition *in vitro* on partially purified AR from rat lens (IC50 = 6.0×10^{-8} mol/L), from rat sciatic nerve (IC50 = 5.2×10^{-8} mol/L) and recombinant human muscle AR (IC50 = 9.6×10^{-8} mol/L) with DL-glyceraldehyde as substrate, and had remarkable improvements of motor nerve conduction velocity and sorbitol accumulation in streptozotocin (STZ)-induced diabetic rats. On the other hand, NZ-314 showed less inhibitory activity to rat kidney aldehyde reductase (ALR), which is implicated in the aldo-keto reductase superfamily as well as AR and the most important physiological role of this enzyme is metabolism or detoxication of aldehyde compounds, especially, 3-deoxyglucosone (3DG). 3DG is a highly reactive intermediate of Maillard reaction and having an important role in advanced glycation end products (AGEs) production and cytotoxicity against various type of cells. 10^{-5} mol/L of NZ-314 has no inhibitory activity on ALR with 3DG as substrate and no effects on LDH leakage from primarily cultured rat hepatocyte by the addition of 3DG, but other AR inhibitors have a strong inhibition on this detoxification of 3DG at the same doses, resulting in enhance the cytotoxicity of 3DG. These results suggest that the highly specific AR inhibitor NZ-314 will be a useful therapeutic agent for preventing and improving some diabetic complications without unsuitable side effects, and therefore, can be discriminated from other AR inhibitors.

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DIGITAL ELECTRON MICROSCOPIC ANALYSIS OF REGENERATING AXON CLUSTERS IN SURAL NERVE BIOPSIES.

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Diabetic neuropathy is characterized by distinct morphological features including thickening of basement membranes, axon degeneration and regeneration. Axon sprouting leads to an increased number of regenerative axon clusters; however, as the disease progresses there is an overall loss of axons. We have employed the most current digital imaging techniques in order to examine the number of regenerating axon clusters in whole nerve fascicles. Human sural nerve biopsies were assessed for EM suitability based on the degree of fixation, absence of mechanical artifacts and size (100,000 - 425,000 μm^2). Based on these criteria, the largest fascicle was selected. Thin sections were collected onto formvar coated slot grids and coated with a layer of carbon. The sections were then viewed on a Phillips CM100 transmission electron microscope equipped with a CompuStage. Digital images were captured with a Kodak digital Megaplus 1.6 camera and stored as individual tiff files. A montage of the entire fascicle was constructed and the resulting virtual image was viewed by trained EM readers. Computer assisted analysis included confirming the identification of individual axons and labeling regenerating axon clusters. A regenerating cluster was defined as two or more myelinated axons within a continuous basement membrane. Quality control was maintained by randomly assigning a sample to different readers and comparing the results. The EM readers were consistent in their analysis of the samples. It is now possible to aquire an accurate count of the total number of axons within an entire nerve fascicle and to further classify those axons into discrete populations based on relevant criteria. Supported by Hoffmann-LaRoche, Canada.

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NERVE FUNCTION AND BIOCHEMISTRY IN DIABETIC PATIENTS TREATED WITH ARI-509, A NOVEL ALDOSE REDUCTASE INHIBITOR

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In early clinical studies, treatment effects of aldose reductase (AR) inhibitors may have been limited by the testing of doses that produced incomplete inhibition of AR in the tissue sites of lesions. To assess its therapeutic potential, ARI-509, a novel inhibitor that has been effective in experimental models of diabetes, was tested in a double blind placebo controlled study with 50 IDDM and NIDDM patients. Placebo or drug at 3 or 20 mg/day was administered for 15 to 17 days. Carbohydrates were quantitated by gas chromatography in RBC samples collected immediately prior to drug administration on alternate days and in sural nerve biopsies collected 24 h after the last drug treatment. Bilateral sural nerve conduction velocity (NCV) was assessed at baseline and on the day preceding the nerve biopsy. Mean fasting plasma glucose, HbA_{1c} and RBC sorbitol levels were not different between patient groups on the first day of the study. On the day of nerve biopsy, RBC sorbitol levels in the ARI-509 low and high dose groups were 70 and 80% lower than those in the placebo group; nerve sorbitol and fructose values were significantly reduced ($p<0.01$) by 87 and 89%, respectively, in the high dose group and by 84 and 74% in the low dose group. NCV significantly improved from baseline by 2.8±0.7 m/s ($p<0.05$) in patients receiving the 20 mg,dose but was unchanged in the other groups. The lack of effect on NCV with the low dose may have been a consequence of the slow accumulation of ARI-509 in tissues. With the 3 mg dose, nerve drug levels on day 16 were 6-fold lower than with the 20 mg dose. These results demonstrate the high potency of ARI-509 in man and suggest that both the level and duration of AR inhibition are important for improvement of nerve function in diabetic patients.

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REPRODUCTION OF HUMAN SYMPATHETIC LEFT VENTRICULAR REGIONAL DENERVATION IN THE STREPTOZOTOCIN-DIABETIC RAT

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Diabetic autonomic neuropathy (DAN) is invoked as a cause of unexplained cardiac death. Our studies in patients with severe DAN using positron emission tomography and the sympathetic neurotransmitter analogue C-11 hydroxyephedrine (HED) have demonstrated left ventricular (LV) sympathetic dysinnervation with proximal hyperinnervation and impaired myocardial blood flow contrasting to distal denervation. This combination could result in potentially life-threatening myocardial electrical instability. Although the streptozotocin-diabetic (STZ-D) rat is extensively utilized as a model of diabetic peripheral neuropathy, no studies have explored its utility as a model for the heterogeneous cardiac denervation observed in man. Therefore the aim of this study was to use HED to determine the time-dependent changes in LV sympathetic innervation in nondiabetic (ND) and STZ-D rats. Changes in regional LV HED retention were assessed after 6 and 9 months. Neuronal heart imaging was performed using a bolus of 200 µCi C-11 HED injected into the femoral vein under halothane anaesthesia. After 30 min the heart was rapidly removed and the LV divided into proximal and distal sections and radioactivity was measured in each segment and corrected for decay. Results are expressed as % kg dose/g tissue/kg body weight.

Group	6 months		9 months	
	Proximal	Distal	Proximal	Distal
ND (n=4)	1.01±0.04	0.96±0.06	1.04±0.08	0.9±0.11
D (n=4)	0.92±0.02	0.64±0.02*†	0.6±0.03*	0.52±0.03*

*p<0.05 vs corresponding ND segment, †p<0.05 vs proximal segment.

In ND rats no difference in HED retention was detected in the proximal vs distal myocardium. After 6 months, compared to ND rats, myocardial HED retention had declined in the proximal segments of D rats by only 9% compared to a 33% ($p<0.05$) decrease in the distal myocardium. By 9 months, HED retention had declined in both the proximal and distal myocardial segments of the D rats by 42% ($p<0.01$) compared to the same segments in the ND rats. Therefore after 6 months of D, the pattern of sympathetic denervation of the LV partially reproduced the pattern observed in man although hyperinnervation was not observed. By 9 months, the denervation has progressed to involve the proximal myocardium. The STZ-D rat myocardium is a model to study the pathophysiology of regional myocardial sympathetic dysinnervation and to assess the efficacy of therapeutic interventions.

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NERVE TAURINE REPLACEMENT ATTENUATES OXIDATIVE STRESS, NERVE BLOOD FLOW AND CONDUCTION DEFICITS IN STREPTOZOTOCIN-DIABETIC RATS.

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Increased oxidative stress has been invoked in the pathogenesis of experimental diabetic neuropathy (EDN). Activation of the adenine nucleotide-linked aldose reductase (AR) pathway (generating sorbitol and fructose from glucose) may exacerbate oxidative stress in part by depleting the ß-amino acid and endogenous antioxidant, taurine. We have previously demonstrated AR-mediated taurine depletion in the sciatic nerve of the streptozotocin-diabetic (STZ-D) rat and that a 5% taurine diet which overcorrects the nerve taurine deficit and exacerbates nerve myo-inositol depletion, does not ameliorate nerve conduction velocity (NCV) slowing. The effects of selective nerve taurine replacement in EDN are unknown. We therefore evaluated the dose dependent effects of 1, 3 and 5% dietary taurine supplementation in order to explore the significance of nerve taurine depletion in EDN. Sciatic motor NCV, composite nerve blood flow (NBF) (by laser Doppler flowmetry), sciatic nerve taurine content (by HPLC) and nerve reduced glutathione (GSH) content (by fluorescence) were determined after 2 wks in nondiabetic (ND) and diabetic (D) animals and D rats given rat chow supplemented with 1% (D+1%), 3% (D+3%) or 5% (D+5%) taurine. All groups N≥6

Group	Taurine	GSH	MNCV (m/s)	Mean BP (mmHg)	Vascular Conductance (Flow/BP)
ND	3.8±0.3	0.36±0.05	57±6	125±20	1.6±0.1
D	2.7±0.4*	0.15±0.02*	44±7*	118±12	1.1±0.2*
D+1%	4.2±0.3†	0.26±0.01*†	54±8†	99±18*	1.3±0.1*†
D+3%	5.4±0.9*†	0.30±0.00†	50±6	110 ±14	1.0±0.1*
D+5%	5.7±1.7*†	0.31±0.02†	49±6	111± 6	1.1±0.1*

Nerve taurine measured as ng/mg wet wt, and nerve GSH as µg/mg wet wt.
*p<0.05 vs ND, †p<0.05 vs D.

Nerve taurine was decreased in D, replaced in D+1%, but over corrected in D+3% and D+5% rats. GSH was decreased in D, increased in D+1% and corrected in D+3% and D+5% rats. MNCV and vascular conductance was decreased in D, partially corrected in D+1% rats but uncorrected in D+3% and D+5% rats. In D+1% rats mean BP was decreased. In D rats, nerve taurine depletion may contribute to NCV slowing and NBF deficits by exacerbating oxidative stress. Correction of NCV slowing in EDN however is not an inevitable consequence of amelioration of oxidative stress.

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PERIPHERAL NEUROPATHY IN VACOR-INDUCED DIABETIC RAT

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Vacor-posioned patients had complained of various neurological symptoms such as burning sensation of foot, urinary retention, areflexia, and orthostatic hypotension. In animal studies, various neruotoxicities of Vacor were also reported in acute toxic phase, however, the cause of neurotoxicity is not well known. We performed electrophysiological studies including measurements of sciatic motor nerve action potential and conduction velocity in Vacor-treated Wistar rats (single oral dose of 10 or 100mg/kg), and ultrastructural studies of neuromuscular junction within the interosseous muscles of the hind foot of Vacor-treated rat (single oral dose of 80mg/kg). The results were as follows. Vacor, one week after a 10 mg/kg and 6 hours after 100 mg/kg, decreased the amplitude the compound muscle action potential with unchanged conduction velocity. Electron microscopic studies showed remarkable loss of presynaptic vesicles and swollen endoplasmic reticulum in axon terminal at 3 days after Vacor treatment. Progressive degenerative changes consisting of marked loss of presynaptic vesicles, focal disruption of membrane in the axon terminal with disappearance of the number of the damaged axon terminal appeared, and flattening of postsynaptic folds was also seen. These results suggest that degenerative changes in axon terminal at neuromuscular junction may contribute to the peripheral neuropathy developed in the early phase of Vacor poisoning.

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PREVENTIVE EFFECT OF CS-045 ON PERIPHERAL NEUROPATHY IN STREPTOZOTOCIN-INDUCED DIABETES RATS WITHOUT DECREASING BLOOD GLUCOSE LEVELS

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We previously reported that N-acetylcysteine and pentoxifylline, free-radical scavengers and inhibitors of tumor necrosis factor-α (TNF) production, significantly inhibited development of peripheral neuropathy in streptozotocin (STZ)-induced diabetes rats. In this study, we examined effect of CS-045 (troglitazone) on serum TNF activity, serum lipoperoxide and motor nerve conduction velocity (MNCV) of the tibial nerve in STZ-induced diabetic rats, because CS-045 is thought to be a free-radical scavenger. Twelve-wk-old male Wistar/Slc rats were divided into the three groups, nondiabetic control (nonDM), diabetic non-treated (DM) and diabetic CS-045-treated (DM-CS-045) and used for a 24 week experiment. Rats were considered to be diabetic when the non-fasting blood glucose levels were more than 16.8 mM (300 mg/dl) at 24 hrs after STZ injection (30 mg/kg). CS-045 (provided by Sankyo Co., Japan) was mixed with regular powder chow at a final concentration of 0.125 or 0.5 % and was given ad libitum to the rats. There is no significant difference between the blood glucose levels of DM and DM-CS-045 rats throughout the experiment. LPS-induced serum TNF activity and serum lipoperoxide levels were significantly (P<0.01) increased in DM rats compared to nonDM rats, whereas the TNF and lipoperoxide were significantly (P<0.05) suppressed in DM-CS-045 rats. At the 16th and 20th week, MNCV were significantly (P<0.01) decreased in DM rats compared to nonDM rats. However, the decreases of MNCV in DM rats were significantly (P<0.05) inhibited in DM-CS-045 rats. In conclusion, CS-045 has a preventive effect on peripheral neuropathy in STZ-induced diabetic rats regardless their blood glucose levels.

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LIPID PEROXIDES IN TYPE 2 DIABETIC PATIENTS WITH NEUROPATHY

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Diabetes and its associated metabolic changes in peripheral nerves combine to cause a decrease of nitric oxide production and diminished nerve blood flow. Since lipid peroxides are thought to be formed by free radicals and may play an important role in the development of vascular disease, we have investigated the possible relationship between lipid peroxides (measured as thiobarbituric acid reacting species (TBARS)) in NIDDM patients with peripheral neuropathy. Seventy-seven NIDDM patients (39 neuropathic and 38 non-neuropathic) and 36 control subjects were studied. The neuropathy study group had significantly lower levels of TBARS, 3.5 µmol/l (2.2-5.6, 95% confidence limits) compared to controls 4.5 µmol/l (3.08-6.8), $p<0.001$ and to diabetics without neuropathy 4.9 µmol/l (3.09-8.05), $p<0.001$. No differences was found in metabolic control between the two diabetic groups. In the neuropathy group there was a negative correlation between the score for nerve dysfunction with the TBARS levels ($r=-0.42$, $p<0.01$). We conclude that in patients with diabetic neuropathy there are abnormalities of TBARS levels. Thus providing further support for the role of ischaemia in the pathogenesis of neuropathy.

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AN EXPERIMENTAL STUDY OF THE THERAPEUTIC EFFECTS OF THE NERVE GROWTH FACTOR ON DIABETIC NEUROPATHY IN RATS

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Objective: To study the function and morphology of nerve in diabetic rats and the therapeutic effects of NGF on it . Methods: Male wistar rats weighting 170-200g were randomly divided into three groups: G-NC; G-DM and G-NGF. The diabetes was induced in late two groups by a single dose (75mg/kg) of Streptozotocin i.p. After inducing the diabetes for two months, the NGF was administed with 200ug/kg/day im. for 1 month. Items of Observation and Results· 1 The function of nerve: 1.1. The thermal pain thresholds by tailfick testing: The thermal pain threshold value in the G-NGF was significantly decrease comparing with its before therapy and the G-DM. P<0.001. 1 2. The ability of learning and memory by water maze testing: The time that completed whole swimming journey and the number of error reaction in the G-DM and the G-NGF were similar, but much higher than that in the G-NC P<0.001 1 3. The sciatic nerve conduction velocity. In the motor nerve conduction velocity, the G-DM and the G-NGF appeared similar , but obviously slower than that in the G-NC. P<0.01. While the sensory nerve conduction velocity in the G-NGF was slower than that in the G-NC, but faster than in the G-DM. P<0.01. 2. The morphology of the nerve tissue: 2.1 The count of hippocampal pyramidal cell in the G-DM and the G-NGF were similar, but were less than that in the G-NC. P<0 01 2 2 The density of the myelinated nerve fiber axons of sural nerve:in the G-NGF was lower than that in the G-NC, but higher than in the G-DM. P<0.01 3. The substance P (SP) and neurofilament (NF) in dorsal root ganglia by the immunohistochemistry: 3.1 The positive reaction of SP in the G-DM was lower than in the G-NC, and the G-NGF was higher than that in the G-NC. 3.2 The positive reaction of NF in the G-NGF was lower than that in the G-NC , but was higher than in the G-DM Conclution: This study showed that NGF had the therapeutic effects on the rats with the diabetic neuropathy of sensory nerve.

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INVOLVEMENT OF CENTRAL NERVOUS SYSTEM IN PATIENTS WITH DIABETES MELLITUS

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The data regarding the disturbances of central nervous system (CNS) in patients with diabetes mellitus are controversial. We investigated time latencies of P_3, N_2, N_3 peaks of visual evoked potentials (VEP) and quantitative parameters of electroencephalogram (EEG) in 89 patients with diabetes aged 17-75 years (mean age 43.3 years, mean duration of disease 13.6 years) and 20 age-matched controls consequently divided to three differently aged cohorts (18-39, 40-59 and 60-75 years) without any overt symptoms of CNS impairment. We found that peak latencies of VEP were significantly prolonged in all cohorts of differently aged patients with diabetes compared to age-matched controls and these differences were the most pronounced in the youngest group (aged 18-39 years). VEP latencies significantly positively correlated with the age of patients, duration of disease, and severity of diabetic retinopathy. Those with diabetes had an increased EEG delta rhythm intensity and decreased intensity of alpha rhythm compared to controls. There were no correlations between VEP and EEG parameters. Thus, revealed abnormalities may reflect CNS impairment in patients with diabetes.

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INSULIN-LIKE GROWTH FACTOR-I (IGF-I) PROMOTES THE MYELINATION OF SENSORY NEURONS.

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IGF-I is an important neuronal mitogen and promotes oligodendrocyte directed myelination in the brain, but the effect of IGF-I on peripheral nervous system (PNS) myelination has not been previously established. E15 dissociated rat dorsal root ganglion (DRG) neurons were cultured in serum free defined medium (SFDM) containing selenium, transferrin, hydrocortisone, β estradiol, putrescine, vitamin E, defined lipids, Neurobasal Medium (Gibco), glutamate, ascorbic acid, glucose, and 10 ng/ml 2.5S NGF. Cultures free of endogenous Schwann cells were obtained by preplating and using 30 μM FUDR/uridine X 6 days. After washing, secondary Schwann cells (SSC) labeled with 10 μM immunofluorescent DiI were added to the neurons. The following culture conditions were used: 10% calf serum, SFDM ± 1-10 nM IGF-I, SFDM/ forskolin ± IGF-I. Changes in the Schwann cells were serially followed and photographed using a rhodamine cube, over 21 days. The cultures were then fixed and stained with 2% osmium and Sudan black and the number of myelinated fibers /square μm measured using a random order grid measuring system. After 24 h, the labeled SSC differentiated and extended along the axons in cultures containing serum or IGF-I, but not with NGF alone, or forskolin. SSC and axons survived well in all conditions except in the presence of forskolin. IGF-I promoted myelination of the DRG, although this was slightly reduced compared to serum treated neurons. Myelination did not occur in DRG cultures treated with SFMM alone, or SFMM/cAMP. Neuronal death was increased in forskolin treated neurons after 1 week in culture. These results imply that 1) IGF-I promotes differentiation of Schwann cells, development of basement membrane, and myelination of sensory neurons in the PNS 2) IGF-I promotes myelination independent of its effect on neuronal and Schwann cell survival, and 3) Additional permissive factors present in serum are required to induce optimum myelin production. Supported by KO8 NS01938, R29 NS32843 and grants from the American Diabetes Association and the Juvenile Diabetes Foundation International.

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Neuropathy – Clinical Picture and Treatment

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CONDUCTION IN CORTICOSPINAL TRACT IN DIABETES MELLITUS.

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The aim of the study was to evaluate the function of pyramidal tract in group of patients with long lasting diabetes mellitus associated with late complications (polyneuropathy, nephropathy, retinopathy). Conduction time was examined using magnetic stimulation of the motor cortex in 20 patient with diabetes (6 women, 14 men, duration of diabetes from 6 to 40 yrs, mean 25.1±9 (SD) and in 10 healthy control subjects. Motor responses from contralateral hypothenar abductor digti quinti (ADV) and tibialis anterior (TA) muscles were recorded and central motor conduction time (CMCT) determined by using F-wave method. Mean CMCT time for TA was prolonged to 14 ms in diabetic patients as compared with 12.6 ms in controls, $p<0.05$. In the subgroup of patients with erectile impotence (11 cases) the difference in CMCT was more marked 14.8 ms, $p=0.02$, as well as the peripheral conduction time for both TA and ADV, $p<0.01$. There was no significant correlation of CMCT with duration of diabetes. These results show impairment of conduction in corticospinal tract in diabetes and suggest that erectile dysfunction in diabetes may have central, not only peripheral origin.

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SUBCLINICAL NEUROPATHY IN NEWLY DIAGNOSED DIABETES.

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Nerve ischaemia resistance (NIR) is common feature of diabetic neuropathy.

We determined whether NIR was present at the time of diagnosis and the influence of glycaemic control on NIR. 420 diabetic patients (DM) mean age 43.2 SD 6.6 (280 males) who attended the clinic within 2 weeks of diagnosis and 420 age and gender matched controls (C) mean age 44.1 SD 5.7 (280 males).

Vibration perception threshold (VPT) was measured using a neurothsiometer before and after 30 minutes of limb ischaemia at diagnosis. An ischaemic index (II) was calculated (30 minute VPT/ baseline VPT) as a measure of ischaemia resistance. Peroneal motor conduction velocities were determined.

Clinical assessment was performed using a neuropathy symptom score (NSS) and a neuropathy disability score (NDS). Peroneal motor and sensory conduction velocities were assessed.

Mean VPT was higher in DM (16 SD 4.3) than in C (8 SD 3.2), p 0.01 and VPT at 30 minutes was lower (22 SD 4.1 vs 31 SD 7.2, p=0.01). II was lower in DM when compared to C (p= 0.01). II corelated with HBA1C at time of diagnosis (r=-0.60, p 0.003). Mean NDS (1.2 vs 1.4) and NSS (12% vs 14%) were similar in DM and C. Nerve conduction velocities were no different in DM (44m/sec) and C (45.2m sec). We conclude that NIR is present at diagnosis.

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PERIPHERAL NEUROPATHY ALTERS GAIT IN DIABETIC PATIENTS

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The aim of this study was to investigate the effect of peripheral neuropathy on gait in diabetic patients. We studied four groups, each of 20 subjects, matched for age, sex and BMI: normal healthy controls, non- neuropathic controls, diabetic neuropathy and diabetic neuropathy with history of foot ulceration. For gait analysis, the VIFOR system was used. Two recordings were taken, one for each leg, in both sagittal and frontal planes. We analysed: walking speed, stance phase duration, joint angles and moment arms for the ankle, knee and hip joints, the components of the ground reaction force (GRF) vector and the ankle, knee and hip joint moments originating from the GRF vector. Two sample t-test, equal variance, was used for intergroup statistical comparisons. Walking speed was found significantly lower ($p< 0.05$) in the two neuropathy compared with the control groups. Joint angles were different in the final part of stance phase. The maximum values of the vertical and the antero-posterior components of GRF were found to be higher in the two control groups compared to the history of ulcers neuropathy group ($p< 0.01$). The maximum frontal plane ankle joint moment was significantly higher ($p< 0.05$) in the two neuropathy compared to the control groups. These alterations in gait parameters found in the neuropathic diabetic patients may be attributed to a proprioceptive deficit but other factors such as altered musculo-skeletal and soft tissue mechanics seen in longstanding diabetes complicated by neuropathy, may also be implicated. They may have clinical significance since they can increase the risk for minor trauma and ulceration.

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POLYNEUROPATHY INCIDENCE IN PATIENTS WITH IMPAIRED GLUCOSE TOLERANCE

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This study is performed in order to find out the incidence of polyneuropathy in patients with impaired glucose tolerance (IGT) and in order to establish whether there is any relation between polyneuropathy and serum calcium, phosphorus, magnesium, sodium and potassium and corrected QT interval on electrocardiogram. 22 patients aged between 28 and 66 years formed the patient group and 11 subjects with normal glucose tolerance formed the control group. We have measured plasma glucose, blood urea nitrogen, serum transaminases, total, HDL-, LDL- and VLDL-cholesterol, triglycerides, insulin, calcium, phosphorus and magnesium; and calculated corrected QT interval in all cases of the study group. Polyneuropathy is assessed with electromyoneurography. The patient group had significantly higher insulin levels ($p<0.01$). We have found mild sensory polyneuropathy in 11 (50 %), and carpal tunnel syndrome in 2 (9 %) of 22 patients with IGT while only one subject (9 %) in the control group had mild sensory polyneuropathy. This difference is statistically significant. Because of this high incidence we propose that existence of polyneuropathy must be assesed with electromyoneurography in every patient with IGT. Further studies must also be planned concerning the effects of diet and drug therapy on polyneuropathy in patients with IGT.

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EFFECT OF TREATMENT WITH ACETYL-L-CARNITINE ON DIABETIC FOOT ULCERATION IN PATIENTS WITH PERIPHERAL NEUROPATHY: A 1 YEAR PROSPECTIVE MULTI-CENTRE STUDY.

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There was evidence to suggest that acetyl-L-carnitine (ALCAR) treatment improves nerve function in diabetes. The safety and efficacy of ALCAR for preventing foot ulceration in diabetic patients with peripheral neuropathy were evaluated in a double-blind, multi-centre trial. 1035 IDDM and NIDDM patients with vibration perception threshold (VPT) ≥25, ≤50V, normal peripheral circulation and no previous foot ulceration were randomised to receive either a daily oral dose of 1000 mg ALCAR (n=686) or placebo (n=349) for 1 year. The primary efficacy endpoint was incidence of first foot ulcer. Secondary parameters included VPT at the hallux and the Michigan Diabetic Neuropathy (MDN) score (sensory impairment by tuning fork, 10 g monofilament and pin prick; muscle strength; and reflexes). The incidence rate of first foot ulcers at 12 months was 6.9% (n=23) for placebo and 7.4% (n=48) for ALCAR. VPT for ALCAR and placebo were 29V (17-50V; median and range) and 28V (16-49V), respectively, at baseline, and were unchanged at 12 months (28V (5-50V) and 28V (6-50V), respectively (p>0.05)). There were no changes in MDN parameters after 12 months. The results indicate that ALCAR is non-efficacious as a preventative treatment for diabetic foot ulceration. VPT, muscle strength, reflexes and 10g monofilament scores at baseline were all significantly associated with risk of foot ulceration (p<0.01). This suggests that VPT, muscle strength, reflexes and 10g monofilament are useful predictors of future foot ulceration and may be used in clinical practice to identify high risk patients.

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DIABETIC NEUROPATHY: POSSIBLE NEW PATHOGENIC ASSOCIATIONS

J.J. Gagliardino (on behalf of PRAMUDIA), Argentina.

The aim of this work was to estimate the prevalence of diabetic neuropathy (N), diagnosed through the evaluation of pain and vibratory sensitivity, and its possible association with variables such as hypertension (H), dislipidemia, microvascular (retinopathy and nephropathy) or macrovascular (peripheral arteriopathy) lesions, and other clinical and biochemical parameters. For this purpose, 798 diabetic patients (49% male) assisted by 20 diabetologists from different provinces of Argentina, were randomly incorporated to a cross-sectional study using an *ad hoc* common protocol. The average prevalence of N was 35% (range 6 to 64% according to diabetes duration). The univariate analysis showed that N was significantly and positively associated with males, H, coronary disease, dislipidemia, retinopathy, nephropathy, peripheral vasculopathy, and diabetes duration. The factor analysis showed a strong link among N, retinopathy, diabetes duration, nephropathy, peripheral vasculopathy and H. The logistic regression determined the significance of the association of N with: retinopathy (adjusted OR= 4.80; 95% CI= 3.19-7.24); age (OR= 1.02 per year of age; 95% CI= 1.005-1.03), male sex (OR= 1.60; 95% CI=1.11-2.29), nephropathy (OR=2.01; 95% CI=1.16-3.49), peripheral vasculopathy (OR = 2.78; 95% CI = 1.34-5.70), diabetes duration (OR = 1.03 per year; 95% CI= 1.001-1.05), dislipidemia (OR= 1.65; 95% CI= 1.03-2.64), H (OR= 1.67; 95% CI= 1.08-2.56).The relative importance of the variables, determined by standardized coefficient ßx' (SD) was: retinopathy (0.73), age (0.34), vasculopathy (0.29), nephropathy (0.25), sex (0.25), diabetes duration (0.22), dislipidemia (0.19), H (0.18). These results suggest that the vascular component (microangiopathy and macroangiopathy), H and dislipidemia, together with the metabolic component, play a complementary role in the patogenesis of diabetic neuropathy.

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LACK OF EFFECT OF EVENING PRIMROSE OIL ON AUTONOMIC FUNCTION TESTS AFTER 12 MONTHS OF TREATMENT

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Beneficial effects of supplementation of the essential fatty acid gamolenolenic acid (GLA) have been demonstrated in both animal models, and clinically in man. At our centre all subjects enrolled in a 2 year double blind multicentre study in painful neuropathy also underwent an additional set of objective quantitative tests to assess the severity of autonomic and peripheral neuropathy. We present the results of an interim analysis at 12 months of treatment with GLA. Of 51 patients recruited, 26 were randomised to GLA 480mg/day, and 25 to placebo. Age (64.6±7.8 vs 60.5±10.1 years, mean±SD), type (9:17 vs 11:14, IDDM:NIDDM), and duration of diabetes (11.7±12.7 vs 16.2±16.2 years) were similar in each group. Compared to baseline, there were no significant changes in: Vibration perception thresholds at the hallux (GLA: 25.9±8.9 vs 24.5±11.4, placebo: 27.7±12.4 vs 23.9±13.4, normal<15 volts), Valsalva ratio (GLA: 1.79±0.49 vs 1.85±0.56, placebo: 1.61±0.6 vs 1.72±0.58, normal>1.21), RR interval variation on deep breathing (GLA: 16.30±9.35 vs 17.69±12.45, placebo: 14.76±11.16 vs 13.50±9.97, normal>15 beatsmin^{-1}), Heart rate response to standing (30:15 ratio: GLA: 1.19±0.31 vs 1.05±0.04, placebo: 1.13±0.21 vs 1.16±0.30 beatsmin^{-1}, normal>1.04), or degree of postural hypotension. We conclude that at 12 months there are no objective improvements in standard tests of severity of diabetic peripheral and autonomic neuropathy. Treatment with GLA may need to be more prolonged to show benefit, and its clinical significance may be limited.

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NATURAL HISTORY OF PERIPHERAL NEUROPATHY IN RELATIONSHIP TO VIBRATION PERCEPTION THRESHOLDS IN DIABETIC PATIENTS

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We followed up 392 diabetic patients who had a baseline diabetic clinic visit at St. Thomas' Hospital between 1982-85 for a mean period of 12 (10-14) years. All patients were reviewed in 1995. Toe vibration perception thresholds (VPT) were performed at baseline and review visits. VPT measurements are converted by our computer (Diabeta) to a 'standard deviation score' by logarithmic transformation. This corrects voltage measurements for age. We defined established peripheral neuropathy as a toe 'standard deviation score' > 1.96. Seventy-eight patients (20.4%) developed peripheral neuropathy by this criterion over a mean 12 year period, so that the calculated annual incidence is 1.7%. Fifteen patients (3.8%) developed foot ulcers (1.8%) or had an amputation (2%). Mean HbA1 over the 12 years interval was higher in patients with neuropathy (11.0 ± 1.4 vs 10.4 ± 1.4; p=0.01). Mean toe VPT in patients developing neuropathy increased from 14.2 ± 3.7 volts at baseline to 35.9 ± 9.5 volts at review and from 10.1 ± 3.7 volts to 14.2 ± 4.7 volts in the rest of the patients. Neuropathy was positively related to retinopathy at baseline (p=0.02), and to Caucasian ethnicity (p=0.02), male sex (p <0.01) and height (p=0.01).

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REVERSAL OF DEFECTIVE NERVE CONDUCTION WITH VITAMIN E SUPPLEMENTATION IN DIABETICS WITH PERIPHERAL NEUROPATHY

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Recent evidence has suggested that diabetic microangiopathy is associated with increased free radical induced oxidative damage. The present study has examined the effect of vitamin E, the principal modulator of free radical activity, on electrophysiologic parameters in patients with diabetic peripheral sensorimotor polyneuropathy. Two groups of noninsulin dependent diabetic patients with mild to moderate peripheral sensorimotor polyneuropathy, matched for duration of disease and metabolic control, received daily vitamin E supplementation of 900 mg and placebo, respectively, for 6 months. Fasting plasma glucose and hemoglobin A_1 (Hb A_1), postprandial plasma glucose and electrophysiologic parameters in the basal state and after 6 months of treatment were studied. Glycemic indices did not show any significant changes during the study, whereas nerve conduction improved significantly after 6 months in patients on vitamin E supplementation. The changes in the electrophysiologic parameters were obvious in the median and tibial motor nerve fibers. Nerve conduction velocity in the median motor nerve fibers (p=0.0051) and tibial motor nerve distal latancy (p=0.0284) improved significantly after 6 months of vitamin E supplementation. This study shows that defective nerve conduction in diabetics with mild to moderate peripheral neuropathy, can be reversed by pharmacological doses of vitamin E supplementation.

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IS VIBRATION PERCEPTION THRESHOLD MEASUREMENT REPRODUCIBLE UNDER DIFFERENT AMBIENT BLOOD GLUCOSE LEVELS AT DIFFERENT HOURS OF THE DAY ?

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Vibration perception threshold (VPT) measurement by biothesiometer is commonly used in the diagnosis of neuropathy in diabetic patients. The results correlate well with the ones of electromyography. The factors known to affect VPT are age and height. Metabolic control through HbA1c was shown to have no affect on VPT measurement. It is not clear whether instantenous blood glucose levels and the hour of the day the test is done (possibly because of neuropathic edema) influence VPT recordings. To adress this question 62 diabetic patients (26 IDDM, 36 NIDDM) with mean age 48.9±13.21, diabetes duration 14.6 ± 4.27, underwent VPT examination by biotheiometer (Biomedical Instrument Co. Newbury, Ohio, USA) at different hours of the day (mainly fasting and late afternoon) right after blood was drawn for blood glucose testing and a neuropathy symptom score (NSS) questionnaire. VPT was measured at both great toes and each time the mean of three successive measurements is recorded. Statistical analyses were done in SPSS for Windows 5.0.1. using Pearson correlation coefficient, independent samples and paired samples t test where required. 34 patients had neuropathy with basal VPT measurement (>25 mvolt). Both sides of the body correlated well (r:0.90, p< 0.001). VPT significantly correlated with age (r: 0.34, p: 0.012), diabetes duration (r:0.30, p:0.02), NSS (r:0.067, p<0.001). No significant correlations were observed between VPT and HbA1c (r: 0.23, p:0.8) and instantenous blood glucose (r:-0.09, p:0.34). Change in the bloood glucose did not correlate with the change in the VPT (r:0.18, p:0.81). VPT measurements gave similar recordings in the morning and late afternoon (p: 0.35). VPT was not different in IDDM and NIDDM patients (p: 0.22). Patients having severe neuropathy (NSS >9) had significantly higher VPT values (p < 0.001) than the others. In conclusion the results of the present study show that VPT measurement is reproducible under different ambient blood glucose values at different hours of the day which confirms that it is an accurate and a rapid method of detecting diabetic neuropathy.

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FIBER-SPECIFIC EFFECTS OF THE ALDOSE REDUCTASE INHIBITOR ZENARESTAT IN MILD HUMAN DIABETIC POLYNEUROPATHY.

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Slowing of nerve conduction velocity (NCV), a hall-mark of large myelinated nerve fiber (MNF) dysfunction in diabetic polyneuropathy (DPN), is corrected by potent aldose reducatse inhibitors (ARI's), yet ARI effects on small MNF have not been well studied. In a 52-wk randomized, double-blind, placebo-controlled multi-dose clinical trial in 208 subjects with mild DPN, zenarestat (Z) (150 mg, 300 mg and 600 mg BID) dose-dependently lowered sural nerve (SN) sorbitol content 70-85% and improved a composite NCV parameter (p=0.003) ~1.5 m/sec. The diameter and the number of MNF's (per mm^2 cross-sectional area = MNF density(D)) were measured in bilateral SN biopsies obtained at baseline and 52 wk. MNFD (±SD) declined in the placebo (PL) and 150 mg BID groups (-239±197 and -175±147 fibers, respectively) and increased in the 300 and 600 mg BID groups (+168±217 and +181±281 fibers, respectively, p=0.05 adjusted for center and glycosylated hemoglobin). The MNF losses in the PL and 150 mg BID groups and MNF gains in the 300 and 600 mg BID groups were largely attributed to changes in A∂ fibers <5μm in diameter: -202±104, -50±108, +135±158 and +249±167 fibers, respectively, p=0.015. Large MNFD of fibers >5μm in diameter was unchanged in the PL and all Z groups. Thus Z, which potently lowered SN sorbitol, improved large MNF function (NCV) and specifically reversed the loss of small MNF in mild DPN. These effects of Z were equal in magnitude to those deemed clinically meaningful and detectable in epidemiological studies (Russell J.W., Karnes J.L., Dyck P.J. *J Neurol Sci* 1996; 135:114-117).

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FACTORS ASSOCIATED WITH FOOT VIBRATION SENSORY THRESHOLD CHANGE OVER 16 YEARS FROM DIAGNOSIS.

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Deterioration in vibration sensory threshold (VST) is related to glycaemia during the 5 years after diagnosis of diabetes: is this true 16 years afterwards? 72 diabetic patients (42 men) had foot VST assessed biothesiometrically 16 years after as well as at diagnosis aged 46 ± SD19 years as non-insulin- dependent (NID), and on 4 occasions between. After a drop from 23.2+10.0 arbitary units initially to 22.7 ± 9.9 (1 year,NS) there was a steady deterioration to 26.8 ± 11.2 (16 years,p<0.01). The 16-year VST increased with age, male sex, various measures over time of total (Body Mass Index) and central (Scapula:Triceps skinfolds) obesity (all p<0.001), as well as (p<0.05) with lower initial fasting growth hormone (GH) levels, 16 year systolic blood pressure and decreased ankle pulses as clinically detected (the two last were N.S.after controlling for the first 3 factors). Glycaemia was not a correlate, nor was it with the deterioration from diagnosis to 16 years, which was however correlated strongly with initial VST, obesity (p<0.01), age and, after controlling for these, the 16-year ankle pulses (p<0.04). The 0-10 year deterioration, after allowing for the associations with initial VST and age, was correlated with initial history of ethanol intake(p<0.01), while the 10-16 year deterioration was also associated with hepatic factors (also GH levels and systolic blood pressure). Glycaemia was not a significant determinant of VST in the later years after diagnosis of NID diabetes; differential death of the more hyperglycaemic may contribute but progressive neuropathy is possibly a 2-stage process.

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LONG - TERM EFFECT OF TOLRESTAT ON HEART RATE VARIABILITY IN PATIENTS WITH SEVERE OR MODERATE DIABETIC AUTONOMIC NEUROPATHY

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Patients with diabetic autonomic neuropathy (DAN) have an increased cardiovascular mortality rate compared with diabetic patients without DAN. Heart rate variability (HRV) time and frequency domain indices are strong predictors of malignant arrhythmias and sudden cardiac death. This prospective, randomised, double-blind and placebo controlled study analysed the long-term effect of tolrestat, an aldose reductase inhibitor, on HRV time and frequency domain variables in 45 patients with diabetes mellitus (DM) and DAN. They were randomised into tolrestat (n=22) or placebo (n=23). HRV was assessed on a 2-chanel 24-hour electrocardiogram with a solid state digital recorder at months 0,3,6,9, and 12. HRV level of the 45 patients was compared with that of 20 matched patients with DM, of analogous glycemic control, without DAN and 20 healthy controls. Tolrestat, compared with placebo, had a beneficial effect on HRV level in 12/22 patients with moderate DAN. At 12th month it improved significantly ($p<0.01$) all HRV time and frequency domain indices. Despite the beneficial effect of tolrestat, the HRV indices remained less than those of controls. The patients with severe DAN (10/22) had a slight, in some indices significant, benefit. No patient at 12th month showed deterioration of HRV indices with tolrestat as seen with placebo. Tolrestat significantly restores sympathovagal interaction in patients with moderate DAN, 12 months after treatment initiation. This might contribute to the reduction of risk for malignant ventricular arrhythmias. The early detection of DAN is imperative for a successful intervention.

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TISSUE OXYGEN TENSIONS IN SKELETAL MUSCLE OF TYPE-1-DIABETIC PATIENTS DURING A COLD PRESSOR TEST.

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Background: The cold pressor test (CPT), a sympathetic stress test, leads to an increase of blood pressure, muscle sympathetic nerve activity and muscle perfusion as well. Several variables of this test may be used to assess autonomic impairment in diabetic patients. We polarographically measured changes of tissue oxygen tensions in the right anterior tibial muscle during CPT which may reflect changes of muscular microcirculation. Research design and methods: Twenty-eight type-1-diabetic patients, 18 males and 10 females, without obvious diabetic late complications (mean age 27±7 years, height 175±9 cm, BMI 22.7±2.8 kg/m^2, diabetes duration 6.8±5.1 years, HbA1c 7.5±1.9%, x±SD) and 34 normal control subjects matched for age, gender and BMI were investigated at rest and during a CPT of 5 minutes duration. Results: During CPT muscular oxygen tensions increased by more than 3 mmHg in 14 out of 28 diabetic patients and in 13 out of 34 control subjects. Mean increases did not differ between groups. Changes of oxygen tensions correlated neither to age, gender, BMI, systolic and diastolic blood pressure, duration of diabetes nor to HbA1c. The changes did correlate inversely to the respective values obtained at rest (in diabetic patients: r=-0.48, p=0.004, in control subjects: r=-0.60, p=0.001). Conclusion: In diabetic patients and control subjects with elevated muscular tissue oxygen tensions at rest there is no further increase during CPT since presumably the extent of sympathetically mediated vasodilation is limited. Basal values may be influenced by pre-test sympathetic activation which has to be taken into account interpreting test results.

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LONG - TERM EFFECT OF QUINAPRIL ON HEART RATE VARIABILITY IN PATIENTS WITH SEVERE OR MODERATE DIABETIC AUTONOMIC NEUROPATHY

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Patients with diabetic autonomic neuropathy (DAN) exhibit an increased propensity for lethal arrhythmias in comparison to patients with diabetes mellitus (DM) without DAN. This is due to increased sympathetic, reduced vagal activity or both. HRV level is a reliable tool to assess DAN. This prospective, randomised, double-blind and placebo controlled study analysed the long-term effect of quinapril, an angiotensin converting enzyme inhibitor, on HRV time and frequency domain variables in 50 patients with DAN (11 with IDDM and 14 with NIDDM). They were successfully randomised into quinapril (n=25) or placebo (n=25). HRV was assessed on a 2-chanel 24-hour electrocardiogram with a solid state digital recorder at months 0,3,6,9, and 12. HRV level of the 50 patients was compared with that of 20 matched patients with DM, of analogous glycemic control, without DAN and 20 healthy controls. Quinapril, compared with placebo, had a beneficial effect on HRV level in all patients with DAN, regardless of severity. In 13/25 patients with moderate DAN this effect was detected early, at month 3, and gradually increased until month 12. In that group quinapril improved significantly ($p<0.01$) all HRV time and frequency domain indices, mainly the parasympathetic related ones, and reduced heart rate. The patients with severe DAN (12/25) had a significant but less pronounced benefit. No patient at 12th month showed deterioration of HRV indices with quinapril as seen with placebo. Quinapril significantly restores sympathovagal balance in patients with DAN, early after treatment initiation. This might contribute to the reduction of propensity for malignant ventricular arrhythmias.

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Gadolinium Enhancement of Peripheral Nerve and Muscle R1 in Human Diabetic Peripheral Polyneuropathy compared to Healthy Subjects

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The aim of this study was to examine if uptake of contrast(gadolinium enhancement) can be detected and estimated in the sural nerve in diabetic human neuropathy compared to healthy controls evaluated by magnetic resonance imaging. Kinetic models of gadolinium enhancement are used for blood flow estimation. Six type 2 neuropathic diabetic patients mean age 54 years ± 4 and 6 healthy control subjects mean age 54 years ± 8 have been studied. The diabetic patients were all classified as having severe symptomatic symmetric polyneuropathy (San Antonio - criteria). MRI was performed at 1.0 Tesla on a Siemens IMPACT MR scanner. A spin-echo sequense was employed. Power of resolution was 0.23 x 0.23 mm^2. Images of the right lower leg was recorded 15 cm proximal to the lateral malleol in an oblique slice. A oblique slice was placed in a plane allowing the sural nerve to transverse the slice perpendicular to the plane. Intially 7 recordings with variable TR was performed to allow estimation of R1. Subsequently an i.v. bolus of Gadolinium-DTPA (Gd) (0,15 mmol/kg) was given. Finally after 30 min the intial 7 recordings were repeated. The difference in R1 of the muscle before and after Gd - bolus was significantly higher in the neuropathic group, $\Delta R1$ mean 1.43 s^{-1} ± 0.7 compared to the $\Delta R1$ 0.13 s^{-1} ± 0.2 in healthy control group ($p < 0.005$). In contrast the increment in R1 in the sural nerve after Gd-bolus was higher in the healthy control group $\Delta R1$ 1.88 s^{-1} ± 1 compared to the neuropathic group; $\Delta R1$ 0.68 s^{-1} ± 0.7. ($p = 0.055$) .We conclude: 1) Gadolinium enhancement in the sural nerve can be detected and tends to be impaired in diabetic neuropathy compared to controls 2) The soleus muscle in diabetic neuropathy accumulates Gd after bolus injection compared to healthy controlls. Whether the observed differences after Gd-bolus are due to differences in blood flow,volume of distribution or Gd extration from the blood remains to be established.

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ELECTRICAL SPINAL CORD STIMULATION: A NEW AND EFFECTIVE TREATMENT FOR PAINFUL DIABETIC PERIPHERAL NEUROPATHY.

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Conventional treatment for painful peripheral diabetic neuropathy is largely symptomatic and frequently ineffective, with unacceptable side-effects. We tested the effectiveness of electrical spinal cord stimulator (ESCS) for the management of chronic neuropathic pain not responding to conventional treatment, in 10 diabetic subjects (mean age ± SD, 51±9.3 years, mean duration of diabetes 12± 6.3 years, mean duration of neuropathy 5±2.1 years). The electrode was implanted in the thoracic/lumbar epidural space. Immediate pain relief was assessed by visual analogue scale (VAS) after connecting the electrode, in a random order, to a percutaneous ESCS and placebo stimulator. 8 subjects had significant pain relief with ESCS compared to placebo and were converted to the permanent system, with a radio-receiver implanted in the anterior abdominal wall. Significant relief of both background and peak neuropathic pain was achieved at 3 months (VAS, n=7, p=0.016), at 6 months (n=7, p=0.03) and at the end of the study (median -14 months, n=7, background pain p=0.06, peak pain p=0.03). One patient died of unrelated cause 2 months after the start of the study while continuing to benefit from ESCS. There was also an improvement ($p<0.05$) in all the 4 components of the McGill pain questionnaire at the end of the study. Exercise tolerance on treadmill also increased significantly at 3 months (p=0.015) and at 6 months (p=0.0007). Electrophysiological tests and glycaemic control were unchanged. ESCS offers a new and effective way of relieving chronic neuropathic pain and improves exercise tolerance, and should therefore be considered in those not responding to conventional treatment.

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CORRELATION BETWEEN PAIN SENSITIVITY AND HYPERGLYCAEMIA A CLINICOEXPERIMENTAL INVESTIGATION

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The aim of the study was to assess the relationship between blood glucose levels and pain perception in 12 streptozotocin induced diabetic rats and in 16 fresh uncomplicated untreated diabetic patients before and after blood glucose control and in 10 healthy normal controls before and after meals. In rats the mean blood glucose before and after streptozotocin injection and after insulin therapy was 55.16 ± 3.23 mg/dL, 118.66 ± 14.42 mg/dL and 63.75 ± 3.26 mg/dL respectively. The corresponding tail flick latencies (in seconds) were 14.99 ± 0.64, 8.3 ± 0.42 and 12.98 ± 0.50 respectively ($p<0.001$). The corresponding tail clamp latencies were 19.17 ± 1.45, 8.88 ± 0.86 and 17.25 ± 1.32 sec. respectively ($p<0.001$). In diabetic patients the blood glucose before and after controlling diabetes was 323.37 ± 28.99 and 110.93 ± 5.48 mg/dL and corresponding ischemic pain threshold and tolerance was 12.37 ± 0.87, 30.87 ± 3.11 and 22.43 ± 1.08, 46.62 ± 3.01 sec. respectively ($p<0.001$). The blood glucose before and after meals in control was 80.10 ± 3.09 and 153.20 ± 3.06 mg/dL respectively. The corresponding ischemic pain threshold was 34.50 ± 3.16 mg/dL and 21.30 ± 1.94 sec. respectively and tolerance was 5.00 ± 2.68 and 39.20 ± 1.83 sec. respectively ($p<0.001$). It is concluded that both in experimental and clinical studies hyperglycaemia produced hyperalgesia and the latter was reversible with control of the blood glucose.

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DOUBLE BLIND TRIAL OF MEXILETINE ON DIABETIC PAINFUL NEUROPATHY

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Acute painful neuropathy may follow either a period of unstable glycemic control or sudden normalization of hyperglycemia, and is most annoying for patients. Mexiletine (MX) is a common anti-arrhythmic agent which has been found to be useful on painful diabetic neuropathy by Dejgard in 1988. The symptomatic effect of MX was studied in a multi-center trial using 118 patients (male:76, female42, age:33-75) with painful neuropathy by double blind method after obtaining written consent. MX 100 mg tablet or placebo was given p.o., t.i.d. for two weeks. A dose finding study conducted previously has proven MX 300 mg/day to be adequate. The effect was evaluated by a 7 grade-score from "extremely severe" to "none" classified by patients. The effect was considered improved when two or more grade improvement was documented. The ratio of improvement on allodynia and shooting pain of the lower extremities was 18/43(41.9%) at the end of the 1st week while in the placebo group 8/46(17.4%) ($p<0.05$), and at the end of the 2nd week 29/55(52.7%) and 11/56(19.6%)($p<0.001$), respectively concerning numbness of the extremities, no significant effect was observed. The incidence of adverse events was not different in both groups. Objective findings, such as motor conduction velocity, vibratory perception threshold and heart beat variation remained unchanged in both groups. From the above results we concluded that MX is useful as a symptomatic agent of controlling acute pain and allodynia accompanied by diabetic neuropathy possibly through its Na-channel blocking mechanism.

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THE ALDOSE REDUCTASE GENE IS STRONGLY ASSOCIATED WITH DIABETIC NEUROPATHY IN PATIENTS WITH IDDM

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The role of the aldose reductase gene (ALR2) in susceptibility to diabetic neuropathy was investigated by analysing a microsatellite marker in the 5'end of the gene in patients with IDDM. DNA from 159 British caucasoid patients with IDDM and 102 normal healthy controls were typed for a (CA) dinucleotide repeat polymorphic marker in the 5' region of ALR2 (5'ALR2) using the polymerase chain reaction. Seven alleles (Z-6 to Z+6) were detected in the patients and controls. The patients were categorised according to the presence or absence of overt neuropathy (symptoms of pain, numbness or parathesiae in the feet and/or hands; loss of vibration and/or temperature; loss of reflexes in legs; history of neuropathic foot ulceration). There was a highly significant decrease in the frequency of the Z+2 5'ALR2 allele in patients with neuropathy - 'neuropaths' (n=78) compared to those with no neuropathy, retinopathy or microalbuminuria after 15 years of diabetes - 'uncomplicated' (n=38) (14.1% vs. 38.2% $\chi 2$ =17.3, $p<0.00001$, Pc=0.0001). There was a corresponding decrease in the frequency of the Z-2 allele in the uncomplicated group compared to the neuropaths (11.8% vs. 30.2% $\chi 2$ =9.3, $p<0.0025$, Pc=0.025). The neuropaths had an decreased frequency of the Z/Z+2 5'ALR2 genotype compared to the uncomplicated group (14.0% vs. 44.7% $\chi 2$ =13.0, $p<0.0005$, Pc=0.005, Odds Ratio 5.0). These results strongly suggest that the ALR2 or an adjacent locus is implicated in the susceptibility to diabetic neuropathy.

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ACUPUNCTURE FOR THE TREATMENT OF CHRONIC PAINFUL PERIPHERAL DIABETIC NEUROPATHY: A LONG-TERM STUDY

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Forty-six diabetic patients with chronic painful peripheral neuropathy were treated with acupuncture analgesia to determine its efficacy and long-term effectiveness. Twenty-nine (63%) patients were already on standard medical treatment for painful neuropathy. Patients initially received up to six courses of classical acupuncture analgesia over a period of 10 weeks, using traditional Chinese Medicine acupuncture points. Forty-four patients completed the study with 34 (77%) showing significant improvement in their primary and or secondary symptoms and in their ability to sleep at night ($p < 0.0001$ for all). These patients were followed up for a period of 13-52 weeks with 67% were able to stop or reduce their medications significantly. During the follow up period only eight (24%) patients required further acupuncture treatment. Although 34 (77%) patients noted significant improvement in their symptoms, only seven (21%) noted that their symptoms cleared completely. The response to treatment was better in white Caucasians (26/31 (84%) compared to Asians and Afro-Caribbeans (8/13 (62%), but this difference was not statistically significant. All the patients but one finished the full course of acupuncture treatment without reported or observed side effects. There were no significant changes either in the peripheral neurological examination scores, VPT or in HbA1c during the course of treatment. These data suggest acupuncture is a safe and effective therapy for the long-term management of painful diabetic neuropathy, although its mechanism of action remains speculative.

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EARLY DETECTION "SMALL FIBERS" NEUROPATHY IN INSULIN-DEPENDENT AND NON INSULIN-DEPENDENT DIABETIC PATIENTS

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Diabetes causes widespread damage to the peripheral nervous system involving both somatic and autonomic fibers. Small nerve fibers abnormalities seem to precede large fibers involvement; nevertheless diabetic neuropathy is generally assessed with electrophysiological tests which reflect only large fibers function. Aim of this study was to evaluate the integrity of small fibers in insulin-dependent (IDDM) and non insulin-dependent (NIDDM) diabetic patients without either clinical or electrophysiological evidence of actual neuropathy. Twentyfour diabetic patients underwent neurophysiological study by means of CO2-laser induced Pain-Somatosensory Evoked Potentials (P-SEPs) and infrared TV-Pupillometry: twelve IDDM (age 33±9 years, duration of disease 15±7 years, %HbA1c 7.1±1.8) and twelve NIDDM (age 55.3±8.6 years, duration of disease 9.5 ±6.5 years, %HbA1c 6.8±1.5) without either nephropathy or retinopathy and with normal Visual Evoked Potentials (VEPs). P-SEPs following hand and foot stimulation were evaluated considering the latency of positive peak of cortical waves (P340 and P400, respectively). With regard to pupillometric exams (ISCAN, sample rate 50 Hz) we measured sympathetic (mydriatic reaction to darkness, MI) and parasympathetic (Latency, L, Amplitude, A, of light reflex) indexes. In both two groups the percentages of abnormalities of P400 and parasympathetic indexes (L and A) were 25% and 8%, respectively. In addition, the percentages of abnormalities of P340 and MI were, respectively, 8% and 42% in IDDM and 17% and 33% in NIDDM patients. Our results show an involvement of autonomic and small unmyelited somatic fibers in diabetic subjects with negative clinical and electrophysiological exams. Abnormalities mainly affect foot P-SEPs and pupillary sympathetic indexes, suggesting a length related pattern. The alterations of pupillometric parameters and P-SEPs did not correlate, indicating that somatic and autonomic nerve fibers may be differently affected by diabetic neuropathy.

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TREATMENT OF DIABETIC NEUROPATHY.

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The aim of this study was to compare the effect of anti-oxidant and bloker serotonin receptors for the treatment of diabetic neuropathy (DN). 94 patients with type I or type II diabetes mellitus and DN were included in this study (mean age 51,80±1,16; disease duration - 11,35±0,56 years). In all patients the expressiveness of DN was carried out with MDNS. The thiobarbituric acid reactive material was determined in the serum of all patients and in the membrane erythrocytes of the patients with IDDM as malondialdehyde (MDA). NCV was determined in the median, peroneal, sural nerves in 60 patients. 47 patients were treated with Tanacan (EGb 761, Beaufour-ipsen)-200 mg, other-Dusodril (Naftidrofuril, Byk Gulden)-120 mg during 6 weeks.

	Dusodril		Tanacan	
	before	after	before	after
MDNS	9,02±0,66	2,64±0,38**	7,73±0,36	2,74±0,28**
MDA in serum	1,07±0,08	0,68±0,04**	1,11±0,15	0,58±0,03**
MDA in membr.	1,17±0,05	1,28±0,05	1,20±0,09	0,84±0,07*
HB A1c	8,81±0,32	7,85±0,29	8,43±0,34	7,74±0,30
SNCV(normal)	81,07±27,38	114,00±30,70*	87,70±35,82	95,50±39,19
MNCV(normal)	102,45±21,2	123,10±18,85*	93,67±20,3	105,59±9,88*

* $p<0,01$, ** $p<0,001$ - compared with baseline values.

The decreased in MDA for the functional state of nerves when using drugs of different mechanism will be discussed.

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DOES MAGNESIUM DEPLETION PLAY A ROLE IN THE NATURAL HISTORY OF POLYNEUROPATHY IN INSULIN-DEPENDENT DIABETIC PATIENTS ? (IDDM)

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Chronic Mg depletion is frequently observed in IDDM and is related to an insufficient level of metabolic control. Since both conditions are also related to the development of microangiopathy and dyslipidemia a possible role in other complications like polyneuropathy (PNP) can be hypothesized. In a first study 154 IDDM (57 W + 97 M) were screened for PNP on the basis of clinical signs and symptoms supplemented by an extensive EMG exploration and repeated measurements of erythrocyte Mg (RBC Mg) were obtained. Group 1 consisted of 89 IDDM (23 W + 66 M) and presented with clear signs of PNP. Group 2 consisting of 65 IDDM (34 W + 31 M) does not have PNP. Group 1 has a higher HbA_{1c} (mean of 4 measurements, $p < 0.05$) a longer duration of diabetes ($p < 0.001$) and was older ($p<0.01$) than group 2. When each group was subdivided on the basis of low RBC Mg (< 2.3 mMol/l) Fisher's Exact Test shows a significant association between this value and the presence of PNP (p=0.0172, Odds ratio=2.389, 95% CI: 1.183 to 4.824). In a second study 23 IDDM (Group A= 14 W + 9 M) with both PNP and low RBC Mg accepted to take oral supplements of Mg during 1 year (300 mg Mg^{++} daily) and were compared to 20 identical patients (Group B = 5 W + 15 M) who did not agree to participate. In Group A 10 patients (9 W + 1 M) showed a significant improvement in the EMG data as compared to 2 patients (2 M) in Group B (Fisher's Exact Test: p=0.0193, OR = 6.923, 95% CI: 1.293 to 37.066). The patients who improved were younger ($p<0.02$) had a shorter duration of diabetes ($p<0.05$) but no differences in metabolic control was observed. The physiopathological mechanisms involved in this observation are unclear but one can speculate on an improvement of the microcirculation, a lesser oxidative stress or a better energy supply in the peripheral nervous system.

2204

THIAMINE STATUS IN NIDDM PATIENTS WITH SOMATIC NEUROPATHY

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Thiamine deficiency can result in neurological damage. In order to asses the thiamine status 75 NIDDM patients (age 38-74 years) and 60 age matched control subjects were investigated. Thiamine status was assessed by measurement of erythrocyte transketolase activity (αETK; normal range: 1.00-1.27). Neuropathy was evaluated by determination of both motor nerve conduction velocity (MNCV; n. peroneus) and sensory nerve conduction velocity (SNCV; n. suralis). The value of αETK was significantly higher in NIDDM patients than that of the control subjects (1.14±0.01 *vs* 1.08±0.02 x±SEM; p<0.01). Optimal levels of αETK (1.00-1.15) were observed in 45 patients (60%) whereas 26 patients (35%) were at moderate risk (αEKT 1.16- 1.27) and 4 patients (5%) at high risk (αEKT>1.27) of inadequate thiamine status. Significant difference was observed between patients with (n=23) and without (n=22) neuropathic symptoms regarding αETK (1.19±0.02 *vs* 1.10±0.01; p<0.001), MNCV (37.4±1.2 m/s *vs* 43.4±0.9 m/s; p<0.001) and SNCV (34.4±2.4 m/s *vs* 40.5±1.5 m/s; p<0.05). Short term use of benfothiamine (320 mg daily for one week) resulted in an improvement of thiamine status (αEKT 1.10±0.02 *vs* 1.01±0.01; p<0.01). Non-optimal thiamine status could be observed in some NIDDM patients with somatic neuropathy.

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NON-ENZYMATIC SERUM ANTI-OXIDANTS IN PATIENTS WITH DIABETIC NEUROPATHY.

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Evidence is accumulating that oxidative stress may play a role in the development of diabetic complications. We therefore studied the relation between nonenzymatic serum anti-oxidants (NSA's) and diabetic neuropathy (DN) in 278 consecutive patients with insulin-dependent diabetes mellitus (IDDM) (153 men ,125 women; mean age 38.1 years (SD 12.0), duration of IDDM 17.2 years (SD 10.2) HbA1c 8.2 % (SD 1.89)). DN was assessed by vibration threshold measurements (both legs and arms) and sensibility testing (legs only). DN was defined by the presence of one or more abnormal tests results. The table gives the NSA's (mean and standard error) in diabetic subjects with (91 men and 55 women) and without DN.

	DN-	DN+
α-Tocopherol (umol/l)	30.7 (7.2)	32.0 (10.9)
γ-Tocopherol (umol/l)	2.93 (1.59)	3.05 (1.99)
β-Caroteen (nmol/l)	299 (184)	370 (422)
Retinol (umol/l)	1.80 (0.48)	1.86 (0.85)
Cerruloplasmin (g/l)	0.39 (0.14)	0.37 (0.12)
Ferritin (g/l)	80.6 (70.2)*	104.0 (103.2)*
Transferrin (ug/l)	2.31 (0.39)*	2.17 (0.40)*
Uric acid (mmol/l)	0.24 (0.06)	0.25 (0.07)

Oneway Anova:* p<0.05

After adjustment for age and gender, no difference was found between patients with and without diabetic neuropathy. These results suggest that non-enzymatic antioxidants are not related to the presence of diabetic neuropathy.

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Neuropathy – Autonomic

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PATHOGENESIS OF AUTONOMIC NEUROPATHY IN MALNUTRITION RELATED DIABETES MELLITUS

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Autonomic neuropathy (AN) is known to be a common complication in both IDDM and NIDDM patients. Although malnutrition related diabetes mellitus (MRDM) constitutes an important class of diabetes in tropical developing countries, the prevalence and pathogenesis of AN have not yet been studied in MRDM patients. The present study was designed to explore the relation of AN with glycemic status, insulin secretory capacity and microvascular damage in MRDM subjects. Seventeen newly diagnosed MRDM (9 PDDM and 8 FCPD) along with 24 NIDDM and 21 non-diabetic Control subjects were studied. Autonomic neuropathy was diagnosed by estimating the Valsalva Ratio (VR), deep breathing test (DBT), hand grip test (HGT) and postural blood pressure test (PBPT). Glycemic status was measured by serum glucose response during a standard OGTT and by HbA_{1c}. Insulin secretory status was measured by fasting serum C-peptide and generalized microvascular damage was estimated by Transferrin-Creatinine ratio (TCR). The Control group showed no case of AN, but 14% of the total diabetic subjects showed this complication. Both DBT and VR ratio were significantly (p<0.05) lowered in patients with AN group. The abnormalities in these two autonomic function tests appeared earlier than the other two tests. PDDM patients showed considerably higher prevalence of AN compared to NIDDM subjects (FCPD 38% and PDDM 22% vs NIDDM 4%). Patients with poor glycemic control showed higher percentage of autonomic neuropathy. Fasting C-peptide levels in MRDM subgroup were much lower (p<0.0001-0.001) than the NIDDM and Control groups. indicating that both glycemic status and insulin secretory capacity may be crucial factors in the pathogenesis of neuropathy in these subjects. From the TCR data (Control-0.04 mg/mmol, PDDM - 0.10mg/mmol and FCPD - 0.06 mg/mmol) the diabetic groups were found to have higher microvascular damage in relation to Control but the three groups did not differ among themselves. It may be concluded that MRDM subjects appear to suffer from increased incidence of AN. Glycemic and insulin secretory status seems to be the most important factors in developing neuropathy in these cases and it may develop independently from generalized microvascular damage.

2207

CORRELATES OF SEVERE DIABETIC AUTONOMIC NEUROPATHY.

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Severe autonomic neuropathy is proposed as being predictive of mortality of diabetes mellitus (DM). We performed each of five non-invasive cardiovascular reflex tests for autonomic function (heart rate responses to deep breathing, Valsalva maneuvre, and tilting; blood pressure responses to isometric hand grip and postural change) in 23 consecutive DM patients (mean age 50.17 years; DM duration 10.9 years) and 25 age and sex matched healthy controls. Based on a standardised scoring system for autonomic dysfunction, DM patients were divided into "Borderline DAN" (B-DAN, n=12 DAN score 2-5) and "Severe DAN" (S-DAN, N=11, DAN score 5). S-DAN (vs B-DAN) was associated with increased age (55 10 vs 45 10 yr; p <0.03) DM duration (12+5.9 vs 9.6+7.4 yr), insulin treatment (63.6% vs 33.4%) and reduced nerve conduction amplitude and velocity (p <0.007 to p <0.02). There was no significant difference in fasting blood glucose and glycated hemoglobin between S-DAN and B-DAN. Thus, non-invasive testing is essential to indicate severity of DAN. S-DAN patients tend to be older, have longer DM duration and higher incidence of peripheral neuropathy.

2208

PANCREATIC POLYPEPTIDE SECRETION AND FASTING SERUM GASTRIN LEVELS IN DIABETICS WITH AUTONOMIC NEUROPATHY

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Test meal-stimulated pancreatic polypeptide (PP) secretion is much lower in diabetics with autonomic neuropathy (AN) than in controls. It is also well established that fasting serum gastrin (FSG) levels are very high in diabetics with AN. These findings are said to result from one pathology: autovagotomy. The aim of this study was to identify the relationship of high FSG levels and blunt PP response to meal stimulus in diabetics with AN. The study subjects were 17 long-standing insulin dependent diabetics with AN (group A) and 18 well-matched diabetics without AN (group B); the latter group serving as controls. Autonomic neuropathy was tested in all the patients, using 5 common tests. FSG and PP concentrations were asessed by radioimmunologic methods. The PP secretion was evaluated for two hours on test meal ingestion and calculated as an area under curve (AUC). The mean FSG level in diabetics with AN was 134.12 ± 50.48 pg/ml compared to 82.0 ± 30.72 pg/ml in the control group ($p<0.001$). Inversely, the AUC of PP secretion values were much lower in group A than in the group B (mean 302.70 ± 72.55 vs 412.94 ± 73.65 pg/ml/2h respectively; $p<0.001$). There was also a significant negative correlation ($r = -0.5531$; $p<0.05$) between FSG and postprandial PP values in the diabetics with AN, whereas no such correlation was found in the controls ($r = 0.024$). In conclusion, high fasting serum gastrin levels in long-standing diabetics with autonomic neuropathy are accompanied by low postprandial pancreatic polypeptide secretion, and there is a negative correlation between these two phenomena.

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AUTONOMIC FUNCTION TESTING: MORNING OR AFTERNOON?

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The cardiovascular tests for the diagnosis of autonomic neuropathy are usually performed in fasting conditions in the morning: however, the morning session is often unpractical in our hospital setting. The study aimed to verify if an afternoon session would lead to substantially different results. Ten patients (5 males, 5 females; 5 treated with insulin, 5 with hypoglicaemic agents, mean age 49.8 years, range 15-68) were submitted to a battery of cardiovascular tests: Deep Breathing (DB), Cough test (CT), Lying to Standing (LST), Postural Blood Pressure test (PBPT), Squatting test (SQv and SQs), corrected QT interval (QTc), Basal Heart Rate (HR). The test were performed at 8.00 AM and 2.00 PM; according to accepted criteria, each test was scored as normal (0), borderline (1) or abnormal (2). Patients with a total score >=4 were considered as affected by autonomic neuropathy . Patients abstained from drugs, insulin and from breakfast or lunch respectively. Mean glycemia was 114.9 and 128.2 mg/dl (NS) prior to the tests. No significant difference was observed in the mean results obtained for each test in the mornings and afternoons. The coefficient of variation (CV) for the repeated measures were as follows: DB 13.4%, CT 4.4%, LST 5.8%, PBPT 29.6%, SQv 6.7%, SQs 6.85%, QTc 2.0%, HR 3.0%. The scores varied in 1 patients from normal to abnormal for SQv, and from abnormal to normal in 2 patients for SQs. The mean total score was 3.9 in both occasions: one patient was considerd neuropathic in the afternoon session only (score 3 in the morning, 5 in the afternoon). In conclusion the CV for repeated measures in morning and afternoon sessions are similar to those published for repeated morning measures in the morning. Cardiovascular testing performed in fasting conditions in the afternoon do not lead to significant difference in patients classification.

2210

PATHOLOGICAL GLUCOSE TOLERANCE TEST: INCIDENCE OF AUTONOMIC SKIN'S NEUROPATHIES.

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It was the aim of this pilot study, to answer the question, if in patients suffering from pathological glucose tolerance test already autonomic skin's dysfunction can be objectivied.

30 subjects suffering from pathological glucose tolerance test since more than 2 years (group A) and 30 age-machted healthy subjects (group B) were studied for their function of autonomic nervous system in skin. Basing on the hyperthermal Laser-Doppler-Flowmetry, a method invented in 1988, it is possible to measure autonomic skin's neuropaties in routine use. The basic consideration of this new method is the fact, that a healthy autonomic nerve function reacts to hyperthermia with an increase of microcirculation after a well defined time without any severe fluctuations. In autonomic neuropathies during the perfusion increase there are severe fluctuations, defined as „Fluctuationindex" (FI) measured in percentage (normal range: ≤20%).

In group A the median of FI was 26% (IR25:11%, IR75:36%), whereas values of group B showed a median of 13% (IR25: 9, IR75:17). The incidence of pathological values (PI >20%) was 26,67% (8 of 30 patients) in group A against 0% (0 of 30 patients) in group B.

The results lead to the conclusion, that patients with pathological glucose tolerance test already develope autonomic neuropathies.

2211

THE VALUE OF COLD PRESSURE TEST IN ASSESSMENT OF SYMPATHOVAGAL BALANCE IN IDDM PATIENTS

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Heart rate variability (HRV) offers a new method in investigating mechanisms of sudden death in diabetic patients with advanced diabetic complications. Our aim was to show the earliest changes in the balance of cardiovascular autonomic system. In 96 patients with insulin dependent diabetes (mean: age: 33.4 years, diabetes duration 10.54 years) who received insulin as the only medication and 30 healthy volunteers (mean age: 31.0 years) the following tests using short-term heart rate variability analysed by ProSciCard were performed: 5 minutes at rest (heart rate - HR-R, standard deviation - SD-R, RMSSD-R, MCR-R, sectral power at: very low frequency VLF-R, low frequency LF-R, high frequency HF-R), and 5 minutes after cold pressor test (HR-CPT, SD-RCPT, RMSSD-CPT, MCR-CPT, VLF-CPT, LF-CPT, HF-CPT). The standard Ewing battery od autonomic tests was also performed. We compared healthy group with IDDM patients without retinopathy (R0), with nonproliferative retinopathy (R1) and with proliferative retinopathy (R2). Retinopathy was assess by ophtalmoscopy.

Results: The following parameters had the highest strenght of discrimination between examined groups: **Group C vs R0:** SD-RCPT (80.68 vs 48.54ms; p=0.0004), ln HF-CPT (0.72 vs -0.10; p=0.01), ln LF-CPT/HF-CPT (-0.38 vs 0.24; p=-0.02), RMSSD-CPT (86.98 vs 40.92; p=0.0001), **Group C vs R1:** LnHF-R (-0.3974 vs -1.53, p=0.002), SDHR-CPT (80.68 vs 46.81, p=0.03), Ewing test: 0.82 vs 0.97, p=0.0002), Ln HF-CPT (0.72 vs -0.62, p=0.04), **Group C vs R2:** HR-CPT (63.57 vs 79.52, p=0.0003), RMSSD-R (60.19 vs 12.95, p=0.00001), RMSSD-CPT (86.98 vs 22.12; p=0.001), Ln HF-CPT (0,72 vs -1.10; p=0.002), **Group R0 vs R1:** ln LF-CPT (0.13 vs -0.75; p=0.001), ln LF-CPT/HF-CPT (0.24 vs -0.12; p=0.04),**Group R0 vs R2:** HR-CPT (68.59 vs 79.52; p=0.0000006), SD-RCPT (48.54 vs 28.53; p=0.0002), ln LF-CPT (0.13 vs -1.38; p=0.0000001), Ln HF-CPT (-0.10 vs -1.10; p=0.0005), ln LF-CPT/HF-CPT (0.24 vs -0.28; p=0.04), RMSSD-CPT (40.92 vs 22.12; p=0.02).

Conclusions: 1. Development of retinopathy is associated with marked changes of high frequency domain of HRV. 2. Spectral analysis of HRV detects more discret disorders in autonomic function than conventional tests.

2212

Moisture measurement of the skin
- A simple test to determine diabetic autonomic neuropathy -
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It is very useful to measure sweating for diagnosis of diabetic autonomic neuropathy. However, there is a few simple test to measure sweating in clinical practice. Therefore, we analyzed the usefulness of moisture measurement of the skin to determine diabetic autonomic neuropathy. 28 diabetic patients and 11 healthy volunteers joined to the study. Mental sweating rate of the thumb and the first toe were measured with Kenz Perspiro OSS-100 (Suzuken, Nagoya, Japan), a devise to measure local sweating rate of patched 1 cm^2 area continuously. Sweating responses to calculation, scratch, standing and deep breathing were examined. Heart rates were also measured continuously. Moisture of the skin were measured by moisture checker (Scalar, Tokyo, Japan), a skin moisture measuring devise. Because the sweating response to deep breathing is a good test to measure autonomic function, subjects were divided into 2 groups according to the response pattern (Group A : good responders, Group B : poor responders). Skin moisture measured by the moisture checker correlated well with sweating rate measured by Kenz Perspiro OSS-100. Skin moisture of the thumb and the toe were significantly decreased in group B compared with that in group A. These results indicate moisture measurement of the skin is useful to determine diabetic autonomic neuropathy.

2213

ALTERED CIRCADIAN BLOOD PRESSURE CHANGE IN IDDM PATIENTS WITH NORMAL BEDSIDE TESTS OF AUTONOMIC FUNCTION

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The presence of hypertension is an important determinant of cardiovascular risk in IDDM patients. A reduced nocturnal fall in blood pressure is a recognised abnormality in BP in IDDM patients and may be related to autonomic dysfunction. The aim of this study was to assess the relationship between circadian blood pressure change, baroreceptor-cardiac reflex sensitivity and sympathovagal balance in patients with normal bedside tests of autonomic function. 31 normotensive, normoalbuminuric IDDM patients and 31age, sex and BP matched control subjects were studied.24hour ambulatory BP was measured using the SpaceLabs 90207 system and circadian change in BP assessed by Cusums analysis. Heart rate and blood pressure variability was assessed,using spectral analysis, from data recorded non-invasively using the Finapres system. The α index, a measure of overall baroreflex gain was assessed from the frequency domain data. There was a significant reduction in high frequency power of heart rate variability in the IDDM group (Mean $\pm$ SEM: 22 ± 3.1 vs 33 ± 3.1 NU $p < 0.002$). In addition, BRS was significantly reduced in the IDDM group (9.7 ± 3.7 vs 18.5 ± 4.7 ms/mmHg $p < 0.001$). Sympathovagal balance (the ratio of low frequency to high frequency power) was increased in the IDDM group (4.6 ± 0.55 vs 2.9 ± 0.52 $p < 0.002$). IDDM patients showed a significant reduction in circadian variation of blood pressure (Cusum Derived Circadian Alteration of Magnitude: CDCAM=15.4 ± 5.7 vs 25.6 ± 7.3 mmHg $p < 0.003$). There was a significant negative correlation between sympathovagal balance and CDCAM ($r = -0.5$ $p < 0.05$). Reduced circadian change in blood pressure is seen in IDDM patients with normal bedside tests of autonomic function and may be due to an increased sympathetic predominance. This would increase cardiovascular risk amongst IDDM patients.

2214

Evaluation of skin vasoconstrictor responses to deep breath in diabetes by using laser Doppler flowmetry

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To elucidate peripheral sympathetic neuropathy in patients with diabetes, we measured the vasoconstrictor responses to deep breath in the feet of 51 non-insulin-dependent diabetes mellitus (NIDDM) and 20 healthy control subjects, using laser Doppler flowmetry. Subjects whose skin temperature was less than 32°C were excluded from our study because a skin temperature of approximately 34°C is an optimal temperature to detect skin vasomotor reflexes to deep breath by laser Doppler flowmetry.

Vasoconstrictor responses to deep breath in the big toe of diabetic patients were significantly reduced compared with healthy controls (26.8±20.0% vs. 48.3±18.5%, P<0.0001). In NIDDM patients, the percent vasoconstriction was positively correlated with diabetes duration, median motor and sensory nerve conduction velocity, coefficient variation of R-R interval on electrocardiogram at rest and postural fall in systolic blood pressure, and inversely correlated with vibratory perception threshold. Vasoconstrictor responses to deep breath in diabetic patients with progressive retinopathy or overt nephropathy were significantly decreased than in those with no retinopathy or normoalbuninuria. These results suggest that vasomotor reflexes to deep breath in the lower limb of diabetic patients is markedly impaired and decreased in parallel with others diabetic neuropathy or diabetic microangiopathy, and that measuring of vasoconstrictor responses to deep breath by using laser Doppler flowmetry is a novel and useful tool for detecting peripheral sympathetic failure in diabetes.

2215

DECREASED PARASYMPATHETIC TONE IN YOUNG IDDM PATIENTS.

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Cardiac autonomic dysfunction is associated with diabetic complications such as nephropathy and retinopathy, and may carry an increased risk of mortality. Using new methods to study cardiac autonomic function an early detection of cardiac autonomic dysfunction is possible. To evaluate the cardiac autonomic function in young diabetic patients we studied 7 (4M/3F) insulin-dependent diabetic and 10 (5M/5F) healthy control subjects aged 14.2 ± 1.3 yr (mean $\pm$ SEM, range 8.1-18.0 yr) and 13.9 ± 0.9 yr (8.6-18.0 yr), respectively. The diabetic patients had a duration of diabetes of 5.1 ± 0.8 yr (1.5-7.3 yr) and HbA_{1c} of 9.6 ± 0.6 % (8.0-12.5%). Heart rate and blood pressure variabilities were registered at rest for 5 min in the supine position, and analysed by power spectral analysis (CAFTS, Medikro Oy, Finland). In addition, conventional tests including the Valsalva, handgrip, and tilt table test were performed. At rest, diabetic patients had decreased heart rate variability in terms of root mean square of successive differences of RR intervals (RRI RMSSD; 42 ± 8 vs 89 ± 19 ms, $p < 0.05$), total power of RRI intervals (TP-RRI; 1952 ± 570 vs 6789 ± 2034 ms^2, $p < 0.05$) and RRI variability in the respiratory band (HFP-RRI; 1076 ± 295 vs 5042 ± 1819 ms^2, $p < 0.05$) compared to healthy controls. During the tilt table test, a decreased acceleration index (19 ± 2 vs 31 ± 3 %, $p < 0.05$), and brake index (13 ± 2 vs 27 ± 5 %, $p < 0.05$) was seen in the diabetic subjects. All these abnormalities reflect mainly an impairment in the parasympathetic tone. There were no differencies in blood pressure variability, Valsalva or handgrip tests between the groups. In conclusion, an easily performed registration of resting heart rate variability reveals autonomic abnormalities even in young IDDM patients. The dysfuntion is seen in parameters mainly reflecting parasympathetic tone.

2216

QUANTITATIVE ASSESSMENT OF DIABETIC AUTONOMIC NEUROPATHY BY ACCELERATED PLETHYSMOGRAPHY

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AIM It is well known that accelerated plethysmogram (APG) is useful for assessing peripheral circulation. In this study, we attempted to evaluate diabetic autonomic nervous dysfunction by the difference (APGD) of APG index (APGI) between before and after cold water stress test .

SUBJECTS Fifty thee healthy volunteers as control group NC (age 54.3± 7.2yr), age-matched 48 diabetics without neuropathy as group DMN- (age 55.4±8.9yr, duration 5.3±3.4yr, HbA1c 8.4±2.6%), and age-matched 46 diabetics with neuropathy as group DMN+ (age 56.2±8.6yr, duration 13.6± 4.2yr, HbA1c 9.2±1.8%) were enrolled into the study.

METHOD First, the subjects were sitting during 5 minutes, and then APGI were measured from the right second finger , using APG-200 (Misawa Co. Ltd.) that automatically calculates APGI from plethysmogram. Second, their left hand was kept dipping in cold water (3℃) and APGI was acquired in the same way. The differences of APGI between before and after cold water test were calculated as APGD.

RESULTS There was no significant difference between NC and DMN- in APGD, but APGD in DMN+ was significantly lower than the other two groups. APGD significantly correlated with coefficient of variation of ECG R-R intervals (CV) in diabetics ($r=0.58$, $p<0.001$). There was significant reverse correlation between APGD and diabetic duration ($r=-0.42$, $p<0.003$). After one month strict blood glucose control of diabetics with relatively short duration (less than 5 years), APGD significantly increased (10.8±5.4 vs 28.7±6.3%, $p<0.01$), but CV did not significantly changed.

CONCLUSION This study shows that APGD is useful to estimate autonomic nervous dysfunction in peripheral circulation and its improvement after strict blood glucose control in diabetics.

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DETERIORATION OF CARDIOVASCULAR AUTONOMIC FUNCTION OF DIABETIC PATIENTS IN TWO YEARS .

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Autonomic neuropathy sometimes carries a poor prognosis and increased risk of mortality from sudden death. The aim of this study was to clarify the natural history of autonomic neuropathy in diabetic patients. Methods: We studied 30 diabetic patients, 8 IDDM and 22 NIDDM, with mean duration of diabetes of 17.3 years (range 10-41) and mean age 54.3 years(range 26-65). Patients aged more than 65 years old or those with overt nephropathy or heart disease were excluded from this study. The HbA1c(%) remained actually unchanged during the study period and was 9.3±0.27(se) the higher and 8.9±0.27 the less(p=NS). We used the apparatus Monitor ONE NDX that helps to perform deep breathing test { Expiration /inspiration ratio(E/I R), standard deviation of R-R(SD), mean circular resultant of vector analysis(MCR), } Valsalva manoeuvre and the changes of R-R (postural index) from lying to standing. We also observed the postural hypotension. All the parients had two or more of the tests abnormal. Results: Deterioration was observed in all the tests performed comparing the baseline with two years. E/I index from 1.073±0.0006(se) in 1.043±0.005, $p<0.0001$, SD from 26.6±2.3 in 17.9±1.9, $p<0.0001$, MCR from 12.6±1.64 in 8.23±1.39, $p< 0.0002$, Valsalva index from 1.3±0.03 in 1.2±0.03, $p< 0.0001$, postural index from 1.12±0.02 in 1.05±0.01, $p<0.0001$ and postural hypotension (mmHg) from 26.2±2.1 in 36.7±2.1, $p<0.0001$.The E/I ratio deteriorated statistically earlier than the other tests. Conclusions: Definite and statistically significant differences in the various tests of cardiovascular autonomic function in all the patients appear in two years of follow up. The E/I ratio is considered as the most sensitive test because it can detect the deterioration of autonomic function earlier. Although the clinical significance of these changes is difficult to be estimated, our findings emerge for early detection and treatment of autonomic neuropathy.

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CAN ACUTE INDIRECT COOLING BE USED TO ESTIMATE PERIPHERAL AUTONOMIC NEUROPATHY?

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Easy non-invasive methods are desired to estimate peripheral autonomic nervfunction. The aim of this pilot study was to examine if an acute indirect cooling-test can be used to diagnose peripheral autonomic neuropathy in diabetic patients. Thirteen patients (5 women) with diabetes duration 16±9 years were examined (age 52±16 years; mean±SD). Five patients had no central autonomic neuropathy, while 4 had suspected and 4 had clear signs of central autonomic neuropathy. Eight patients had clinical signs of peripheral autonomic neuropathy (dry skin, loss of hair, impaired sweating). Eight healthy controls (25-65 years) were also examined. The skin microcirculation at the pulp of the left great toe was measured by laser Doppler fluxmetry (LDF) before and during cold exposure of the right hand for 90 s in icewater (4-6°C). LDF was measured in arbitrary units (AU). In 7 patients and 5 controls the skin microcirculation was simultaneously measured at the pulp of the left thumb and at the pulp of the left great toe. Before cooling, LDF in the left great toe was similar (ns) in patients (65±53 AU) and controls (42±24 AU). During cooling, LDF decreased ($p<0.05$) to 35±21 AU in the controls, while LDF in the patients was unchanged (62±47 AU). However, in 4 patients without autonomic neuropathy LDF decreased ($p<0.05$) during acute cooling. LDF in the left thumb decreased ($p<0.05$) during cooling in all patients and controls. In conclusion, in diabetic patients with central autonomic neuropathy reflective vasoconstriction was lost in the contralateral foot, but intact in the contralateral hand and in patients without autonomic neuropathy. Consequently, acute indirect cooling of the hand seems to be a useful method to investigate peripheral autonomic neuropathy in the feet.

2219

A MODIFICATION OF THE 30:15 AUTONOMIC FUNCTION TEST-EFFECT OF SQUATTING

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The classical 30:15 autonomic function test consists of the measurement of the RR cardiac intervals at the 30th and 15th cardiac beats immediately after standing up from the lying position. The present study deals with similar measurements (30:15) after resuming the standing position following a two-minute interval during which examined persons remained squatting. In order to investigate this, 105 diabetic persons (type 1: n=55; type 2: n=50) as well as 32 controls, serially matched (by age and sex) were examined. They underwent the standardized battery of the 5 Ewing tests and also the squatting test (SqT). Autonomic neuropathy was diagnosed if 2 of the 5 Ewing tests were abnormal. There were 40 diabetic persons (DP) without (AN-) and 65 with autonomic neuropathy (AN+). Sensitivity (S), specificity (Sp), positive (PPV) and negative predictive value (NPV) of the classical 30:15 (Clr) and SqT ratios (SqTr) are shown on table along with 95% confidence intervals (CI):

	Clr		SqTr	
	%	95% CI	%	95% CI
S	60.31*	51-69	68.25*	59-77
SP	54.36[1]	45-63	77.50[1]	77-78
PPV	44.70[2]	38-57	82.26[2]	74-79
NPV	69.13*	60-77	60.78*	51-70

(*: $p>0.05$, [1]: $p=0.0005$, [2]: $p<0.0001$)

The coefficients of variation were 6.91 and 6.94% for Clr and SqTr, respectively. The passage from squatting to the standing position caused greater tachycardia than the classical lying to standing test in all subjects. Until 15th beat all DP showed a similar response to heart rate. At the 30th beat, although the heart rate of AN- and controls was restored, AN+ continued to have tachycardia. The resulted low SqTr clearly indicates parasympathetic dysregulation. So, the greater cardiac stress after squatting seems to unmask an underlying parasympathetic damage to a greater degree than the classical test. Conclusions: 1) The proposed SqTr can discriminate between AN- and AN+ DP to a greater extent than the Clr. 2) SqTr gives information on parasympathetic activity.

2220

THE VARIABILITY OF THE PHOTOPLETHYSMOGRAPHIC SIGNAL - A MEASURE OF AUTONOMIC NEUROPATHY

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The heart rate and the arterial blood pressure show spontaneous low frequency oscillations, which are associated with the sympathetic nervous system activity. Similar fluctuations were also found in the photoplethysmographic (PPG) signal, which measures the tissue blood volume increase during systole. The latter depends on the compliance of the arterioles, which is affected by the sympathetic nervous system. The aim of the study was to compare the PPG signal variability in diabetic patients to that in non-diabetic subjects. The PPG signal was measured simultaneously on the index fingers of both hands of 16 non-diabetic and 7 diabetic patients. None of the diabetic patients showed clinical manifestations of neuropathy. The correlation coefficient of the PPG signal amplitude between the right and the left hand was 0.87 ± 0.08 for the non diabetic subjects, while four out of the seven diabetic patients had correlation coefficients of 0.47 – 0.75. The low correlation coefficient is probably due to autonomic neuropathy. The difference in the PPG amplitude between the two fingers was $9.4 \pm 10.2\%$ for the nondiabetic subjects, while four of the diabetic patients showed differences higher than 40%. In conclusion, the PPG variability provides a potential means for the assessment of the sympathetic neuropathy.

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AUTONOMIC NEUROPATHY AND HYPERTENSION IN INSULIN-DEPENDENT DIABETES MELLITUS: THERE IS A RELATIONSHIP

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Follow-up studies revealed poor prognosis of autonomic neuropathy (AN) in diabetic patients. Silent myocardial ischemia / infarction and major arrhythmias are thought to be most important in this respect. However, factors leading to increased mortality of AN have not been definitely identified up to now. Aim of our study was to evaluate a connection between AN and blood pressure in 32 patients with IDDM (mean age: 37,8 ± 12,7 ys, mean duration of diabetes: 17,9 ± 10,1 ys, males: 5, females: 27). The five standard reflex tests of cardiovascular autonomic function were applied. Twenty four hours blood pressure monitoring (ABPM) was performed by Meditech ABPM 02 device. Severity of AN was characterized by the number of abnormal reflex indices on patient and correlated significantly with systolic ($p<0,001$) and diastolic ($p<0,01$) hypertensive time indices as well as with systolic ($p<0,01$) and diastolic ($p<0,05$) hyperbaric impact (mmHgxh). Analysing the relationship between ABPM parameters and the five autonomic tests separately, a significant correlation was found with two tests only, 30/15 ratio and Valsalva ratio - both tests reflect mainly parasympathetic function. These data suggest that a relative sympathetic overactivity due to predominantly parasympathetic neuropathy might be responsible for the higher ABPM indices observed. Severity of AN correlated also with microalbuminuria ($p<0,001$.) Autonomic neuropathy as well as microalbuminuria were independently associated with hypertension. Severity of AN did not correlated significantly with diurnal blood pressure indices. Conclusion: autonomic impairment and hypertension seems to be associated in IDDM. Hypertension itself may contribute to the poor prognosis of autonomic neuropathy.

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SKIN BLOOD FLOW RESPONSE TO HEATING DURING A 75-g oGTT IN HEALTHY INDIVIDUALS

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Capillary blood flow response to a thermal stimulus is expected to be affected by glucose intake, since systemic catecholamine secretion occurs after administration of the substance. During a 75-g oGTT of ten right-handed and overnight fasted healthy volunteers, we recorded the capillary blood flow on the left forearm using Laser-Doppler flowmetry (Periflux model 4001, Perimed Ltd, Sweden) and raising the local temperature to 44^0 C for three minutes, at 0, 30 and 60 minutes (EXP1). We repeated the same procedure on a different day using water instead of glucose solution (EXP2). Three double-peaked curves were obtained for each individual on each experiment. Peak flow absolute values (APF) and areas under the curve (AUC) were significantly decreased in EXP1 as compared to EXP2. In EXP1, Friedman Two-way ANOVA revealed a significance of $p=0.02$ for AUC values, and a $p=0.06$ for APF values, while it failed to show any correlation in EXP2. Repeated measures ANOVA (MA) of APF revealed a significance $F=0.059$ in EXP1 but no significant correlation in EXP2. MA of AUC showed a trend for decrease ($F=0.083$) in EXP1, and no change in EXP2. Recovery of blood flow to baseline was faster in the second and in the third curve. No difference was noted in the percentages of changes of the peak flow rates over baseline levels as well as in the time elapsed from baseline to peak flow. Thus, in healthy individuals, glucose load results in a decreased capillary blood flow response to short and acute thermal stimuli in healthy individuals.

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DIVERGENT DEVELOPMENT OF AUTONOMIC AND PERIPHERAL SOMATIC NEUROPATHIES IN NIDDM

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There is no information on the mutual occurrence and the development of autonomic and peripheral somatic neuropathies based on long-term follow-up of patients with NIDDM. We studied a representative group of patients with newly diagnosed NIDDM (n = 133, aged 45-65 yr) at baseline and 5 and 10 years later. Parasympathetic autonomic neuropathy was diagnosed on the basis of conventional criteria for heart rate variability during deep-breathing (E/I ratio $\leq$ 1.10) and sympathetic autonomic neuropathy on the basis of fall in systolic blood pressure ($\geq$ 30 mmHg) while changing from supine to standing. Polyneuropathy was diagnosed on the basis of both clinical criteria (pain and paresthesias) and electrodiagnostic studies (nerve conduction velocity and response-amplitude values). We investigated the relation between the changes in autonomic function values and electrodiagnostic values, and the relation between occurrences of autonomic neuropathy and somatic peripheral polyneuropathy. Altogether 36 patients died during the follow-up and 78 patients completed the study on autonomic nerve function tests and electrodiagnostic tests for polyneuropathy. When the study ended, the number of patients with parasympathetic autonomic neuropathy was 61.3 % of those with polyneuropathy and 66.7 % of those without. Likewise, the number of patients with sympathetic autonomic neuropathy was not different in those with polyneuropathy (21.9 %) and those without (26.5 %). The worsening of parasympathetic and sympathetic autonomic function values was not related to the worsening in sensory or motor nervous function with time. **In conclusion**, the development of autonomic and peripheral somatic neuropathies are divergent in patients with NIDDM suggesting different pathopysiological processes for these neuropathies.

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LASER DOPPLER BLOOD FLOW IN IDDM PATIENTS BEFORE AND AFTER PANCREAS TRANSPLANTATION

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To investigate the effect of a long-term glucose normalization on peripheral blood 74 IDDM patients with severe secondary complications were studied: **Group A** (n=10): patients on the waiting list for pancreatic-renal grafting (age 39.7±4.3 yrs, duration of diabetes 24.1±3.2 yrs, duration of dialysis 47.9±46.6 m, Hb 10.0±2.8 g/dl, HbA1c 7.0±0.9 %); **Group B** (n=41): patients after successful simultaneous pancreas and kidney transplantation (age 41.9±7.8 yrs; duration of diabetes 29.2±5.4 yrs, duration of dialysis 41.8±23.3 m, posttransplant 59.7±46.4 m, Hb 13.5±1.8 g/dl, HbA1c 4.6±0.7 %) ; **Group C** (n=23): posttransplant patients with functioning kidney graft, under insulin therapy (age 42.3±9.3 yrs, duration of diabetes 29.1±8.7 yrs, duration of dialysis 33.4±24.5 m, duration of renal graft functioning 83.4±56.7 m, Hb 12.8±2.0 g/dl, HbA1c 6.7±1.0 %). Data were compared to 19 control persons (**Group D**): age 30.6±11.5 yrs, HbA1c 3.7±1.4 % (mean±SD). Laser Doppler blood flow was measured in supine position on the dorsum of the foot using Periflux 4001 Master, Perimed AB, Sweden. Skin temperature was very similar in the groups at room temperature of 22±2° C. Basal blood flow was calculated as mean of 2 min measurement. After arterial occlusion by a cuff with 240 mmHg for 3 min maximum hyperemia was assessed. The biological zero flow was subtracted from measured values. Blood flow on the middle finger was measured before and during 30 seconds of a cold pressure test by putting the contralateral hand into ice water. Veno-arteriolar response was assessed by calculation of the decrease of blood flow on the foot after reaching standing position using a tilting table. Basal blood flow was lowest in Group B (5.0±3.4 Perfusion Units) and Group D (6.1±3.2 P.U.) followed by Group C: 9.3±6.9 P.U. and Group A: 14.7±11.2 P.U. With the exception of the comparison between Group B and D all groups differed significantly ($p<0.05$). Maximum post-occlusive increase was highest in Group D with 569±300 %, followed by 338±290 % in Group B; Group C: 194±250% ($p<0.01$ vs. all other groups); Group A: 149 ±200%. Decrease in blood flow during cold pressor test was highest in Group D with 67±20 %. Group C 31±30 %, Group B 30±16 %, Group A 18±20 % ; D vs A,B,C $p<0.001$). The veno-arteriolar response was similar in all groups. These data suggest that long-term glucose normalization has a positive influence on several functional parameters of microcirculation.

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QUONTITATIVE EVALUATION OF CARDIOVASCULAR AUTONOMIC REFLEXES FOR DIABETIC COMPLICATIONS.

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In order to clarify the relationship between diabetic complications and cardiovascular autonomic nervous functions, cardiovascular autonomic reflexes in diabetic patients were quantitatively evaluated by means of a deep breathing test and active postural change test with the Finapres (a method of non-invasive continuous blood pressure measurement). It revealed that both sympathetic and parasympathetic nervous system in the diabetic patients were deteriorated. The coefficient of variation of the pulse rate (CV-PR) during deep breathing in the sitting position and the time required for the recovery of blood pressure (Δ Time) in the active postural change test showed significant differences between the patients with major diabetic complications (retinopathy, peripheral neuropathy or nephropathy) and healthy subjects. Moreover, the critical point for the appearance of each complication was about 2% for CV-PR and about 30 sec for Δ Time. The present method enables the separate examination of sympathetic and parasympathetic nervous functions of patients by quantitatively evaluating their cardiovascular autonomic reflexes, which appears to be useful for detecting complications and investigating of correlations between autonomic nervous function and both somatosensory and peripheral circulatory functions.

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IMPAIRED MICROVASCULAR RESPONSES TO GLUCOSE IN FRUCTOSE-INDUCED DIABETIC RATS.

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Abnormal reactivity of resistance vasculature may contribute to the pathogenesis of diabetic microangiopathy, inducing alterations in regional haemodynamics. The purpose of our study was to determine whether microvascular responses to elevated blood glucose and insulin, mimicking the postprandial state, are impaired in a model of type II diabetic rats. Studies were conducted on 3-months fructose (10 % in drinking water/FRU)-treated rats and age-matched controls. Intravital microscopy was used to examine diameter changes of arterioles (< 40 µm) in spinotrapezius muscle of fasted, anesthetized rats, before and 15, 30, 45 and 60 min after glucose infusion (1.5g/kg; i.v.) or NaCl 0.9 %. Blood glucose and insulin levels were determined before and 10, 40 and 70 min after infusion. FRU rats were hyperglycemic (7.5 ± 0.4 mM vs 6.6 ± 0.3 mM for control rats, $p < 0.05$) and hyperinsulinemic (184 ± 23 UI/ml vs 88 ± 11 UI/ml for control rats, $p < 0.01$) in the basal state. Intravenous glucose infusion produced a peak of glycemia and insulinemia which returned to basal in control rats at 40 min, but not in FRU rats. NaCl infusion did not modify arteriolar diameter. After intravenous glucose infusion, an arteriolar vasoconstrition was observed (14 % at 30 min vs NaCl, $p < 0.05$) in control rats but not in FRU rats. In contrast, glucose infusion produced an arteriolar vasodilation (15 % at 30 min vs NaCl, $p < 0.05$) in FRU rats. Our results indicate that in FRU rats, there is a lack of vasoconstriction in microvascular responses to intravenous glucose infusion. This impaired vasoconstriction could result in hyperperfusion and subsequent microvascular damage which might contribute to the development of diabetic microangiopathy.

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CARDIOVASCULAR EFFECT OF DIABETIC AUTONOMIC NEUROPATHY.

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AUTONOMIC NEUROPATHY, a late complication of Diabetes Mellitus, also effects CVS. In 1995 an outpatient based prospective study was designed to see this effect in Diabetic patients at JPMC diabetic clinic,Karachi.First 100 adults (50 male 50 female)diabetics for more than 5 years were selected, 45 were IDDM and 55 were NIDDM. Follow-ingparameters were recorded.On ECG heart rate changes (RR-interval)during deep breathing(48%) Immediate response to standing (41%) and Valsulva maneuver(26%). Fall in systolic blood pressure on standing (12%)and an increase in diastolic blood pressure by sustained handgrip(8%). And accord-ingly these patients were divided into four groups. All tests normal (44), mild neuropathy with only one test abnormal(28), definite neuropathy with more then one test abnormal(29) and denervated heart with any one test reflecting sympathetic damage and another test showing parasympathetic damage (10). The results suggest that Diabetic Autonomic neuropathy effects CVS.in a large number of diabetics and is directly related to the age and duration of the disease. Simple bedside non-invasive tests are important for early detection and control of this complica-tion.

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IMPROVEMENT OF NUTRITIVE CAPILLARY BLOOD FLOW DURING INTRAVENOUS C-PEPTIDE INFUSION IN IDDM PATIENTS

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It is postulated that C-peptide substitution increases blood flow, oxygen uptake and capillary diffusion capacity in forearm muscle in IDDM patients. The purpose of the present study was to investigate total skin microvascular blood flow and nutritional capillary blood flow during C-peptide administration in the feet of IDDM patients. Eight male IDDM patients without severe secondary complications (age 29 - 45 years, duration of diabetes 2.5 - 40 years) participated in this investigation. Following a randomized double-blind crossover protocol, C-peptide (8 pmol/kg/min) or placebo (0.9 % NaCl) were infused intravenously for 60 min. Observation parameters, measured after 0, 15, 30, 45, 60, and 90 min, were capillary blood flow at the toe nailfold (CBV; television-microscopy), total skin blood flow at the pulp of the toe (LDF; laser-doppler-flux), blood pressure, blood sugar and plasma C-peptide levels. In the verum arm, C-peptide levels increased directly after start of injection to reach a maximum of 6.6 ± 1.0 ng/ml after 45 min, but remained below 0.5 ng/ml during placebo treatment. No significant difference was found in CBV between the two treatment periods at baseline (C-peptide 150 ± 6 nm/s ; NaCl 148 ± 8 nm/s). In parallel with the increase in C-peptide levels, a significant increase in CBV was observed ($p<0.05$), reaching a significant difference in CBV between C-peptide and NaCl after 45 min of infusion (C-peptide 164 ± 7 nm/s; NaCl 136 ± 5 nm/s; $p<0.001$). LDF signal slightly decreased during NaCl infusion and remained unchanged during C-peptide infusion. The results were not biased by changes in systemic haemodynamic parameters (blood pressure; heart rate). Our findings suggest that there is a change in the distribution of microvascular blood flow during C-peptide administration, which may be important in the development of microvascular complications in IDDM.

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THE RELATIONSHIP BETWEEN DURATION OF DIABETES AND AUTONOMIC NEUROPATHY.

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Onset of many diabetes complictions such as retinopathy and peripheral neuropathy is related to duration of diabetes. We determined the relationship between cardiac autonomic denervation (CAD) and duration of disease in 171 diabetic patients with CAD, 1000 patients with retinopathy and 500 patients with neuropathy attending the national diabetes centre, National Hospital, Sri Lanka and Faculty of Medicine Colombo. Autonomic function was assessed using standard cardiovascular reflex tests. Sensorimotor neuropathy was assessed using biothesiometry and electrophysiology. Retinopathy was assessed by fundus examination. There was no correlation betweer duration of disease and the presence of autonomic neuropathy or any of the cardiovascular reflex tests (p 0.19). No significant differences were observed in CAD in subgroups of patient with diabetes for 1-5 years (21%), 6-10 (27%) years, 11-15 (28%) years, 15-20 years (22%). The prevalence of peripheral neuropathy ($p=0.01$) and retinopathy ($p = 0.01$) were significantly higher in those with a longer duration (10 years). Mean duration of diabetes was similar among those who had CAD and those without CAD ($p=0.6$) Duration of diabetes was not related to onset of CAD but was related to onset of neuropathy and retinopathy.

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DIFFERENTIATED ANALYSIS OF PLETHYSMOGRAPHY IN DIABETES MELLITUS

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[AIM] Disorder of peripheral blood flow is an important problem in diabetes mellitus, as it is said to be responsible for its complications. In the present study we assessed the usefulness of differential calculus analysis of plethysmography in diabetes mellitus.

[SUBJECTS & METHODS] Plethysmography was done in 40 control subjects, and 52 diabetics whose average age was 61.8 years and average duration of diabetes was 7.1 years. Plethysmogram was taken at both forefingers using the system composed of recording part and analyzing program (FUKUDA DENSHI Co.,Ltd., Tokyo, Japan). Obtained plethysmogram was successively differentiated as much as five times and arbitrary parameters of each portion of the plethysmogram were set up. The effect of autonomic blocking agents was also studied in healthy subjects.

[RESULTS] Each parameter of the differentiated plethysmogram was negatively correlated to age. Twice differentiated plethysmogram was revealed a lower response in subjects taking β-blocker in healthy subjects. No significant difference of the parameters was observed between diabetics and control subjects in terms of the effect of the modality of treatment and control state of blood glucose. In diabetics aged over fifty years with complications parameters of plethysmogram were lower than those without complications.

[CONCLUSION] Differentiated plethysmography seems to become an indicator of diabetic complications irrespective of modality of treatment and blood glucose level.

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PROGNOSTIC SIGNIFICANCE OF CORRELATION BETWEEN PERIPHERAL NEUROPATHY AND CARDIAC AUTONOMIC NEUROPATHY IN NIDDM

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Fifty one cases of NIDDM were examined for the Peripheral Neuropathy (PN) and Cardiovascular Autonomic Neuropathy (CAN). Clinical scoring method of Valk-et-al was adopted for evaluation of PN and bed side clinical tests advocated by American Diabetic Association were performed to detect CAN. Mean age of the patients was 52.2 years and mean duration of Diabetes was 7.4 years. Peripheral Neuropathy was present in 56.7% cases of which 27.4% had mild, 19.6% moderate and 9.8% had severe neuropathy. Cardiovascular Autonomic Neuropathy was present in 23.1% cases. Statistical analysis showed that PN score had a strong positive Correlation with CAN score (0.7113, $p < 0.0001$). Peripheral Neuropathy correlated best with Postural hypotension test (0.6273) followed by 30:15 ratio (0.6233), hand grip test (0.4938) and deep breathing test (0.4937). No correlation was found with resting heart rate and Valsalva maneuver. The severity of symptoms of PN demonstrated a significant rise in the number of test positive for CAN. This positive correlation can be explained on the basis of similar pathogenic mechanism involving Microvascular function defects. We conclude that the presence of severe Peripheral Neuropathy in NIDDM indirectly indicate presence of Cardiovascular Neuropathy and therefore these patients are at a increased risk of Sudden Cardiac death.

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RENAL TRANSPLANTATION: A CURE FOR DIABETIC GUSTATORY SWEATING

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Gustatory sweating (GS) is very common in diabetic nephropathy, and complete resolution after renal transplantation has recently been reported in a few cases. We studied patients who had undergone renal transplantation for diabetic nephropathy to determine how common the phenomenon is. 31 subjects were questioned about gustatory sweating and had an assessment of neuropathy at a mean (range) time after transplantation of 36(2-108) months. 20/31 reported gustatory sweating prior to transplantation and in 11/20, GS completely disappeared post-transplantation. A further 3/20 noted partial improvement. In 1 subject who reported improvement, sweating in response to food was measured before and after transplantation by measuring the increase in weight of absorbent dressings. Prior to transplantation, GS was 9.3mg/cm^2, falling to 1.5mg/cm^2 post transplant. There were no differences between improvers and non-improvers with regard to age at transplantation, diabetes duration or type, sex, sympathetic function or post-transplant creatinine or immunotherapy. There was a trend towards worse somatic neuropathy in the improvers, as measured by the median (IQR) vibration perception threshold (38V(23-47) vs 16V(8-49)) and modified neuropathy disability score (8(6-10) vs 2(0-9)), but these differences did not reach statistical significance. Although GS is common in nephropathy, complete or partial resolution occurs in 70% of patients undergoing renal transplantation. The mechanism of this change remains unclear, but the observation indicates that aberrant nerve regrowth is untenable as a cause of GS.

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COEXISTENCE OF AUTONOMIC NEUROPATHY AND MICROANGIOPATHY IN DIABETIC SUBJECTS

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The purpose of the present study was to investigate whether microangiopathy (retinopathy or microalbuminuria) is associated with autonomic neuropathy (AN) in diabetic subjects. For this purpose data from 101 diabetic persons (type 1: n=57; type 2: n=44) were analyzed. The battery of the five standardized autonomic function tests (Valsalva test, deep breathing test, heart rate and blood pressure responses from lying-to-standing position and handgrip test) was used. Retinopathy was assessed using the criteria of the Hammersmith protocol. Microalbuminuria was measured by RIA in 3 separate urine collections. Values in the range of 30-299 mg/24-hrs were considered as abnormal. No case exceeding 299 mg/24-hrs was observed. No person had hypertension, clinical and/or ECG findings of coronary heart disease and no one received medications other than antidiabetic drugs. Logistic regression analysis controlling for sex, age, type and duration of diabetes, AN, smoking, blood pressure, HBA_{1c} and type of treatment, showed that the risk for retinopathy significantly increases with AN (odds ratio: 3.66, p=0.03) and systolic blood pressure (odds ratio: 2.44, p=0.02). No other significant association was observed. Several potential explanations could be offered of a common etiopathogenetic mechanism for AN and retinopathy and/or that AN may predispose to the development of retinopathy. No such conclusion can be drawn about microalbuminuria. In addition, we confirmed the association between systolic blood pressure and retinopathy. Thus, the strong association of AN with retinopathy possibly indicates a common etiopathogenetic mechanism or that AN may be a risk factor for retinopathy.

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RELATIONSHIP OF AUTONOMIC DIABETIC NEUROPATHY AND PROSTAGLANDIN E1 RESPONSE IN IMPOTENT DIABETIC MEN

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The aim of the study was to explore the relationship of autonomic diabetic neuropathy (ADN) and response to intracavernosal application of prostaglandin E1 (PGE1) in impotent diabetic men. It is assumed that ADN does not play an important role in the erection response to PGE1 application and that it is mainly influenced by the vascular status of the penis. We examined 71 diabetic patients (18 Type 1, 53 Type 2), median age 51.5 years, median diabetes duration 9.5 years.All patients were assessed by the standard battery of cardiovascular tests (ProSciCard system) of which 6 tests of R-R interval were evaluated (coefficient of variation at rest, power spectrum at low and mid frequency bands in the supine position, mean circular resultant during deep breathing, max/min 30:15 ratio, and Valsalva index) as well as fall in systolic blood pressure on standing. All patients were tested by intracavernosal aplication of PGE1 starting with 10 ug and increasing up to 40 ug until sufficient erection for intercourse was achieved (positive reaction). During this procedure 54 patients achieved sufficient erection. The number of pathologic autonomic function tests correlated significantly with the response to PGE1 (Gamma statistic=-0.321, p=0.03). However, when age, diabetes type, diabetes duration and number of pathologic tests were entered in a logistic regression model with the positive or negative reaction to PGE1 as the outcome variable, none of the regression coefficients quite reached statistical significance at the 95% level (age B=-0.08,p=0.19; duration B=0.06, p=0.23; type B=-0.19, p=0.86; number of pathologic tests B=-0.32, p=0.09). Only 11% of the variance in the outcome variable was explained by the selected set of predictors. However, possibly through its' relation to variables not measured in this study, the significant correlation and the p-value of the autonomic tests regression coefficient (close to the conventional level of 0.05) indicate that it is likely that a higher number of pathologic autonomic tests is related to a negative response to PGE1.

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EFFECT OF METABOLIC CONTROL ON PROGRESSION OF DIABETIC AUTONOMIC NEUROPATHY

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The role of strict metabolic control in the treatment of established diabetic autonomic neuropathy remains an important but controversial issue. The DCCT report has shown delay in onset and retardation in the progression of complications of diabetes in all groups of diabetic patients by intensive and meticulous glycemic control. 50 patients of diabetes mellitus (both IDDM and NIDDM) were selected with typical symptoms, signs and positive bed side tests of autonomic neuropathy. All the patients were followed for three months during which strict metabolic control was achieved by routine treatment with oral hypoglycaemic agents and/or insulin, simply by change in their previous treatment dosages and better attention to diet and physical activity. 22% patients showed significant improvement in symptoms of autonomic neuropathy. 42% showed partial improvement and 36% patients did not show any improvement. Improvement in objective test score was significant in 18%, partial in 46% and insignificant in 36%. Improvement in neuropathy did not correlate with HbA1C levels. 36% patients did not show any subjective or objective improvement in autonomic neuropathy inspite of good glycaemic control as indicated by normal HbA1C levels in them.

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BLADDER DYSFUNCTION IN DIABETIC MEN WITH AUTONOMIC NEUROPATHY. A.Z.Ibrahimov.Azerbaijan medical university,Baku,Azerbaijan Republic.
The aim of this study was to examine the functional state of urinary bladder in men with IDDM Three groups of subjects were examined:gr.1-healthy men(n=7);gr.2-5 men with IDDM without autonomic neuropathy(AN) and gr.3- 6 IDDM patients with AN.Mean age of three groups and average duration of IDDM in gr.2 and 3 were practically same.Diabetic AN was diagnosed with battery of tests(Valsalva,deep breathing,active ortostatic test,heart rate variation in rest).The bladder function was assessed with uroflowmetry, retrograde cystometry and profilometry of urethra.All diabetic patients(gr.2 and 3) had reservoir disorders:decrease of detrusor tonus(gr.1-1,65+-0,140;gr.2-0,95+-0,144;gr.3-0,95+-0,121; p<0,001);increase of threshold sensibility to fill(gr.1-144+-12,4 ml,gr.2-282+-62,5 ml,gr.3-326+-36,4 ml,p<0,001)and increase of the maximal bladder volume(gr.1-286+-13,3 ml,gr.2-733+-66,3 ml,gr.3-897+-98,8 ml,p<0,001).Empty disorders was revealed in gr.3.Diabetic men with AN had decrease maximum(Qm)and average(Qa)urinary flows(Qm-13,98+-1,92 ml/sec,Qa-7,2+-1,45 ml/sec, p<0,01),increase voiding time(52+-8,5 sec,p<0,01 flow time(49+-8,4 sec,p<0,05)and time to peak flow(18,0+-4,11 sec,p<0,05).Thus,diabetes mellitus leads to reservoir dysfunction of bladder. Diabetic autonomic neuropathy results in reservoir-empty disorders of urinary bladder.

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Cardiovascular Reflexes in Children and Adolescents with Insulin Dependent Diabetes Mellitus

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Objective: To assess the usefulness of specific cardiovascular reflex test (CVRs) in children and adolescents with IDDM and to estimate the prevalence of cardiovascular reflex abnormalities among young IDDM patients.

Abstract: The study included 100 young IDDM patients. Their age ranged from 4,42 to 30.25 years (mean 12.53 ± 4.5) of whom 55 were males and 45 females. Five non-invasive CVR tests namely HR-deep breathing mean, HR 30/15 ratio, valsalva ratio, blood pressure, response to standing and hand grip tests were done to detect cardiac autonomic neuropathy. The 5th percentile for 42 non-diabetic children (age and sex matched) was chosen as the lowest limit of normal for the tests. In 100 patients who had the full battery of investigation, 49% showed abnormality; 24% one test abnormal, 13% two tests abnormal and 12% three tests abnormal. None of our patients showed abnormality in four or five tests at the same time. 28% of diabetic patients showed abnormalities in HR-DRm, 20% in the 30/15 ratio and the hand grip test, 13% in the valsalva ratio and only 5% in the postural blood pressure difference. Symptoms of autonomic neuropathy was present in 37 patients (37%), 27 of which had abnormal cardiovascular reflexes. Autonomic neuropathy symptoms was highly related to the results of CVRs. The results of this study also showed that CVRs was highly related to age of the patient (P value - 0.001), accordingly age dependent indices would be needed in children. Also duration of illness was related to results of CVRs (P value = 0.005). Sex did not show any difference between those with normal or abnormal reflexes. Glycemic control as assessed by mean HBA,C showed significant relation to CVRs.

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THE EVALUATION OF ESOPHAGEAL MOTOR DISORDERS IN IDDM

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Esophageal motor abnormalities are an early sign of neuropathy in diabetic patients. Early diagnosis and treatment are essential. The clinical symptoms, which are correlated with radiological, manometric and scintigraphic data, may diagnostiċ esophageal motility disorders. Twenty patients with an over 5 year insulin dependent diabetes presenting clinical signs of episodic esophageal syndrome – dysphagia, retrosternal chest pain, heartburns, esophageal reflux – has been investigated. Contrast esophagogram has been used for excluding an ulcer, cancer, hiatal hernia and resected stomach in these patients. Esophageal manometry studies entailed the recording of esophageal peristalsis, lower esophageal sphincter (LES) pressure, and swallow response. Esophageal sequential scintigraphy (ESS) with choloidal ^{99m}Tc – labeled liquid bolus – was used for calculating total and segmental esophageal transit time and residual radioactivity. A 90% correlation index between the manometric changes and prolonged esophageal transit time was found, thus confirming the sensitivity of ESS. The mean pressure in LES was 20.5±8.1 mmHg, and an incomplete swallow relaxation was found in 5 patients. Within the esophageal body, the manometric studies have revealed the absence of deglutitive contractions in the distal esophagus, segmental contractions, multiple pressure peaks in response to a single swallow. With ESS, a prolonged esophageal transit time, especially in the distal esophagus (11.5± 1.3sec, normal 8.5±1.5sec) with TE_3 10.1±1.3sec (normal 7.6±0.8sec) was recorded. The use of ESS, a cheap and reproductible technique, as a screening test for early diabetic neuropathy is suggested.

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DIAGNOSTICS OF THE AUTONOMIC DIABETIC NEUROPATHY IN CHILDREN.

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Autonomic diabetic neuropathy (ADN) is well known complication of Diabetus melitus (DM). But a diagnostics of this complications in children have not worked out up to now.The aim of our investigation was elaborating of a simple and reliable method for revealing autonomic disorders of children with DM. It was investigated 116 practically healthy children and 48 with IDDM(age from5to 15 years old). It was carried out, the following tests on children:Valsava maneuver; deep dreathing test;HR variations in supine resting position(50 intervals RR); HR response to standing up(30 intervals RR after standing up).The investigations showed impossibility of the conducting the Valsava test in the majority of children under 10 years old.On the base of the other test data, we elaborated the complex ECG-index that is the sum of differences of maximal and minimal RR of the three tests. The index more than 410 should consider as normal one, less than 350 as patology. All the others indicies are the border ones.ADN was diagnosed in 15 children with IDDM from 48 investigated ones(31 6,7%). Thus, it was elaborated a simple method of ADN diagnostics in the children.

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HYPERINSULINEMIA AND ESOPHAGEAL MOTILITY DYSFUNCTION IN PATIENTS WITH SCHISTOSOMIASIS AND DIABETES MELLITUS

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In this study patients with hepatosplenic schistosomiasis and DM were studied for esophageal motility and serum insulin level. It comprised 30 patients and 7 control normal subjects. Patients were classified into: (a) Group with DM (b) Group with hepatosplenic schistosomiasis (with and without ascites). (c) Group with both hepatosplenic schistosomiasis and DM. There was significant decrease in LES pressure in patients with Type 1 or Type 2 DM. compared to the control group (P 0.016). There was significant decrease in the velocity and distal amplitude of peristalsis in hepatosplenic schistosomiasis with ascites compared to control group (P= 0.021 and 0.043 respectively). Also, significant increase in the percent of the retrograde waves in hepatosplenic schistosomiasis with ascites and DM compared to hepatosplenic schistosomiasis without ascites (P= 0.034). There was significant decrease in percent of full relaxation of LES in hepatosplenic schistosomiasis with DM compared to DM. There was significant decrease in the LES pressure in hepatosplenic schistosomiasis (with and without ascites) with DM compared to the control group (P= 0.014). There was significant elevation of insulin levels in hepatosplenic schistosomiasis with ascites than without ascites (P=0.032).The study revealed significant positive correlation between insulin level and LES pressure in hepatosplenic schistosomiasis (without ascites) with DM & highly significant negative in hepatosplenic schistosomiasis (with ascites) with DM . There was highly significant positive correlation between insulin level and mid amplitude in hepatosplenic schistosomiasis without ascites & significant positive correlation with proximal amplitude in hepatosplenic schistosomiasis with ascites We concluded that ascites decreases LES pressure ,velocity, distal amplitude of peristalsis and the percent of primary peristalsis. DM decreases LES pressure, combined DM & Bilharziasis produce more abnormalities than each disease alone. Hyperinsulinemia in those patients increases proximal and mid amplitudes of peristalsis and percent of tertiary peristalsis and increases LES pressure in absence of ascites.

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EFFECT OF NITRATES AND CALCIUM CHANNEL BLOCKERS ON AUTONOMIC REFLEXES IN NIDDM PATIENTS

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Effect of nitrates and calcium channel blocking drugs on cardiovascular autonomic reflexes was studied in ten NIIDM patients who were newly detected to have hypertension (HT) and ischemic heart disease (IHD). Five standard tests for autonomic reflexes were performed before starting isosorbide dinitrate (10 mg 6 hourly) and nifedipine (10 mg 8 hourly). The tests were repeated after 2 days. The results were as follows. 1. Rise in diastolic blood pressure (DBP) on isometric hand grip (Mean±SD in mmHg): 18.4±15.3 (pretreatment) Vs 9.8±7.3 (on treatment), (p=0.05) 2. Fall of DBP on 70 degree head up tilt (mean±SD in mmHg): 5.4±9.8 (pretreatment) Vs 9.8±8.0 (on treatment), (p<0.05). 3. Expiratory inspiratory ratio (on deep breathing), Valsalva ratio, 30:15 ratio & systolic blood pressure response on head up tilt as well as DBP response to cold immersion didn't change significantly. However, the overall autonomic status, as assessed by Ewing's score, worsened from 4.80±2.70 (pretreatment) to 5.80±2.04 (on treatment), (p<0.05). Thus calcium channel blockers and nitrates, given together, can cause significant worsening of autonomic reflexes in NIDDM patients with HT and IHD.

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MULTIPLE-SITE GASTROINTESTINAL MOTILITY DISORDER IN DIABETIC AUTONOMIC AND SENSORY NEUROPATHY

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The aim of this study was to determine the pattern of motor dysfunction at different levels of the gastrointestinal (GI) tract in patients with long-standing diabetes mellitus (DM) and to evaluate the influence of autonomic and sensory diabetic neuropathy on GI motility. *Methods*: 17 diabetic patients were studied (duration of DM: 20.6±3.3 years, BMI: 26.1±1.6, age: 53±3.7 years, mean±SE). GI motility was investigated by means of manometry. The motilities of the pharynx, upper and lower esophageal sphincters, esophageal body, stomach, and external and internal anal sphincters were measured. To determine the severity of autonomic neuropathy, five standard cardiovascular tests were performed and a score was calculated. The sensory nerve function was studied with a Neurometer (Neurotron Inc., Baltimore MD), using constant sine wave transcutaneous nerve stimulation. *Results*: The mean autonomic score of the patients was 5.6±0.5 and all had increased current perception thresholds at 2 kHz and 250 or 5 Hz (median nerve: 3.57±0.49, 3.20±1.07 and 2.21±1.07; peroneal nerve: 5.64±1.02, 4.77±1.26 and 4.12±1.27 mA, respectively). The presence of an esophageal body dysfunction of varying severity was detected in all patients (17), while abnormalities of lower esophageal sphincter relaxation were observed less frequently (11). Ano-rectal motility disturbance was found in 13 patients. There was an impaired gastric motor function in 12 cases, 7 of these involving the absence of a migrating motor complex phase III, i.e. diabetic gastroparesis. Analysis of the motor functions tested revealed at least three concomitantly impaired regions in most patients (14). *Conclusions:* High frequencies of pathologic esophageal body, gastric and ano-rectal motility parameters were found in diabetic patients with moderate autonomic and severe sensory neuropathy. The data confirm the hypothesis that a multiple-site motility disorder is present in long-standing DM. The observed coexistence of long-term DM, diabetic neuropathy and GI motility disorders emphasizes the necessity for further clinical and experimental investigations in this field.

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FACTORS DETERMINING THE 24H BP PROFILE IN NORMOTENSIVE IDDM AND NIDDM PATIENTS

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To evaluate the relative influence of clinical data, autonomic neuropathy (AN), and albuminuria on 24h blood pressure (BP) profile in IDDM and NIDDM, we studied 43 IDDM (age: 40±11, duration: 18±9 yr), and 31 NIDDM (age: 53±6, duration: 14±7 yr) normotensive non-proteinuric patients. Cardiovascular AN, 24h BP, and urinary albumin excretion rate (UAE) on simultaneous urine collections timed day and overnight were measured. IDDM patients with AN showed lower day-night difference in systolic (ΔsBP: 3±9 vs 9±5%, p<0.01) and diastolic BP (ΔdBP: 8±10 vs 15±5%, p<0.01) than those without AN. Microalbuminuric IDDM patients had higher night BP (119±16/74±6 vs 107±12/64±7 mmHg, p<0.01), and lower ΔsBP (2±17 vs 8±6%, p<0.05) and ΔdBP (6±11 vs 14±7, p<0.01) than normoalbuminuric IDDM patients. In NIDDM patients with AN, ΔdBP was significantly lower than in those without AN (4±8 vs 10±4%, p<0.05), while no difference was found in relation to the presence of microalbuminuria. In IDDM patients ΔsBP and ΔdBP were related to cardiovascular tests and to autonomic score, index of tests impairment (r= -0.56, p<0.0001), but also to night UAE (r=-0.44, p<0.01). In NIDDM patients both ΔdBP and ΔsBP were related only to cardiovascular tests and to autonomic score (r=-0.50, p<0 005). We performed a stepwise regression analysis including sex, age, body mass index, smoking, diabetes duration, HbA_{1c}, serum cholesterol, autonomic score, and night UAE as independent variables, and ΔBP or 24h BP as dependent variables. We found that in IDDM autonomic score and night UAE were the variables of primary importance for ΔsBP and ΔdBP, while HbA_{1c}, cholesterol, body mass index, and diabetes duration were all related to 24h BP. In NIDDM autonomic score was the major determinant not only of ΔsBP and ΔdBP, but also of 24h BP. In conclusion, in both IDDM and NIDDM normotensive patients the day-night variation in BP is dependent on autonomic function. In IDDM the strong relationship between AN and nephropathy does not allow a full discrimination of their relative role. In NIDDM the weaker relationship between BP and albuminuria makes more easily detectable the influence of AN on 24h BP pattern.

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THE USEFULNESS OF RADIOPAQUE MARKER (KG-S10) TO EVALUATE THE DIGESTIVE TRACT MOVEMENT IN NIDDM.

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AIM : Diabetic gastroenteropathy is speculated to induce postprandial hyperglycemia in diabetics. The aim of this study was to access the usefulness of radiopaque maker, KG-S10, and to elucidate impaired digestive tract movement in patients with NIDDM. METHODS : KG-S10 is a gelatin capsule including 20 pellets of polyvinyl chloride rings (4.5×1.0mm). The KG-S10 was given orally just before intake of a test meal to 12 patients with NIDDM who had a symptomatic diabetic gastroenteropathy (age 75±9 yrs ; duration of DM 20±3 yrs; HbA_{1c} 9.2±1.3%). The serial abdominal plain radiograms were taken until the marker could not be detected by radiography. The markers were counted at 7 parts on the abdominal radiograms. RESULTS : Diabetics showed a shorter calculated segment passing time(hour) in stomach (1.4±0.6vs.4.1±2.5), longer times in upper small intestine, pelvic part of small intestine, ascending colon, transverse colon, and descending colon (3.3±2.3vs.1.9±1.7, 8.3±10.4vs.5.7 ±4.7, 10.2±8.0 vs.6.6±5.3, 9.4±7.9vs.6.6±5.3, and 21.1±21.2vs.6.6± 3.1, respectively) , and comparable time in sigmoid colorectum (5.9±5.8vs.5.9 ±5.8), as compared with those of healthy subjects. The patients with constipation had a longer segment passing time in colon than the patients without constipation. No adverse effect was observed except X-ray radiation on abdomen. CONCLUSION : The KG-S10 makes it possible to quantitatively and less-invasively evaluate the movement at each digestive tract part through stomach to rectum without specific facilities. Compared with healthy subjects, NIDDM with symptoms of diabetic gastroenteropathy showed shorter segment passing time in stomach and longer times in upper small intestines and in the subsequent parts, which might worsen postprandial hyperglycemia.

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24-H-BLOOD PRESSURE BEHAVIOR WITH INCREASING AUTONOMOUS NEUROPATHY OF THE CARDIOVASCULAR SYSTEM

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Autonomous neuropathy of the cardiovascular system (ANP) is connected to a high cardiovascular mortality rate. The present study examines secondary changes in blood pressure behavior, in relation to the severity of ANP, using 24-hour ambulatory blood pressure monitoring (ABPM). 94 type I and type II diabetics with a mean diabetes duration of 22±10 years ($\bar{x}$±SD) and without pre-existing antihypertensive medication or ouvert nephropathy underwent ABPM, with statistical attention being paid for the first time to the actual time of falling asleep, as well as a standardized test battery for ANP (heartbeat variation at rest, heart rate analysis, deep respiration and the Ewing test). With an increase in the number of pathological ANP tests (group 1 to 5), the proportion of non-dippers (<10% blood pressure day/night drop) increased from 29 via 40, 58, 70 to 73%. The systolic day and night mean values differed significantly in group 1 from those in group 5 (132±9 vs. 135±17 and 116±10 vs. 130±10 mmHg respectively; $p<0.05$), as did the subsiding reduction in pulse rate at night (-24±12 vs. -14±9 bpm; $p<0.005$). The ANP degree of severity did not correlate with the duration of diabetes, but with metabolic quality (HbA1c), existing retinopathy and peripheral sensitive neuropathy ($r>0.34$; $p<0.001$). ANP can lead to an increase in blood pressure and pulse rate at night, indicating principal damage to the parasympathetic nervous system. Long term, the predominance of adrenergic stimulation could be partly responsible for cardiac damage with diabetes mellitus. The occurrence of ANP seems more likely to be linked to metabolic control than to the duration of diabetes. Regarding secondary prevention with existing diabetic ANP, importance should be attached to thorough diagnosis, a consistent reduction in blood pressure, restoration of physiological dipping, as well as optimum metabolic control, in order to lessen the cardiovascular risk to these patients.

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HYPERGLYCAEMIA SLOWS GASTROINTESTINAL MOTILITY IN PATIENTS WITH DIABETES MELLITUS

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It has been supposed that gastric emptying time is affected by blood-glucose-concentration. We measured the effect of different blood-glucose-concentrations (glc) on gastric emptying (GE), gallbladder motility (GBM) and small bowel transit time (SBTT).

10 patients with diabetes mellitus were tested (8 IDDM, 2 NIDDM; 2 women, 8 men; age 38.2y (28y-56y), HbA1c 7.6%+/-1.25, duration of diabetes 15.8y in mean). Using glucose-clamp-technique (Biostator) glc was 5.5mmol/l during one and 14mmol/l during the other test and an observation period of 4 hours postprandial each. GE was measured by ultrasound using antral cross sectional area. GBM was determined by ultrasound using maximal area in longitudinal axis. SBTT was measured by breath hydrogen analysis. Test meal was a solid-liquid-multicomponent meal of 1692kJ plus 20g lactulose.

In analyses of multivariance GE was delayed significantly when glc was 14mmol/l ($p<0.05$; emptying time about 180min postprandial with glc=5.5mmol/l vs. 240min with glc=14mmol/l). Postprandial GBM was different ($p<0.001$), especially refilling was slower with glc=14mmol/l. SBTT also was significantly different ($p<0.01$): SBTT was about 95min with glc=5.5mmol/l vs. about 135min with glc=14mmol/l.

These results demonstrate a strict dependence of the described parameters of gastrointestinal motility on blood glucose in patients with diabetes mellitus. Reducing postprandial blood glucose, f.e. by using a short acting insulin analogue, could be an alternative to prokinetic drugs, which often fail especially in long time treatment of gastroparesis diabeticorum.

(partly supported by Institut Danone für Ernährung, Munich, Germany)

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BAROREFLEX DURING AN ORTHOSTATIC TEST (SQUATTING) IN DIABETIC PATIENTS WITH CARDIAC AUTONOMIC NEUROPATHY

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We studied the influence of diabetic cardiac autonomic neuropathy (CAN), assessed by decreased respiratory arrhythmia during deep breathing, on the baroreflex during an orthostatic test. Changes in mean arterial blood pressure (MAP) and heart rate (HR) were measured continuously with a Finapres^R device (Ohmeda, USA) during an active orthostatic test (successively 1 min standing; 1 min squatting; 1 min standing) in three groups of subjects similar for sex, age, height and body weight : 41 healthy controls, 32 diabetic patients without CAN (R-R E/I : 1.32 ± 0.02) and 23 diabetic patients with CAN (R-R E/I: 1.07 ± 0.01). In initial standing position, HR was higher ($p < 0.01$) in both diabetic groups (87 ± 2 and 92 ± 3 min^{-1}, respectively) than in controls (79 ± 1 min^{-1}). In squatting position, MAP increase was greater ($p < 0.01$) in diabetic patients with CAN. After standing up, diabetic patients with CAN were characterized by a greater (39 ± 2 mm Hg, $p < 0.05$ *versus* 31 ± 1 in controls and 32 ± 2 in diabetic patients without CAN) and more prolonged ($p < 0.02$) drop in MAP and by a markedly decreased reflex tachycardia (10 ± 1 min^{-1}, $p < 0.0002$ *versus* 20 ± 1 min^{-1} in controls and 19 ± 2 min^{-1} in diabetic patients without CAN). The baroreflex gain calculated during the hypotension phase (HR increase/MAP drop, expressed in beat/mm Hg) was markedly dampened in diabetic patients with CAN (0.27 ± 0.03, $p < 0.0001$) when compared to that of diabetic patients without CAN (0.74 ± 0.10) and that of controls (0.75 ± 0.08). In conclusion, non-invasive beat-to-beat monitoring with the Finapres^R device during a squatting test allows to clearly demonstrate major disturbances in the baroreflex of diabetic patients with cardiac autonomic neuropathy.

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INSULIN PER SE MODIFIES GASTROINTESTINAL SENSORY RESPONSES TO DUODENAL DISTENSION IN NORMAL SUBJECTS.

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Recent studies have demonstrated that in healthy individuals physiological hyperglycaemia (~10mM) modulates visceral sensations arising from the gastrointestinal tract. Concurrent hyperinsulinaemia may be the putative mediator of this phenomenon. The present study investigated the effect of physiological changes in blood insulin concentration on the perception of duodenal distension in 7 healthy volunteers. Measurements were performed during normoinsulinaemia (50pM) and mild hyperinsulinaemia (300pM) in randomized order and cross-over fashion, i.e. hyperinsulinaemia-normoinsulinaemia (H/N) vs. normoinsulinaemia-hyperinsulinaemia (N/H). Gastrointestinal sensory responses were evaluated at baseline and after repetitive proximal duodenal distension which was performed with a non-compliant ultrathin bag during phase I activity. Intrabag volumes increased by 4 mL at each distension step (from 12 to 48 mL), which lasted 2.5 min in 10 min intervals. Perception of duodenal distension was scored from 0=no perception to 6=pain. Gastrointestinal sensations were assessed using a visual-analogue scale after each distension. Perception threshold for duodenal distension was lower during hyperinsulinaemia: 18.7±1.5/20.0±1.6 (H/N) vs. 22.7±2.4/18.6±1.2 (N/H); $p<0.05$. At all intrabag volumes perception of duodenal distension was higher during hyperinsulinaemia, e.g. at 48mL: 5.7±0.2/4.7±0.5 (H/N) vs. 4.8±0.3/5.5±0.2 (N/H); $p<0.05$. The gastrointestinal sensation fullness was experienced more intensively during hyperglycaemia, e.g. at 48 mL: 10.8±5.3/7.5±4.4 (H/N) vs. 12.2±5.6/17.0±7.6 (N/H); $p<0.05$), hunger less intensively, e.g. at 48 mL: 3.2±1.9/20.0±8.3 (H/N) vs. 10.8±6.5/2.5±1.6 (N/H); $p<0.05$. Physiological changes in blood insulin concentration have a major impact on the gastrointestinal sensory responses to duodenal distension in healthy individuals.

2249

IMPAIRED BLOOD PRESSURE RESPONSE TO STANDING IS ASSOCIATED WITH DIMINISHED DIURNAL BLOOD PRESSURE INDICES IN IDDM

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Cardiovascular reflexes are essential in the diagnosis of autonomic neuropathy. Blood pressure (BP) responses to standing and sustained handgrip (SH) reflect mainly sympathetic function. However, there are no data whether changes in BP during standing and SH are related to diurnal variations of BP and to hypertension itself or not. To evaluate these connections 60 patients with IDDM (mean age: 40,7 ± 14,2 ys; mean duration of diabetes: 20,4 ± 12,1 ys) were studied. The five standard reflex tests of cardiovascular autonomic function were applied. Twenty four hour long blood pressure monitoring (ABPM) was performed by Meditech ABPM 02 device. Decrease of systolic BP after standing correlated significantly positively with systolic ($p<0,01$) and diastolic ($p<0,05$) diurnal BP indices that is higher diurnal indices were associated with higher increase or diminished fall of systolic BP after standing. Mean casual BP values were 122,7±3,9/78,5±10,9 mmHg while mean ABPM values were 116,6±15,7/73,9±11,2 mmHg. The degree of orthostatic hypotension correlated significantly negatively ($p<0,05$) with higher casual systolic BP not as with systolic ABPM values. No significant correlations were found with SH test. Conclusions: impaired BP response to standing is associated with diminished diurnal BP variations in IDDM. ABPM is suggested to perform in patients with orthostatic hypotension and vice versa: "non-dippers" should be screened for postural hypotension. Impaired BP regulation to standing as well as diminished diurnal BP indices may contribute to poor prognosis of autonomic neuropathy in IDDM.

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THE EFFECT OF EUGLYCAEMIC HYPERINSULINAEMIA ON GASTRIC EMPTYING IN IDDM

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Hyperglycaemia (blood glucose~15mmol/l) delays gastric emptying in both normal subjects and in patients with diabetes but whether hyperinsulinaemia has an effect remains debatable We looked at the effect of two different levels of hyperinsulinaemia, with concomitant euglycaemia, on gastric emptying in 7 male patients with IDDM None had evidence of autonomic neuropathy. Mean diabetes duration was 14.6±10.7 years and mean HbA_{1c} was 6.9±0.7%. After an overnight fast the subjects were infused with insulin, at 40mU/m^2/min on one occasion and at 80mU/m^2/min on the other, with 20% glucose given simultaneously to maintain euglycaemia. Steady-state glucose infusion rate was ensured before the subjects ate a meal consisting of a pancake (labelled with 3MBq non-absorbable ^{99m}Tc) and milkshake (labelled with 0.33 MBq non-absorbable ^{111}In-DTPA), providing 400 kcal (57% carbohydrate) Gamma-scintigraphic images were then obtained at 20 min intervals for the next 3-4 hours to quantify gastric emptying. There was no difference in the solid t_{50} or the liquid t_{50} between visits (132.5±10.0 min v 124.0±9.6 min for solid and 34.3±7.1 min v 38.6±6.0 min for liquid respectively, $p>0.05$, paired t-test). CCK and GLP1 hormone responses were similar ($p>0.05$, ANOVA for repeated measures) following the meal on the two occasions. Thus our data show that there is no effect of the two different levels of hyperinsulinaemia on gastric emptying of a solid and liquid meal in IDDM subjects.

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MEALTIME SC INSULIN INJECTION CAUSES ORTHOSTATIC HYPOTENSION IN DIABETIC AUTONOMIC NEUROPATHY DESPITE ADAPTATION TO PROLONGED ORTHOSTATISM.

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To assess whether the therapeutic prandial subcutaneous (sc) insulin injection, or the meal *per sè*, has appreciable haemodynamic effects in patients with diabetic autonomic neuropathy (DAN) adapted to prolonged orthostatism, 11 patients without DAN (DAN-), 6 with DAN but no orthostatic hypotension (DAN+OH-), 6 with DAN and OH (DAN+OH+) and 6 nondiabetics (N) were studied on 4 occasions (S1-S4) at 2 week intervals. In all studies subjects remained seated for 2 hours before, and for 3½ hours after 0.2 U/kg insulin injected sc 30 min prior to ingestion of a standard mixed meal (in N no insulin was injected) (S1). In S2, all subjects fasted (diabetics had 0.15 mU/kg/min insulin infused to prevent ketosis). In S3 (diabetics only) meal, but no sc insulin was given (i.v. insulin as in S2). In S4, all subjects had sc insulin but glucose was infused to maintain euglycaemia. In S1, mean blood pressure (MBP) did not change neither in DAN-, nor in N due to simultaneous *increase* in forearm peripheral vascular resistances (PVR) by 4.23± 0.03 mmHg/ml/100ml/min. In contrast, in DAN+OH- and DAN+OH+, MBP decreased between 60-210 min by 9.7±0.07 and 12.4±0.08 mmHg (patients experienced symptoms of faintness), because PVR *decreased* by 5.21±0.03 and 6.38 ±0.05 mmHg/ml/100ml/min ($p<0.05$ vs DAN-). Plasma noradrenaline (NA) increased to 2.17±0.04 nmol/l in DAN-, 2.62±0.06 nmol/l in DAN+OH-, but only to 1.33±0.05 nmol/l in DAN+OH+ (N 1.76±0.03 nmol/l, $p<0.05$). In S3 MBP did not decrease in DAN-, whereas it decreased by 4.55±0.03 mmHg in DAN+ without changes of FVR. In S4, MBP did not change in N and DAN-, but it did in DAN+ as in S1. Thus, in DAN+ prolonged adaptation to orthostatism does not prevent the haemodynamic effects of therapeutic hyperinsulinaemia which unmasks overt or latent defects of NA release. The meal *per sè* may contribute to decrease in MBP, likely by reducing splanchnic vascular resistance.

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GASTRIC EMPTYING IN INSULIN-DEPENDENT DIABETES MELLITUS: LONG-TERM EFFECTS OF CISAPRIDE VERSUS PLACEBO.

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In many patients with insulin-dependent diabetes mellitus, slow gastric emptying makes the delivery of ingesta into the small intestine and the time of their absorption unpredictable. Hence, insulin is administered at inappropriate time points and poor glucose control ensues. Prokinetic agents were found to accelerate emptying, but controlled studies in long-term effects are scarce. We investigated the effects of 10 mg cisapride QID for 8 weeks, as compared to placebo, on gastric emptying and glucose control under random, crossover, double-blind conditions. Ten males and 13 females, age 25-67 years (median (M) 45 yr), Body Mass Index 19.0-32.8 kg/m^2 (M 23.9 kg/m^2), illness duration 3-48 yr (M 20 yr), completed the study. None took other drugs affecting gastrointestinal function. Emptying of a radiolabelled semisolid 1168 kJ meal was recorded before and after 8-weeks' drug intake as well as after a 4-week wash-out followed by another 8-weeks' drug intake. At the study's start, 14 of the 23 patients had a delayed emptying, their residual radioactivity (RRA) 50 min after meal ingestion exceeding 48 healthy subjects' mean RRA+1.5 SD, i.e. 73.2 %. Of these 14 patients, 7 had cardiovascular autonomic neuropathy (CANP) as compared to 1 of the 9 with normal emptying. Cisapride accelerated emptying in 8 of the 14 patients with delayed and 7 of the 9 with normal emptying. Emptying was enhanced by cisapride in 11 of the 15 patients without CANP, but only 4 of the 8 with CANP. In those without CANP, the RRA 120 min after meal ingestion was 19.0 %±3.4 SE following cisapride and 24.3± 2.4 % following placebo; in the patients with CANP, these figures were 30.5±9.5 % and 30.6±7.5 %. Glycemic control as revealed by glycosylated hemoglobin was unaffected by cisapride. Conclusion. Cisapride intake over 8 weeks tended to accelerate gastric emptying in patients without but not with CANP, and had no effect on glucose control.

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POSTURAL STABILITY IN DIABETIC NEUROPATHY: IS IT A DEVICE FOR EARLY DIAGNOSIS ?

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Our previous papers have shown that diabetic patients affected by peripheral neuropathy show a typical postural pattern, charactherized by mean sway speed, paths and areas much higher than in normal subjects and not neuropathic diabetics. Altogether, the speed diagrams of the sway show frequent "escapes" from the mean values, and this shape seems to be related only to sensitive defect.
In the present work insuline dependent diabetic patients have been studied, in order to define if the lack of the postural stability is an early symptom of an asymptomatic neuropathy. Twenty patients (13 F, 7 M) and ten healthy subjects (6 F, 4 M) have been submitted to stabilometry by postural platform, Nerve Conduction Velocity, Vibration Perception Threshold by biothesiometer, Neurological Examination performed by a single neurologist to avoiding intra-observers variability, Autonomic Nervous System Functional Evaluation by DB, PH, VR beside methabolic common parameters control. The data of patients are shown in the table. Our results suggest that stabilometric parameters seems to be more sensitive than VPT in discriminating neuropathic patients.

	Normal subjects	Not Neuropathic Diabetics	Neuropathic Diabetics
Age	32 ± 10	37 ± 12	45 ± 6
Sway Path (mm)	139 ± 28	142 ± 24	203 ± 46
Sway Area (mm^2)	168 ± 77	172 ± 105	251 ± 57
Mean Velocity (mm/sec)	18 ± 3.6	21 ± 5.8	49 ± 23
Nerve Conduction Velocity	Normal	Normal	Abnormal
VPT (Volt)	5 ± 2	10 ± 2	15 ± 4
Neurological Examination	Normal	Normal	Abnormal
Autonomic Test	Normal	Normal	30% Abnormal
Duration of Desease (years)		12 ± 6	19 ± 4

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SLOWER, NOT FASTER, GASTRIC EMPTYING OF SEMISOLID MEAL IN NON-INSULIN-DEPENDENT DIABETES MELLITUS THAN IN HEALTH.

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In patients with recently diagnosed as well as with more longstanding non-insulin-dependent diabetes mellitus (NIDDM), the gastric emptying of liquids and solids has been reported to be faster than in healthy subjects. Others, however, found gastric emptying in NIDDM patients either not to differ from the one in healthy individuals or, in a substantial number of cases, to be delayed. The present study was carried out to shed more light on this controverse but, for therapeutic consequences, important topic. Thirteen patients (7 females and 6 males; age 44-68 years, median (M) 56 yr; Body Mass Index (BMI) 22.9-41.3 kg/m^2, M 27.1 kg/m^2; NIDDM duration 1-23 yr, M 5 yr) took part. The gastric emptying of a semisolid meal (1168 kJ) as well as of 250 ml 10 % dextrose solution (420 kJ) was studied scintigraphically at 1-week intervals. A single-headed gamma camera and a correction procedure for posterio-anterior movement of gastric contents were employed. The patients emptied the semisolid meal significantly ($P<0.001$) slower than 55 healthy subjects, the mean residual radioactivity (RRA) in the stomach 50 min after ingestion being 73.3 %±18.0 SD versus 54.0±13.3 %. After the semisolid meal, the RRA in 5 of the 13 patients was greater than the healthy subjects' mean RRA ± 2 SD, i.e., >80.6 %. Two patients had a RRA slightly lower (48.2 % and 40.8 %, respectively) than the healthy subjects' mean RRA. The glucose solution was emptied slightly faster by the patients than by 11 healthy subjects (age 23-52 yr, M 34 yr; BMI 19.4-33.7 kg/m^2, M 22.2 kg/m^2), the mean RRAs 30 min after drinking amounted to 59.0±10.7 % vs 68.1±15.1 %. In the patients, the RRAs for the semisolid meal and the glucose solution were correlated with neither the blood-glucose levels before the emptying studies nor with the levels of glycosylated hemoglobin indicative of glycemic control. Conclusions. The gastric emptying of semisolids but not the one of liquids is slower in patients with NIDDM than in healthy subjects and both are unrelated to actual blood-glucose levels and glycemic control.

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BAROREFLEX SENSITIVITY IN NORMAL AND IMPAIRED GLUCOSE TOLERANCE AND NIDDM

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The deep breathing expiration-inspiration difference in heart rate (EI-value) is used to diagnose diabetic autonomic neuropathy. Easily overlooked is the fact that this EI-value is largely caused by respiration-induced changes in blood pressure (BP, mmHg), which may differ among persons. However, baroreflex sensitivity (BRS, ms/mmHg) is an estimate of the change in heart rate caused by these BP variations and possibly a more sensitive parameter of autonomic function than conventional EI-values, which has recently been claimed to be the case in IDDM. Therefore BRS-estimates and EI-values were studied in relation to glucose tolerance in a population based study. Subjects (50-74 yr-old) were categorized (after two OGTTs) as normal (NGT, n=199), impaired glucose tolerant (IGT, n=125), newly diagnosed diabetes (NDM, n=62) or known diabetes (KDM, n=46). RR-intervals (ms) and systolic BP (SBP, Finapres®) were recorded in supine position during spontaneous breathing (3 min) and during deep breathing at 6/min (1 min). BRS_{spont}, $BRS_{6/min}$ (both transfer function gain), $\Delta SBP_{6/min}$ (absolute changes in SBP) and $EI_{6/min}$ were calculated in a narrow frequency band around 0.1 Hz, including only those spectral points having a squared coherence $\gamma^2>0.5$.

Group	BRS_{spont}	$BRS_{6/min}$	$\Delta SBP_{6/min}$	$EI_{6/min}$
NGT	7.1 ± 4.2	9.1 ± 4.6	19.2 ± 8.9	177 ± 98
IGT	6.1 ± 3.6 #	8.3 ± 4.7	18.6 ± 12.6 #	155 ± 97 #
NDM	5.2 ± 3.4 *	7.6 ± 4.1 †	18.1 ± 7.4 †	146 ± 98 †
KDM	5.3 ± 2.6 †	7.5 ± 3.9 †	16.9 ± 7.7	132 ± 89 *

given are mean ± SD; different from NGT group with * $p<0.01$, † $p<0.05$, # $p<0.1$

BRS_{spont} was significantly lower than $BRS_{6/min}$ (paired t-test, $p < 0.0001$). After transformation, analysis of variance showed that, adjusting for age and gender, BRS_{spont} ($p=0.04$), $BRS_{6/min}$ ($p=0.05$) and $EI_{6/min}$ ($p < 0.01$) decreased with decreasing glucose tolerance while ΔSBP only showed a trend ($p=0.07$). However, BRS did not show a stronger association with glucose tolerance than EI-values did.

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SEX DIFFERENCES IN THE RELATIONSHIP BETWEEN THE QT INTERVAL AND DIABETIC COMPLICATIONS IN IDDM PATIENTS

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QT interval prolongation is thought to be a risk factor for mortality. We examined the relationship of corrected QT (QTc) prolongation with Ischemic Heart Disease (IHD), nephropathy, and retinopathy in people with IDDM. QT intervals were measured and corrected for heart rate in 3147 patients from EURODIAB, which was a study of 3250 men and women from 31 clinics across Europe. IHD was defined by ECGs which were Minnesota coded, nephropathy was assessed using albumin excretion rate (AER), which was measured centrally from a single 24 hours urine collection, and retinopathy was assessed by centrally graded retinal photographs. Mean QTc was 0.412s in men and 0.422s in women ($P<0.001$), and QTc was positively related to HbA1c and age. Therefore all subsequent analyses were adjusted for these confounding factors. In men the mean QTc for those without IHD was 0.410s and was 0.423s for those with IHD ($P<0.001$). In women these values were 0.422s and 0.429s ($P<0.01$). Thus the relationship between QTc and IHD was stronger in men than in women ($P<0.05$ for the interaction). A similar pattern was seen for nephropathy. For men mean QTc for those with AER < 20 mcg/min was 0.411s, for those with AER 20-200 mcg/min was 0.413s and for those with AER > 200 mcg/min 0.418s ($P<0.001$), and the equivalent figures for women were 0.421s, 0.425s and 0.425s ($P<0.08$) ($P<0.09$ for the interaction). Ther was no relationship between retinopathy and QTc in men or women. We show that Qtc is higher in women than men, and the relationship of Qtc with IHD and nephropathy is not as strong in women as in men. Further studies need to be performed to determine the importance of Qtc and it's impact on mortality in both men and women.

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DISTURBANCES OF HEART RATE VARIABILITY AND SPATIAL AND CIRCADIAN QTc INTERVALS IN DIABETIC PATIENTS WITH CARDIAC AUTONOMIC NEUROPATHY

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Increased cardiac mortality due to ventricular arrhythmias has been demonstrated in diabetic patients with cardiac autonomic neuropathy (CAN). The aim of this study was to evaluate the disturbances of heart rate variability (HRV) and spatial and circadian QTc intervals in diabetics with CAN. *Patients and methods:* CAN was assessed by means of five standard cardiovascular reflex tests. 8 healthy controls and 18 insulin-treated diabetics (7 CAN- [score$\leq$1] and 11 CAN+ [score$\geq$2]) were studied. 24-h HRV parameters (mNN, SDNN, SDNNi, SDANN, rMSSD and pNN50; and low-frequency [LF], high-frequency [HF] and total power [TP] spectra) were calculated. The QTc interval dispersion (QTc-d) was defined as the longest *interlead* difference of the standard 12-lead ECG. QTc intervals during a sampling period of 6 s were analyzed from the Holter ECGs every hour and the diurnal fluctuation of QTc was characterized by a circadian index generated by a custom-made computer program. One-way ANOVA and Scheffé's test were applied for statistics. *Results:* No differences in HRV indices and QTc intervals were found between the control and CAN- groups. The two diabetic groups (*CAN- vs CAN+*) differed significantly in SDNN, SDNNi, SDANN, rMSSD, pNN50, HF, LF and TP, but there was no difference in mNN. The circadian fluctuation of QTc was diminished in the CAN+ group. Moreover, CAN+ diabetics displayed an increased QTc-d relative to the CAN- subset. *Conclusions:* 1) CAN+ diabetics exhibit an enhanced spatial inhomogeneity and a depressed temporal variability of the ventricular repolarization (QTc) time; 2) CAN+ diabetics have decreased non-spectral and spectral HRV parameters.

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CLINICAL BENEFIT OF THE SPECTRAL ANALYSIS AND ITS SEGMENTAL INTEGRATION TO ASSESS THE AUTONOMIC NERVE FUNCTION IN DIABETIC PATIENTS

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The clinical usefulness of simultaneous guantitative assessment of sympathetic and parasympathetic function by spectral analysis using the maximum entropy method (MEM) was evaluated. ECG tracings were obtained from 235 normal individuals and 776 patients with diabetes mellitus, and R-R interval variations over 100 beats were subjected to spectral analysis using an MemCalc 1000. In normal individuals, segmental integration in the high frequency range (HF) was increased significantly by deep breathing and was well correlated with other parameters of parasympathetic function based on R-R interval variation. Parasympathetic activation by deep breathing was associated with sympathetic activation, as indicated by a significant increase of segmental integration in the low frequency range (LF). Compared to normal individuals, patients with sympathetic dysfunction had a smaller resting LF segmental integration value. In this diabetic group, segmental integration also showed a significatly smaller increase upon deep breathing. In both normal individuals and diabetic patients, segmental integration values decreased with ageing. In patients with obvious diabetic neuropathy, both the HF and LF segmental integration values were reduced at rest and only increased slight upon deep breathing. In conclusion, HF and LF segmental integration values obtained by MEM spectral analysis of R-R interval variation were correlated well with other parameter of sympathetic and parasympathetic nerve function. In particular, LF segmental integration may be of clinical value for assessing sympathetic function in patients with diabetes mellitus.

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CARDIAC AUTONOMIC NEUROPATHY IN DIABETIC PATIENTS UNDER HEMODIALYSIS THERAPY FOUND BY POWER SPECTRAL ANALYSIS OF 24 HOUR R-R INTERVAL

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AIM: Diabetics under hemodialysis showed poorer prognosis and more frequent episodes of sudden death than non-diabetics under hemodialysis.

METHODS: In order to analyze the poor prognosis, we detected episodes of arrhythmia and evaluated cardiac autonomic nerve functions in hemodialysed diabetics without peripheral neuropathy (HD group), age-matched diabetics without peripheral neuropathy nor autonomic neuropathy (NN group), and age-matched diabetics with symptoms of autonomic neuropathy (AN group). The averaged (± SEM) low frequency (LF; 0.03-0.15Hz) and high frequency (HF; 0.15-0.4Hz) components, which represent cardiac sympathetic nerve function and parasympathetic function, respectively, were calculated from 24 hour RR-intervals by means of power spectral analysis for 24 hours (24h: 8h-8h), daytime (12h-18h), and midnight (0h-6h).

RESULTS: HD group showed significantly lower 24h-LF (41 ± 16 vs 322 ± 83 msec2, $p<0.05$) and 24h-HF (51 ± 8 vs 162 ± 34 msec2, $p<0.05$) than NN group, which were comparable to those of AN group (27 ± 8 for 24h-LF and 43 ± 9 for 24h-HF). Daytime (12h-18h) averaged sympathetic function (LF) was significantly lower in HD and AN than that in NN group (36 ± 15, 29 ± 6, 340 ± 98, respectively). Midnight (0h-6h) averaged para-sympathetic function (HF) was significantly lower in HD and AN than in NN group (56 ± 17, 47 ± 12, 200 ± 40, respectively). The episodes of arrhythmia were comparable among HD, NN, and AN groups.

CONCLUSION: In spite of non-symptoms of autonomic neuropathy, hemodialyzed diabetics showed impaired cardiac autonomic functions comparable to those of diabetics with symptomatic autonomic neuropathy, which might result in their poor prognosis.

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POWER SPECTRUM ANALYSIS OF R-R INTERVALS UNDER 24-HOUR HOLTER ELECTROCARDIOGRAM IN PATIENT WITH NIDDM WHO DEVELOPED INCREASED BLOOD PRESSURE AT NIGHT

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[Objective] The mechanism of an abnormality in diurnal variation of blood pressure seen in patients with diabetes mellitus was studied by means of power spectrum analysis of R-R intervals under 24-hour Holter electrocardiograms. [Subjects and Methods] The subjects were 69 in-patients with NIDDM. Each patient wore a portable noninvasive automatic blood pressure meter and were administered a Holter electrocardiogram at about 2:00 p.m. Based on their 24-hour blood pressure pattern, the patients were classified into three groups: a first group in which blood pressure decreased at night (the decreased group), a second group in which blood pressure flattened at night (the flattened group) and a third group in which blood pressure increased at night (the increased group). The R-R intervals obtained from Holter electrocardiogram were analyzed every 512 seconds by means of fast Fourier transformation to draw a power spectrogram. In some patients urine was divisionally sampled during the day and at night to measure catecholamine. [Results] The coefficient of variation (CV) of R-R at rest in the increased group was 1.7%, and 1.2% in the flattened group, both of which were lower than the CV R-R of the decreased group at 2.6%. The excretory amount of catecholamine in the urine of the increased group decreased during the day and at night in comparison with that of the decreased group. In the decreased group, low frequency (LF) was superior during the day and high frequency (HF) was superior at night under the power spectrum. In the increased group, both the LF and HF components decreased, and some patients actually showed very few HF components. [Discussion] Increased blood pressure at night seen in patients with diabetes mellitus is profoundly associated with the level of autonomic disorder. Especially, in consideration of the results of the power spectrum of R-R intervals, the disappearance of any change in the vibrational amplitude of the maximum frequency as well as the disappearance of the HF components alone could be associated with the disappearance or reversal of diurnal variations in blood pressure. It also is possible that increased blood pressure at night might be attributable to parasympathetic hypoactivity rather than sympathetic hypoactivity, taking their mechanisms into account.

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MYOCARDIAL PERFUSION, ECG-INDICES AND CARDIAC REFLEX TESTS IN NEWLY DIAGNOSED AND LONG-TERM IDDM

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Myocardial perfusion of 20 newly diagnosed and 40 long-term IDDM patients without clinical evidence for coronary artery disease was assessed with a Tc-99m-methoxy-isobutyl-isonitrile (Tc-99m-MIBI) scintigraphy with regard to ECG-based indices (PQ segment, QRS complex, QT and QTc interval). Global and regional (anterior, lateral, posterior, septal and apical) mean tracer activities were grouped into a myocardial uptake score (MU-score: 1-6). Five cardiac reflex tests were performed to assess cardiac autonomic neuropathy (CAN). None of the diabetic patients demonstrated significant myocardial perfusion defects (MU-score > 3). In newly diagnosed IDDM, QRS-complex correlated with global ($p < 0.05$) and posterior ($p < 0.01$) 99mTc-MIBI uptake. QTc interval was related to global ($p = 0.02$), posterior ($p < 0,05$) and septal ($p < 0.05$) 99mTc-MIBI uptake. In long-term IDDM, a correlation between apical 99mTc-MIBI uptake and QTc interval was observed ($p < 0.05$). One patient (5 %) with newly diagnosed and 17 (42.5 %) patients with long-term IDDM demonstrated ECG-based CAN (≥ 2 abnormal reflex tests). No significant differences in global and regional myocardial Tc-99m-MIBI uptake, PQ segment, QRS complex, QT and QTc interval were observed between diabetic patients with and without CAN. The study demonstrates that CAN in IDDM patients without clinical evidence for coronary artery disease occurs in the absence of myocardial perfusion defects. In this group, ECG indices are not strongly related to myocardial perfusion.

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QT DISPERSION IN PATIENTS WITH CARDIAC AUTONOMIC NEUROPATHY DURING INSULIN - DEPENDENT DIABETES MELLITUS.

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Patients with diabetic cardiac autonomic neuropathy are particullarly at risk of sudden death as the result of ventricular arrythmias. Our objective was the measurement of QT and QTc dispersion and the assesment of influence of cardiac autonomic neuropathy on QT and QTc dispersion in patients with insulin - dependent diabetes mellitus. Patients were tested for cardiac autonomic neuropathy using ProSciCard system. We carried out four tests: resting pulse, deep breathing pulse, Ewing test, Valsalva maneuwer. QT interval was measured manual on the basis of 3 tests on each of 12 - lead ECGs. We studied 44 patients (23 men, 21 women) in which 21 with cardiac autonomic neuropathy (14 men, 7 women) and 23 without cardiac autonomic neuropathy (9 men, 14 women). Mean QTc interval by Bazett's formula in the group with cardiac autonomic neuropathy was $0.4099 \sqrt{sek} \pm 0.0300$ and was significantly higher than in the group without cardiac autonomic neuropathy ($0.3767 \sqrt{sek} \pm 0.0294$) ($p < 0.001$). The QT dispersion is the difference between the maximum and minimum QT across the 12 - lead ECG. QT dispersion in the group with cardiac autonomic neuropathy was statistically higher than in the group without cardiac autonomic neuropathy (mean $0.0511 \text{ s} \pm 0.0159$ vs $0.0319 \text{ s} \pm 0.0104$; $p < 0.001$). In the group with cardiac autonomic neuropathy QTc dispersion was significantly statistically higher (mean $0.0620 \sqrt{sek} \pm 0.0219$ vs $0.0501 \sqrt{sek} \pm 0.0134$; $p < 0.05$). Our result show higher QT and QTc dispersion in patients with cardiac autonomic neuropathy which can be the reason of higher risk of ventricular arrythmias.

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Cognitive Function in NIDDM Patients before and after Glycemic Adjustment

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Several studies have found cognitive impairment in Type II -Diabetes subjects (DS). However, data are not consistent on all accounts. This study was conducted to determine cognitive impairment in DS with neuropsycholoical methods. We hypothesized that glycemic state and diabetic late complications affect cognitive performance.

54 DS inpatients (58,8 ± 6,0 years) were compared at admission (t1: GHb 13,3 ± 1,6%; FPG 205,4 ± 40,1mg/dl) and after glycemic correction, mainly by insulin (t2: GHb 11,6 ± 1,3%; FPG 111,2 ± 17,8mg/dl), with 30 age-matched non-diabetic controls, tested in the same interval of 14 days. Cognitive function was assessed on memory, learning, processing speed and reaction time in a focused-attention task. Scores of depression and intelligence, relevant variables of diabetes and general health status were controlled.

First results of ANOVA show: reaction time is prolonged in DS ($p<.01$), they reproduce fewer words ($p<.05$), and recall fewer words from immediate memory ($p<.01$) than controls. In the two latter tasks DS decline from t1 to t2 ($p<.05$). DS with the greatest reduction of FPG (237,1 ± 27,3 to 97,1 ± 9,5mg/dl) show a decrease in reproduced words ($p<.05$). DS with microangiopathic and neuropathic late complications reproduce fewer words ($p<.05$) and have a lower intelligence score for abstract reasoning ($p<.05$) than those without late complications.

Cognitive impairment in DS were found primarily in the encoding of new information and in reaction time. According to our data late complications have important impact on cognitive performance. The impact of FPG on performance is seen as a transient effect of metabolic adaptation. The results of the 3-month follow-up study may confirm these findings. Information on neurophysiological processing is expected from the data of evoked potentials.

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The evaluation of the frequency of asymptomatic central diabetic neuropathy in insulin dependent diabetic children with auditory, visual and somatosensorial evoked potentials.

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To investigate the frequency of asymptomatic diabetic encephalopathy, we examined the somatosensorial (SEP), visual (VEP) and auditory (BAEP) evoked potentials in insulin dependent diabetic children whose visual, auditory and somatosensorial functions were normal. 36 diabetic patients, 21 female, 15 male and 10 normal children at the same age and sex were included in the study. Diabetes duration varied between 6 months and 11 years (average 4.6 ±2.6 years), with average control of disease (HbA1C: %10.97± 4.02) and ages varied between 6 years and 17 years (average 11.1 ±3.24 years). Supraspinal (central) somatosensorial conduction was normal whereas median nerve (wrist-C7: $p<0.001$, wrist -scalp: $p< 0.001$) and tibial nerve (ankle -scalp: $p<0.05$) SEP latencies were significantly delayed as compared to control group.

Right eye, left eye and intraocular VEP (P100) latencies were also significantly delayed as compared to control group (for each one $p<0.001$).

Evaluation of auditory pathways by BAEP revealed normal latencies at I., II., and III. waves, however IV. wave latencies (right $p<0.001$, left $p<0.05$) and V. wave latencies (right: $p<0.05$, left: $p<0.001$) were found to be delayed. Interpeak latencies were also delayed between I-V (right: $p<0.05$, left: $p<0.001$) and between I-III (left $p<0.05$).

Age, sex, duration and control degree of diabetes did not correlate with SEP, VEP or BAEP abnormalities.

Perjpheral SEP pathology was found in 13 patients (36.1%), central BAEP pathology in 14 patients (39%) and VEP abnormalities were seen in 9 patients (25%) whose somatosensorial , auditory and visual functions were intact, respectively. 17 patients (47.3%) had either abnormal BAEP or abnormal VEP results.

These electrophysiological results revealed that 47.3% of the patients included in this study had asymptomatic central encephalopathy and 39% of the patients had asymptomatic peripheral sensorial neuropathy unrelated to age, sex, duration and control degree of diabetes.

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CENTRAL MOTOR INVOLVEMENT IN TYPE I DIABETIC SUBJECTS: A NEUROPHYSIOLOGICAL STUDY.

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The aim of this study is to evaluate, in a group of type 1 diabetic patients, most without clinical symptoms of neuropathy, the presence of neurophysiological signs of peripheral and central nervous system involvement. Thirtyone well compensated patients (HbA1c<6,5% ; aged 18-45 years ; duration of diabetes 1-20 years) were enrolled in the study. The following neurophysiologic tests were performed: surface neurography of median and tibial nerves, "near nerve" sensitive neurography of sural nerve; Motor Evoked Potentials (MEPs) to magnetic brain Trans-Cranial Stimulation (TCS) through a regular coil applied on the scalp at optimal positions for hand and foot motor areas; sudomotor palmar responses to electric median nerve, brain TCS and cervical roots stimulation (skin test). Significant differences between controls (25 healthy age-matched subjects) and diabetics in the excitability threshold of MEPs (THR lower limbs=56.3±6.8 vs 70.6±12 % - $p<0.001$; THR upper limbs=39.4±3.6 vs 48.9±9.1 % - $p<0.001$), but not in the central conduction time (CCT lower limbs=12.7±1.2 vs 13.4±2.7 msec; CCT upper limbs=5.6±0.8 vs 6.1±1.2 msec) were found. Twenty-two subjects had a mixed, mainly axonal, neuropathy in the lower limbs and 4 in all four limbs. MEP parameters showed, in the lower limbs, increased THR in 11 patients and prolonged CCT in 5 patients. Motor central involvement for the upper limbs was found in 16 patients lacking sign of peripheral neuropathy: increased THR was detected in 12 patients and prolonged CCT was found in 4 patients. The skin test was absent in 12 diabetics, 3 of whom without peripheral involvement. Five out of nine patients, suffering from diabetes for less than three years, presented signs of polyneuropathy and involvement of central motor pathways, whilst three out of nine had only a pathologic skin test. The present study shows a subclinical central motor involvement in some patients with was not correlated with the occurrence of peripheral neuropathy, possibly suggesting a distinguished pathophysiology responsable for central and/or peripheral involvement.

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HEART RATE VARIABILITY AND MORTALITY. THE ARIC STUDY

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Low HRV is a marker of sympathetic predominance, and is associated with mortality risk in patients with heart disease. We studied the predictive value of HRV in the Atherosclerosis Risk in Communities Study, a prospective study of 16,000 men and women, aged 45-64, using a case-cohort design.

At baseline, in 1987-1989, 2-minute rhythm strips were recorded. HRV was determined in a random sample of 900 subjects without CHD, and in all deaths through 1993 (423). Subjects were categorized according to the tertile-distribution in the random sample. Relative rates of total- and cause-specific mortality, and 95% confidence intervals, were computed using Poisson regression for case-cohort design, adjusting for age, race and field center. For cancer analyses, subjects with a history of cancer were excluded.

Subjects with low HRV had an adverse cardiovascular risk profile and higher risk of mortality from all causes (see table). The associations could not be attributed to other risk factors, or to prevalent diseases. These results suggest low HRV may be an indicator of poor general health.

HRV	All causes	CVD	cancer	other
SDNN\# cases:	423	134	162	89
< 23.7	1	1	1	1
23.7-34.7	0.6 (0.5-0.9)	0.5 (0.3-0.9)	0.9 (0.6-1.3)	0.5 (0.3-0.9)
≥ 34.8	0.6 (0.5-0.9)	0.4 (0.2-0.6)	1.2 (0.7-1.8)	0.4 (0.2-0.8)
pNN50				
< 0.8	1	1	1	1
0.8-5.5	0.4 (0.3-0.5)	0.3 (0.2-0.5)	0.5 (0.3-0.7)	0.3 (0.2-0.9)
≥ 5.5	0.6 (0.5-0.9)	0.4 (0.2-0.6)	1.0 (0.7-1.5)	0.6 (0.4-1.1)

SDNN: standard deviation of all RR intervals (msec)
pNN50: % successive RR intervals differing ≥50 msec

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CARDIAC SYMPATHETIC DYSINNERVATION IN DIABETES: AN EXPLANATION FOR ENHANCED CARDIOVASCULAR RISK?

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Regional cardiac sympathetic hyperactivity predisposes to malignant arrhythmias in non-diabetic (ND) cardiac disease. Conversely, cardiac sympathetic denervation predicts increased mortality in severe diabetic autonomic neuropathy (DAN). Diabetic patients however experience proportionally greater cardioprotection from β-blockade. We propose that in diabetes, regional cardiac denervation may induce regional sympathetic hyperactivity and ischemia which may act as a focus for chemical and electrical instability. We have used positron emission tomography and C-11 hydroxyephedrine (HED) to explore regional changes in cardiac sympathetic neuronal density and tone and N-13 ammonia to measure regional changes in myocardial blood flow (MBF) at rest and after intravenous adenosine (140 µg/kg/min) in diabetic patients with and without DAN. Cardiac scans were performed in 10 diabetic control subjects without DAN (DAN-), 9 subjects with DAN (4 mild and 5 severe) and 14 healthy subjects. 40% of DAN(-)subjects had abnormalities of cardiac HED retention. In mild DAN, only distal inferior left ventricular (LV) tracer defects were observed but in severe DAN, defects also involved the distal and proximal anterolateral walls. Absolute tracer retention was paradoxically increased by 63% ($p<0.05$) in the proximal segments of severe DAN subjects compared to the same segments in mild DAN subjects (60% $p<0.05$ greater than ND subjects) while distally tracer retention was decreased in these subjects by 42% ($p<0.05$). No washout of tracer was observed in the proximal segments, consistent with normal regional tone but increased sympathetic innervation. Coronary flow reserve was decreased only in severe DAN subjects who achieved only 49% ($p = 0.0004$) and 51% ($p = 0.01$) of the values measured in ND and DAN(-) subjects, respectively. Reduction of myocardial vascular resistance on adenosine infusion during severe DAN subjects was identical to DAN(-) subjects in the distal denervated myocardium, but was markedly blunted in the proximal hyperinnervated segments, where vasodilation was attenuated by 35% ($p = 0.04$). Diabetes may result in LV sympathetic dysinnervation with proximal hyperinnervation complicating distal denervation. The hyperinnervated segments also demonstrate elevated vascular resistance. These findings could result in potentially life-threatening myocardial electrical instability and explain the enhanced cardioprotection from β-blockade in these subjects.

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IODINE-123-METAIODOBENZYLGUANIDINE IMAGING AND CORONARY ARTERY DISEASE IN PATIENTS WITH NIDDM.

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It has been reported that abnormality in I-123 metaiodo benzylguanidine (MIBG) uptake is closely related to sympathetic nerve dysfunction in diabetics . But, in some cases, asymptomatic coronary artery disease is coexisted in abnormal MIBG accumulation . In this study ,the patients with non-insulin dependent diabetes mellitus (NIDDM) is undertaken to examine the relationship between abnormality in MIBG uptake imaging and coronary artery disease.We compare the H/M ratio of MIBG between 68 NIDDM patients (Group N, male:36,female:32, Age 62+/-6 y.o.) and 7 non diabetic patients (Group C, male:5,female2, Age 60+/-10 y. o.). We studied a coronary angiogram in 37 NIDDM patients who had abnormality of MIBG imaging (Group NA, male:17cases, female: 20cases, Age:65.6+/-8.6 y.o.). (1)The group N had a lower H/M ratio than those in group C(3.1 +/-0.3 vs. 2.74+/-0.42, $p<0.05$). (2) In group NA, 7 cases had detected more than 75 % stenosis of coronary arteries (Group A), 12 cases had 25% or 50% stenosis (Group B) and remains 18 cases are normal coronary angiogram (Group C). We found that the more severe stenosis of coronary artery, the lower the H/M ratio, but not significantly ((A) 2.38+/-0.52, (B) 2.38+/-0.54, (C) 2.66+/-0.32, (A)vs. (C), $p = 0.11$, (B) vs.(C), $p=0.06$). Group A were patients with asymptomatic myocardial ischemia. And 2 cases of them had decreased MIBG uptake in corresponding to areas perfused by severe stenosis of coronary arteries. In conclusion, the H/M ratio in diabetics tends to lower than those in non diabetic patients . But, if we will find abnormality of MIBG uptake imaging in diabetics, we must role out asymptomatic coronary artery disease.

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LEFT VENTRICULAR MASS AND MYOCARDIAL SYMPATHETIC INNERVATION IN TYPE I DIABETICS WITH AUTONOMIC NEUROPATHY.

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Myocardial uptake of 123 I- metaiodobenzylguanidine (MIBG) is decreased in type I diabetics (IDDM) with autonomic neuropathy (AN). Moreover, we previously demonstrated increased left ventricular mass (LVMI) in diabetics with autonomic neuropathy. Aim: to investigate the impact of diabetic AN on LVMI and its relationship to circadian rhythm of blood pressure (BP), and on myocardial sympathetic innervation in normotensive IDDM. We studied 8 IDDM, 4 with autonomic neuropathy (AN+) and 4 without (AN-) (cardiovascular tests), basally and after 6 months of evening treatment with an α-blocker (doxazosin). patients underwent 24-h BP measurement, M-mode echo- cardiographic recording, planar scan and single-photon emission tomography with ^{123}I-MIBG and ^{99m}Tc-MIBI to assess myocardial sympathetic innervation and perfusion. The two groups were comparable for all the main clinical characteristics. Before drug administration, BP although similar in the two groups during the day, was significantly higher in AN+ during the night (Δ mean BP AN-: 8.9±1.5 vs AN+:-5.3±1.7, $p<0.05$). LVMI was in the normal range in both groups and slightly, but not significantly increased in AN+. Posterior wall was significantly increased in AN+ (AN-: 7.4±0.2 vs AN+: 8.7±0.5 mm, $p<0.03$). The heart/mediastinum uptake ratio of 123 I-MIBG (H/M) was significantly reduced in AN+ (2.04±0.1 vs AN-: 2.34±0.1, $p<0.04$). H/M significantly correlated with autonomic score ($r^2=0.6$, $p<0.05$), deep breathing ($r^2=0.8$, $p<0.005$), orthostatic hypotension ($r^2=0.6$, $p<0.05$). Preliminary results suggest that doxasin treatment decreases LVMI (basal: 109.3±9.8 vs 6 month: 97.9±5.1 g/m^2, $p<0.05$) and increases Δ mean BP (basal: -5.3±1.7 vs 6 month: 5.5±2.5, $p<0.05$). In conclusion, 123 I-MIBG allows to detect sympathetic innervation abnormalities in diabetic neuropathy, which in turn may play a role in determining those myocardial abnormalities largely known to be involved in the pathogenesis of sudden deaths.

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SENSITIVITY OF HEART SCINTIGRAPHIC IMAGING USING (123 I) MIBG COMPARED TO EWING TESTS IN CARDIAC AUTONOMIC NEUROPATHY IN DIABETES

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Cardiac autonomic neuropathy is a frequent complication of diabetes leading to resting tachycardia, postural hypotension, painless myocardial ischaemia, rhythm disturbances and sudden cardiac death. The aim of the study was to evaluate in a diabetic population the sensitivity of two exploration modes of autonomic neuropathy in diabetics : the Ewing tests which are, at present time, the reference method and the (123 I) meta-iodo-benzylguanidine (MIBG) single photon emission computed tomography (SPECT) which evaluates the cardiac sympathetic innervation. Patients and methods : 9 male insulin-dependent diabetes mellitus patients were studied. Mean age was 40.7 ± 15 years and diabetes duration was 10.8 ± 6 years. None had hypertension or macroangiopathy as demonstrated by patient's history, clinical examination, rest and exercise electrocardiography and ambulatory blood pressure monitoring. The complications observed were background retinopathy in 2 patients, incipient nephropathy in 3 and a peripheral neuropathy in 4 patients. Ewing tests, i.e. Valsalva maneuver, beat to beat heart rate variation during deep breathing and standing, blood pressure response to standing and to sustained handgrip, were performed. The results were considered as pathologic when the score was over 2. After injection of 10 mCi (123-I) MIBG, planar imagies were realized at times 1, 2 and 4 hours and SPECT imagies after 2 hours. The heart/mediastinum uptake ratio was calculated. Results : We noted abnormalities of planar imagies in 3 patients, SPECT imagies in 1, and both in 1 patient. None was positive for EWING tests. Conclusion : although MIBG SPECT will explore only the sympathetic innervation, these preliminary findings suggest that this technique could be more sensitive for the evaluation of cardiac autonomic neuropathy. Nevertheless cost and lack of disponibility of this technique should limit its use.

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HEART RATE VARIABILITY DURING EUGLYCEMIC, HYPERINSULINEMIC CLAMP IN OFFSPRING OF NIDDM PATIENTS AND CONTROL SUBJECTS

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Sympathetic activation has been considered as a link between insulin resistance, hyperinsulinemia and hypertension. However, little is known about the association between insulin sensitivity and autonomic regulation as well as the effect of acute hyperinsulinemia on cardiac sympathovagal balance. The aim of this study was to investigate the effects of insulin sensitivity and insulin infusion on heart rate variability (HRV) during euglycemic, hyperinsulinemic clamp study. We studied 35 non-diabetic offspring of non-insulin dependent diabetes (NIDDM) patients and 14 controls. The probands were chosen from a 10-year follow-up study of patients with well-characterized NIDDM according to their fasting C-peptide level (selected from the both ends of the distribution) and from control subjects to form three groups: group with parental history of insulin resistant NIDDM (phR, n=18), other with parental history of insulin sensitive NIDDM (phS, n=17) and a control group with parental history of normal glucose metabolism (phN, n=14). In phR-group whole body glucose uptake (M-value) was lower than in phS- and phN-groups (7.5±2.4 vs 10.1±1.9 and 11.3±3.1 mg/kg/min, $p<0.05$). In the pooled population M-value correlated with low frequency (LF, $r=0.34$, $p<0.05$) and high frequency (HF, $r=0.29$, $p<0.05$) spectral components of HRV. In all groups heart rate increased significantly during insulin infusion. In phR-group insulin infusion increased also total power of HRV (from 2695±414 to 3804±590 ms^2, $p<0.01$) and LF/HF ratio (from 2.19±0.30 to 4.11±0.81, $p<0.01$) and decreased power of HF spectral component (from 391±66 to 276±42 ms^2, $p<0.01$), whereas in other groups changes in HRV were non-significant. In conclusion, insulin resistance was associated with decreased cardiac vagal activity. Exogenous insulin changed cardiac autonomic balance toward sympathetic predominance. In addition, HRV-response was modulated by the parental history of the NIDDM phenotype.

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AUTONOMIC NEUROPATHY IN INSULIN DEPENDENT PATIENTS ASSESSED BY SPECTRAL HEART RATE VARIABILITY ANALYSIS

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In diabetic patients with advanced diabetic neuropathy heart rate variability (HRV) is known to be depressed. To evaluate the pattern of cardiovascular autonomic dysbalance 96 insulin dependent patients (mean age: 33.4 years, diabetes duration 10.54 years, treated only with insulin four time a day) and 30 healthy volunteers were studied. The following short-term heart rate variability (HRV) measures were assessed by a computer assisted technique (ProSciCard, Linden, Germany): standard deviation (SD), coefficient of variation (CV), root mean square successive difference of RR intervals (RMSSD), expiration/inspiration ratio, MCR, Valsalva ratio (VR), max/min R-R ratio on standing. Absolute and relative (%) spectral analysis HRV power in 3 standard frequency bands: VLF (0.01-0.05 Hz), LF (0.05-0.15Hz), HF (0.15-0.50 Hz) was assessed by Fast Fourier Transform. We compared healthy group with IDDM patients without retinopathy (R0), with nonproliferative retinopathy (R1) and with proliferative retinopathy (R2). **Results:** The following parameters had the highest strenght of discrimination between examined groups: **C vs R0:** Ln ratio LF/HF (-0.55 vs0.33; p=0.001), RMSSD (60.19 vs 39.55 p=0.04), **C vs R1:** LnHF (-0.40 vs -1.52; p=0.002), Ln total power (1.69 vs 0.51; p=0.03), **C vs R2:** LnVLF (0.41 vs -1.23; p=0.001), LnLF (0.25 vs -1.96; p=0.0006), LnHF (-0.40 vs -1.74; p=0.002), Lntotal power (1.69 vs -0.39; p=0.00004), RMSSD (60.19 vs 12.96; p=0.00001), HR (69.92 vs 84.35; p=0.03). **Conclusions:** 1. The gradually more pronounced difference between the diabetic patiens with retinopathy and control group seems to be inseparably connected with the degree of diabetic complication. 2. As compared to time domain measures, spectral analysis seems to be more precise in differentiation diabetic patients without complications from control group.

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HOLTER SPECTRAL ANALYSIS : A TECHNIQUE WITH A FUTURE FOR CARDIAC AUTONOMIC NEUROPATHY ASSESSMENT IN DIABETES

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The frequency of diabetic cardiac autonomic neuropathy (DCAN) is probably underestimated and its assessment is still based on Ewing tests. New techniques could help to earlier and easier detection. Patients and methods: 30 patients with insulin-dependent diabetes were studied, 20 males and 10 females, aged 20 to 66 years (mean : 38.1 ± 11 yrs). None of these patients had a cardio-vascular treatment. The five Ewing tests have been performed for each patient by the same operator, in standardized conditions, the results beeing expressed as a score from 0 to 1 for each maneuver. Heart rate variability (HRV), which reflects autonomic control, has been studied by spectral analysis of 24 hour continuous ECG record (ECG Holter Elatec, HRV Software, Ela Medical, France). Spectral analysis was performed using a nonparametric method by calculation of Fast Fourier Transform values (FFT). A particular study was done for day time (sympathetic tract prevalent) and night time (parasympathetic tract prevalent). Results: a severe DCAN (total score >2) was detected in 5 patients. A significant correlation (p<0.001) has been found between total score of Ewing tests and FFT values, showing a depressed heart rate variability (HRV) in case of DCAN. The correlation is especially apparent between night FFT and the parasympathetic tests : Valsalva maneuver (r : 0.603), heart rate response to deep breathing (r : 0.664) and heart rate response to standing up (r : 0.448). In opposition, the correlations of FFT with other maneuvers (sustained handgrip, BP response to standing up) have a borderline statistical significance.
Conclusion : the 24 hour continuous ECG record appears to be a relevant technique in diabetic patients for the detection of DCAN and may help in coronary heart disease. Spectral analysis of ECG Holter is a simple method which does not require the patient's contribution as it does in Ewing tests. But it is now necessary, by the mean of large studies, to define reliable criteria for DCAN quantization in spectral analysis.

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THE CIRCADIAN ASSESSMENT OF HEART RATE VARIABILITY IN DIABETIC SUBJECTS.

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The aim of this study was to investigate the circadian rhythm of heart rate variability (HRV) and to evaluate its clinical significance in diabetic subjects. One hundred and thirty-six diabetic patients and forty-eight non-diabetic subjects underwent 24 hour ambulatory ECG monitoring. HRV was evaluated hourly using the spectral variables of total frequency (0.01-1.00 Hz: TF), low frequency (0.04-0.15 Hz: LF), high frequency components (0.15-0.40 Hz: HF), and LF/HF ratio. The mean TF, LF, and HF for 24 h were significantly lower (p<0.0001) in diabetic subjects than in controls (21.7±8.5ms vs. 29.4±6.2ms, 11.2±5.0ms vs. 15.9±4.3ms, and 7.9±3.5ms vs. 10.3±2.9ms; respectively). The mean LF/HF ratio for 24 h was lower in diabetic subjects than in controls, but the difference does not reach statistical significance (1.46±0.48 vs. 1.60±0.40, p=0.08). The midnight (1:00am-6:00am) / daytime (1:00pm-6:00pm) ratio (N/D ratio) of TF, LF, or HF does not show any differences between diabetic subjects and control subjects. The N/D ratio of LF/HF ratio was higher in diabetic subjects than in controls, but the difference was not statistically significant (94±27% vs. 88±30%, p=0.10). During the daytime the mean LF/HF ratio was significantly lower in diabetic subjects than in control subjects (1.47±0.48 vs. 1.72±0.58, p<0.02). We concluded that parasympathetic TF and HF were significantly lower throughout 24hr period, while the sympathetic LF/HF ratio was significantly lower only in daytime, suggesting that sympathetic nervous function analyzed by the HRV should be evaluated by the day-time recordings of Holter monitoring in diabetic subjects.

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CARDIAC AUTONOMIC DYSFUNCTION IN LONG-STANDING TYPE-I-DIABETES WITH AND WITHOUT NEPHROPATHY

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The increased mortality risk in type-I-diabetic patients has repeatedly been linked to cardiac autonomic neuropathy (CAN). CAN may contribute to myocardial electrical instability and an increased risk of sudden death. We have studied CAN in 66 consecutive patients with type-I-diabetes (>10years duration). 24 had no diabetic nephropathy, 19 incipient and 23 overt nephropathy (diabetes duration 21, 32, 31 years; HbA1c 7.4%, 7.4%, 8.0%; hypertension was present in 5%, 25%, 96%; mean values). A cohort of 184 healthy subjects served as control group. Standardized assessment of CAN was performed with short-term spectral analysis (Variapulse TF3), spectral power was used as an index of autonomic activity in two frequency-areas. In the low-frequency area (0.06-0.15 Hz) which mainly represents sympathetic activity, spectral power was predominantly reduced in patients with nephropathy (incipient: median 138 ms^2 [upper;lower quartile: 80;335], overt: 43 ms^2 [18;229]) in comparison to patients without nephropathy (457 ms^2 [275;713]) and controls (521 ms^2 [197;1000]; p<0.0001). In the high frequency area (0.15-0.50 Hz) which is considered as an index of vagal activity, diminished spectral power was observed in all patients (no nephropathy: 462 ms^2 [267;1078], incipient: 144 ms^2 [59;337], overt: 88 ms^2 [30;234]) as compared to controls (707 ms^2 [273;1476]; p<0.0001). These results suggest that longstanding type-I-diabetes is associated with CAN and that its pattern differs depending on the stage of nephropathy. Patients without nephropathy show an impaired neural function with a predominantly vagal damage. Patients with incipient and overt nephropathy show a considerable loss of both vagal and sympathetic activity. These results probably explain the higher risk of sudden death in patients with diabetic nephropathy.

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Disorders of Bone Metabolism

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BONE LOSS IN INSULIN DEPENDENT DIABETES MELLITUS PATIENTS WITHOUT DIABETIC NEPHRTOPATHY

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Insulin-dependent Diabetes Mellitus (IDDM) is thought to be one of risk factors for secondary osteoporosis - diabetes osteopathy. The aim of the study was to assess the bone mineralisation in diabetes type I patients in age< 40 years old. The examined group consisted of 99 patients (45 women and 54 men), with IDDM without diabetic nephropathy and without another risk factors for osteoporosis. The control group consisted of 113 healthy controls matched for age, sex, weight, height and calcium diet. The evaluation of bone mineral density (BMD) was performed with the LUNAR DPX-L apparatus in the AP projection for lumbar part of vertebral column (BMD L2-L4), left femur neck (BMD-neck) and total skeleton (BMD-total). Bone tissue metabolism was evaluated with the aid of some biochemical tests. The results were related to: sex, age in which IDDM was diagnosed, duration of IDDM, and metabolic control of diabetes. In patients with IDDM the BMD and Z-score were significantly lower when compared to healthy subjects ($p<0.001$), for all measured points. Bone loss was higher in diabetic men then diabetic women. For all patients bone loss was higher when diabetes appeared before 21 years of age. BMD seems to be dependent to duration of IDDM and metabolic control of IDDM. In IDDM patients a higher level of: total serum hydroxyproline ($p<0.05$), hydroxyproline in 3 hours and 24 hours urine collection ($p<0.01$) and urine calcium in 3 hours morning collection ($p<0.05$) were found. One the most important bone loss patomechanism in IDDM seems to be a dominance of bone resorption over bone formation. The proof to it are biochemical results of bone markers and the presence of statistically important correlation coefficients between these parameters and BMD and Z-score in IDDM patients.

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Bone changes in patients with insulin - dependent (type I) diabetes mellitus

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The aim of the study is to evaluate bone changes in patients with insulin-dependent diabetes. We studied 60 healthy subjects and 112 diabetics (mean age-31,3±8,9 years, BMI-24,5±2,4 kg/m^2, sex-ratio M/F- 44/68). Patients were subdivided into 2 groups matched for age, gender and BMI: group 1-60 diabetics with poor control of diabetes and chronic diabetic complications; group 2-52 diabetics with good control without complications. Patients were subjected to a clinical examination, bone densitometry, spinal radiography, electromyography, bone scintigraphy, investigation of vibration perception threshhold, doppler ankle/arm systolic pressure, microalbuminuria, blood level of calcium, phosphorus, alkaline phosphatase. Bone mass density (BMD) is lower in type I diabetics than in controls (0,405±0,020 vs 0,520±0,025g/cm^2). BMD is lowest in patients with poor control and chronic complications-diabetic foot, retinopathy, neuropathy, nephropathy. A positive correlation between the degree of neuropathy and BMD reduction is found. The estimated frequency of osteopenia in group 1 and 2 is 33,4% vs 23% and for osteoporosis 24,2 % vs 12,4%. Insulin-dependent diabetics reveal lower BMD than healthy subjects. Reduction of BMD is more frequent and is positively correlated with diabetic control and complications- diabetic foot, neuropathy, retinopathy, nephropathy. Index of Tc99-MDP fixation in foot bone scintigraphy is higher in group 1 and is correlated with decrease of BMD. Bone densitometry could be one of prognostic tools for assessment and prevention of diabetic foot.

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LIMITED JOINT MOBILITY IN SUBJECT WITH INSULIN DEPENDENT DIABETES MELLITUS.

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The frequency of limited joint mobility (LJM) was investigated in 104 (55 girls, 49 boys) insulin dependent diabetic patients and 82 (35 girls, 47 boys) control subjects. In diabetic patients, mean age was 12.4 years (range: 3.6-28 years) and mean duration of diabetes was 4.5 years (range: 2 months - 16 years). LJM was observed in 32 patients (30.7 %) and 2 (2.4 %) control subjects. Mean age of patients with LJM was 15.6 ±4.4 years while that in patients without LJM was 10.9±3.9 years ($p< 0.001$). Mean duration of diabetes in patients with LJM was also longer than in those without LJM (6.7±4.4 years vs 3.5±2.3 years, $p< 0.001$). LJM was related with age, puberty and duration of diabetes. In patients with LJM, 64.5 % have a duration of diabetes greater than 5 years, 94 % of them were older than 10 years of age and 87 % were pubertal. No relation was found between LJM and sex, HbA1c and height and weight SDS and microvascular complications. In conclusion, duration of diabetes, age, and puberty were important factor influencing joint contractures.

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BONE METABOLISM IN IDDM PATIENTS WITH INCIPIENT AND OVERT DIABETIC NEPHROPATHY.

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We investigated the bone metabolism in IDDM patients with low-range normoalbuminuria and high-range microalbuminuria or proteinuria. We studied 25 patients (age<55yrs, females with regular menstruation): Group A (n=18, AER<10 µg/min), Group B (n=7, AER 100-1000 µg/min, serum creatinine<150 µmol/l)). Serum parathormone (PTH) and osteocalcin (OC) were assessed by RIA, serum bone alkaline phosphatase (ALP-B) and tartrate-resistant acid phosphatase (ACP-TR) by photometry. The groups A and B did not differ in diabetes duration (medians: 21vs22yrs), HbA1c (7.1vs 8.2%), creatinine (80vs95µmol/l). There was a difference between two groups in ALP-B ($p<0.05$)(380vs560 nkat/l). No significant difference was found in PTH (0.01vs0.2 ng/ml), OC (9.6vs13.7 ng/ml) and ACP-TR (160vs174 nkat/l), although the values were slightly increased in group B. We conclude that slightly increased bone turnover expressed mainly by biochemical parameters of bone formation occurs in nonazotaemic IDDM patients with proteinuria.

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THE EFFECTS OF CHRONIC PANCREATITIS AND DIABETES MELLITUS ON BONE AND MINERAL METABOLISM.

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Chronic pancreatitis (CP) due to alcohol has become common in urban black South Africans and often causes diabetes. We examined bone histomorphometry and the biochemical changes in 13 black male patients, aged 27-55, with alcohol-induced chronic pancreatitis - 6 diabetic(D) and 7 nondiabetic(ND). Serum Ca,P,Mg, PTH, vitamin D metabolites, osteocalcin, testosterone, leukocyte ascorbic acid, urine Ca and hydroxyproline were compared to those of 12 healthy controls(C) or available control data. Transiliac bone biopsies following tetracycline double-labelling were obtained for histomorphometric analysis and compared to those of 26 age-matched healthy controls(C) for static measurements, and to 8 controls for the dynamic data. TBV (% trabecular bone vol/total vol), TbTh (trabecular thickness), OTh (osteoid thickness) and EDe (erosion depth) were all significantly reduced in CP and the D (diabetic) subgroup vs C (see Table).

	TBV(%)	TbTh(μm)	OTh(μm)	EDe(μm)
C(n=26)	21.2(12.4-32.8)	164(122-242)	10(4.1-16.1)	8(4.8-13.1)
CP(n=13)	13.3(5.8-35.3)^c	94(58-302)^a	8.5(3.1-10.3)^e	5(4-11.4)^c
D(n=6)	9.9(5.8-19.0)^c	87(58-107)^b	5.8(3.1-10.3)^d	4.7(4-6.2)^d
ND(n=7)	18.3(8.1-35.3)	97(82-302)^c	8.8(4.4-11.9)	5.7(4.4-11.4)

Results are median (range). ^{a}p<0.0001; ^{b}p<0.0002; ^{c}p<0.005; ^{d}p<0.02; ^{e}p<0.04 vs C. In ND,however, only the TbTh was significantly reduced. There were more haemosiderin laden macrophages/mm^2 bone marrow in CP: 8(0-140)vs C:0.5(0-26), p<0.005. Leukocyte ascorbic acid levels were subnormal in 10 subjects. There were no differences in Ca, P,Mg, PTH and osteocalcin between groups. 25OH vitaminD (nmol/l) was reduced in CP (33.8±5.0, mean ± SEM) and D(25±4.5) vs C(54.4±3.7), p<0.005 for both. 1,25$(OH)_2$D (pmol/l) was reduced in D alone (52.8±7.8) vs C(95±4.4), p<0.03. In conclusion, CP patients have reduced TBV, TbTh, OTh, and EDe, consistent with low bone mass and turnover, especially in the diabetic subgroup. Apart from diabetes, other contributing factors include alcohol, poor nutrition, vitamin C deficiency and dietary Fe overload.

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Increased uptake of ^{99m}Tc - tetramethylene phosphonic acid in sacroiliac joints of adolescents with diabetes mellitus.

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A wide range of musculoskeletal syndromes have been described in association with IDDM. The aim of this study was to investigate the status of the sacroiliac joints (SIJ) in adolescents suffering from IDDM.22 diabetic patients (12 boys,10 girls; mean age 13,8 ± 1,3 yrs.,range12-16 yrs.) were divided into 3 groups. Group 1: 6 patients without limited joint mobility (LJM),with diabetes duration from 1 to 2 yrs.(mean 1,6 ± 0,5 yrs.).Group 2A: 7 patients without LJM, with diabetes duration from 6 to 10 yrs.(mean 7,9 ± 1,4 yrs.).Group 2B: 9 patients with LJM, with diabetes duration from 6 to 12 yrs.(mean 8,2 ± 1,9 yrs.). 9 age- and sex- matched healthy adolescents were studied as controls. SIJ were investigated using scintigraphy with ^{99m}Tc-tetramethylene phosphonic acid (5 MBg/kg.). The parameter for measuring joint activity was a ratio of peak SIJ to peak sacrum counts (SIJ index). **Results** (means ±SD): The diabetic patients showed increased SIJ uptake (mean SIJ left index 1,44 ± 0,20 , mean SIJ right index 1,44 ± 0,20) in comparison with controls (mean SIJ left index 1,19 ± 0,32, mean SIJ right index 1,25 ±0,20;p< 0,05).The mean SIJ index in group 2A and in group 2B had the same value (mean SIJ left index 1,43 ± 0,21,mean SIJ right index 1,42 ± 0,21 and mean SIJ left index 1,44 ± 0,22,mean SIJ right index 1,42 ± 0,21; respectively) that's why group 2A and group 2B were combined. Comparing group 1 and group 2 (A,B) we found to diminished of the increased SIJ index in group 2 (A,B) (not significant) and inversion ratio SIJ left / SIJ right index (p< 0,01).**Conclusion:** This study shows that the adolescents with IDDM frequently have increased uptake of ^{99m}Tc - tetramethylene phosphonic acid in SIJ, and it could be a radiological sign of subclinical sacroiliitis. These results are preliminary and need further observation .

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BONE METABOLISM IN THE PATIENTS WITH NON-INSULIN DEPENDENT DIABETES MELLITUS :EFFECT OF INSULINTHERAPY

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Because of the controversy findings so far published in the patients with non-insulin dependent diabetes mellitus (NIDDM), we assessed bone mineral content (BMC) and bone metabolism in 17 NIDDM patients (10 males and 7 females) and healthy controls matched for sex and age (±2 years). We selected NIDDM patients who were very poorly controlled due to insulinopenia and considered to require insulin therapy. First, BMC was compared in lumber vertebrae and in separate parts of body, using dual energy x-ray absorptiometry (DEXA) between the cases and the controls. Second, calcium and vitamin D metabolism were examined in NIDDM patients before and after 3 months of insulin therapy with the comparison with those in the controls. No significant differences in the bone mass was found between the cases and the controls. In terms of bone metabolism, the serum level of 1,25(OH)2vD among the patients was significantly lower than that in controls. With the improvement of glycemic control, moreover, the level of 1,25(OH)2vD and serum calcium elevated significantly by insulin therapy. From these observations, we concluded that hyperinsulinemia might cause the bone increase in NIDDM patients and that insulinopenia may cause the bone decrease through a deficit of 1,25(OH)2vD.

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BONE MINERAL DENSITY (BMD): CORRELATION WITH METABOLIC CONTROL AND VIT D RECEPTOR GENE POLYMORPHISM (VDR) IN IDDM

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Among the genetic factors related to the development of osteoporosis, a possible causal association with VDR may exist. Literature reports low BMD in IDDM population and our aim was to verify the correlation of BMD values with glycemic control and with presence of different vitamin D receptors genotypes. In this way, 78 patients with IDDM were studied (45M, 33F, 23.3 ± 5.5 years old, median of 21 years). Mean age at diagnosis of IDDM was 12.0 ± 3.9 years (median of 13 years), mean duration of the disease was 11.3 ± 5.9 years (median of 10.5 years). None of these patients had any other disease or condition that could interfere in bone mass. Fasting blood was drawn for the measurement of ionized calcium, HbA1, creatinine and for DNA analysis. DNA was extracted from peripheral leucocytes and amplified by PCR. PCR products were digested by the *Bsm*I restriction enzyme and genotypes were detrmined by agarose gel electrophoresis. Isolated urinary albumin was also measured. BMD at lumbar spine and femoral neck was evaluated by a LUNAR DPX densitometer (DEXA). Statistic analysis was performed by using Student t test and Pearson correlation test. The VDR distribution was: 33.3% bb (26/78), 50% Bb (39/78) and 16,7% BB (13/78). Patients with bb genotype disclosed a higher BMD at femoral neck and lumbar spine when compared to BB genotype (p = 0.02). Bb genotype was also associated with a better lumbar spine BMD than BB (p = 0.01). A negative correlation between BMD at both sites and urine albumin loss was observed. No significance was found when comparing HbA1 and urine albumin levels among the different genotype groups. Duration of disease was significantly lower in the BB genotype group (median of 7 years) than in the bb patients (median of 12 years). These results suggest an independent influence of VDR (besides metabolic control and duration of the disease) in BMD in IDDM patients.

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OXYDEVITE AS A METHOD OF EFFECTIVE DIABETIC OSTEOPOROSIS THERAPY.

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The purpose of this issue is to study the influence of $1_{\alpha}-$ oxivitamine D_3-oxydevite on bone tissue mineralization process in diabetic osteoporosis. Active metabolite of vitamin D_3-oxydevite was used for 45 insulin−dependent diabetes mellitus (IDDM) patients and 78 non−insulin−dependent diabetes mellitus (NIDDM) patients. The existence of osteoporosis was evaluated clinically: by a dynamics of bone pains, a capacity of motive activity; by results of rentgenogramme of feet bones and rentgendensitymetric data. Treatment using oxydevite had been carried out during 6 months. For the starting period it was prescribed during 2−4 weeks with a dosage of 0.5−2 mkg/a day, and thereafter during 6 months with a dosage of 0.25−1 mkg/a day simultaneously a calcium gluconat with a dosage of 1−1.5 g/a day. In a month after treating IDDM and NIDDM patients, there were signs of a clinic improvement, expressed by lessening of pains in bones, increase of number of motions, increase of ability to work. An increase in mineralization of bone tissue was noted when it was applied not less than 3 months ($P<0.02$). After 6 months of treatment, mineral saturation in bones of IDDM patients was increased 1.9 times ($P<0.001$), and in bones of NIDDM patients 1.3 times ($P<0.002$). Thus, the treatment using oxydevite should be carried on with a dosage of 0.25 up to 1 mkg/a day with combination of calcium preparations up to 1.5 g/a day, continuously during 3 up to 6 months. Analysis of the results proves that when using oxydevite for IDDM and NIDDM as per the said scheme a clinical improvement can be noticed after one month, and increase of mineralization of bone tissue according to rentgendensitymetric data can be observed only after 6 months.

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BONE MINERAL DENSITY OF THE CALCANEUS MEASURED BY ULTRASOUND DENSITOMETRY IN DIABETIC NEUROPATHY

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Diabetic patients are known to develop osteoporosis. We have previously demonstrated that bone mineral density is lower in the lower limb of diabetic patients with neuropathy. Ultrasound has recently been suggested to give information regarding the bone structure as well as bone mass. We therefore conducted a study using ultrasound to assess the calcaneus in diabetic patients with and without neuropathy. Control (C), diabetic patients without neuropathy (D) and diabetic patients with neuropathy(DN) were matched for age, sex, type and duration of diabetes (6 per group). All patients had ultrasound of the heel using CUBA clinical (McCue ultrasonics); measurements of the os calcis including broadband ultrasound attenuation (BUA) and velocity of sound (VOS). There was no significant difference in BUA or VOS between C vs D or D vs DN; although it was lower in diabetic subjects. However, the difference in BUA between C (102 $\pm$ 8.9 dB/Mhz) and DN (78.9 $\pm$ 17.9 dB/Mhz) was significant ($p<0.05$). This indicates that in addition to influencing bone mass diabetic neuropathy may have an effect on bone architecture.

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The Evidence for Uncoupling between Bone Formation and Resorption in NIDDM Patients

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Diadetes mellitus has been known to be chronically associated with abnormal calcium(Ca) metabolism and bone mass reduction. To clarify the nature of metabolic bone disorder in NIDDM, alterations in mineral metabolism and biological markers of osteoblast/osteoclast were studied. Age matched 99 male NIDDM patients and 55 non-diabetics were compared. Serum level of intact osteocalcin(i-OC) in NIDDM(3.4±1.7ng/mℓ) was significantly lower than that of non-diabetics(5.9±1.8ng/mℓ)($p<0.01$). Serum level of intact-PTH (i-PTH) in NIDDM(20.4±3.4pg/mℓ) was significantly lower than that of non-diabetics(29.1±3.0pg/mℓ)($p<0.01$). There were no significant difference in serum Ca and calcitonin levels. Level of serum tartaric acid-resistant acid phosphatase (TRACP) in NIDDM(11.0±1.2U/l) was significantly higher than that of non-diabetics(10.0±0.9U/l)($p<0.01$). There were positive correlation between i-PTH and i-OC ($p<0.01$). However, there was no clear correlation between i-PTH and TRACP or between i-OC and TRACP. In NIDDM, therefore, bone formation is thought to be decreased due to the reduction of osteoblastic function, meanwhile bone resorption increases. The absence of correlation between i-OC and TRACP suggests the uncoupling between bone formation and resorption. The positive correlation between i-PTH and i-OC may suggest that the reduction in serum i-PTH level reflecting reduced parathyroid function might be a causative factor of decreased osteoblastic function.

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DOES IMPROVEMENT OF METABOLIC CONTROL INFLUENCE THE MARKERS OF BONE TURNOVER IN DIABETIC PATIENTS?

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Some recent data suggest that intensification of treatment in patients with insulin-dependent diabetes mellitus (IDDM) may alter bone metabolism, as expressed, amongst the others, by a significant decrease of markers of bone formation. The aim of study was to evaluate the effects of improved glycemic control of diabetes assessed by serum HbA_{1c} on some markers of bone turnover: osteocalcin (OC), total alkaline phosphatase (ALP) as well as pyridinoline (PYD) and deoxypyridinoline (DPD) crosslinks in patients with long-standing IDDM. Serum OC was assessed by RIA (Incstar Corporation) and ALP by enzymatic method. PYD and DPD in urine were assessed by EIA (Metra Biosystems). The study was performed on 50 patients (23 F, 27 M), aged 38.4 ± 9 years and mean duration of IDDM 16.7 ± 8 years. OC, ALP, PYD, DPD and HbA_{1c} were measured at the baseline and in 6-month intervals during 3 years. The improvement of metabolic control was achieved by dietary advices, increased physical activity and the intensification of insulin therapy. At the baseline mean HbA_{1c} was 8.4 ± 1.2% and significantly decreased to 7.5 ± 1% after 1 year ($p<0.0005$), 7.3 ± 1.3% after 2 years ($p<0.0001$) and 7.2 ± 1.2% after 3 years from the baseline ($p<0.0001$). The diminution of HbA_{1c} was not accompanied by marked changes of markers of bone turnover during 3-year follow-up: at the end, OC decreased by 0.42 ± 1.7 ng/ml and DPD by 0.17 ± 1.5 nM/mMcreat; PYD increased by 0.63 ± 5.3 nM/mMcreat and ALP by 10 ± 41 IU/l, and all the values were within normal range. There was no significant correlation between markers of bone turnover or between HbA_{1c} and particular markers. Moreover, the markers were not correlated with age, sex or duration of IDDM.

In conclusion, the improvement of metabolic control in IDDM patients does not influence markedly the bone metabolism, frequently altered in diabetes, assessed by markers of bone formation and bone resorption. We also conclude that the interpretation of results obtained from repetitive measurements of markers of bone turnover is difficult and warrants further studies.

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EFFECT OF OBESITY ON BONE DENSITY IN PATIENTS WITH NIDDM AND INCREASED CORTISOL LEVEL.

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In NIDDM associated with obesity the density of bone is only mildly altered Protective factors include hyperinsulinism, insulin resistance, insulin-like growth factors, slow bone turnover. Cortisol accompanying obesity is a resorptive factor **Aim of the study:** 1) assessment of bone density in NIDDM with obesity and increased cortisol. 2) assessment of the effect of androgenes (metabolites) on bone density. 3) assessment of the effect of diabetes normalization on bone density. **Material and methods:** 44 patients with NIDDM treated with insulin (n=31) or orally (n=13). Bone density was measured by ultrasound with Lunar (USA). The patients were divided into a group without osteoporosis I (- 1≤ Tscore < 1), risk II (-2 ≤ Tsc <-1) and with osteoporosis III (Tsc <-2). Body weight was assessed according to BMI. Biochemical determinations: cortisol, endogenous insulin (in orally treated NIDDM), 17-ketosteroids, 17-ketogenic steroids in Tscore groups **Results:** Group III had the highest BMI (mean=30.1 SD=4,68) and cortisol level (mean=22.2 SD=9.73). In group I and II 17-ketosteroid level (mean=37.4 SD=10.95, mean= 41.1 SD=19.71) was higher than that in group III. Endogenous insulin was highest in group I (mean=10.5 SD=3.77). Significant correlations were noted: negative SOS with cortisol (r=-0.30, p<0.05) and positive SOS with insulin (r=0.80, p<0.05). BUA with 17-ketosteroids (r=0.44, p<0.05) **Conclusions:** The lowest bone density in NIDDM with the highest body weight and increased cortisol, as well as correlations between cortisol, SOS and BUA suggest that in these patients the balance between protective and resorptive factors is impaired. The lowest 17-ketosteroid level in patients with the lowest bone density, obesity and the correlation between 17-ketosteroids and BUA may indicate a reduced anabolic effect of androgenes in this group The highest level of endogenous insulin in patients with NIDDM and normal bone density and obesity confirms its contribution into bone protection.

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AMINOGUANIDINE TREATMENT INCREASES BONE MINERAL DENSITY IN STZ INDUCED DIABETIC RATS.

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Osteopenia is a recognised complication of long term diabetes mellitus. Advanced glycation endproducts (AGEs) are believed to contribute to chronic diabetic complications in various sites. Aminoguanidine (AG) is a potential therapeutic agent to prevent diabetic complications which acts to inhibit AGE formation. We have previously reported that there is accelerated accumulation of collagen AGEs in bone tissue in STZ induced diabetic (DM) rats *in vivo* and altered osteoblastic cell function *in vitro*. These findings suggest that advanced glycation may play a role in diabetes related osteopenia. In the present study, we evaluated the effects of diabetes and AG treatment on AGEs formation and bone mineral density (BMD) in STZ (55mg/kg) DM rats with and without treatment of AG (1g/l in drinking water). In addition, a group of DM rats were rendered euglycaemic by insertion of insulin silastic implants (DI). Plasma glucose and glycated haemoglobin levels were increased in DM but not in DI rats and were not affected by AG treatment. In the DM rats, the BMD of femora was significantly reduced by 37% (control, 0.45±0.02 vs. diabetic, 0.28±0.01g/cm^3, mean±SEM, p<0.01). AG treatment significantly attenuated but did not normalise the reduced BMD in DM rats (0.38±0.03 g/cm^3, p<0.05 vs DM). DI rats had BMD levels similar to control rats (0.44±0.02g/cm^3). Levels of Plasma Bone Gla Protein, a marker of bone formation, were reduced in diabetic rats (control, 24.8±2.4 vs diabetic, 10.7±0.6ng/ml, p<0.001) and were not influenced by AG treatment (8.8±0.7ng/ml). Levels of urinary deoxypyridinoline, a marker of bone resorption, were reduced in diabetic rats (control, 3.2±0.3 vs diabetic, 0.6±0.1nmol/day, p<0.001) and were not influenced by AG treatment (0.5±0.1nmol/day). Immunohistochemistry revealed increased AGE staining in tissues from DM rats which was reduced by AG treatment. These data suggest that diabetic osteopenia may be related to AGE accumulation in bone and that AG may have a therapeutic role in preventing osteoporosis in this population.

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DIABETIC TREATMENT REGIMENS AND BONE REMODELING

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The influence of diabetic treatment regimens on bone remodeling is still object of debate.In our study bone mineral density,calcium regulating hormones(PTH,vitamin D metabolites), bone turnover markers(bone GLA protein,deoxypyridinoline) and glucose metabolism parameters(fasting glucose,HbA1c, C-peptide)were measured in 93 NIDDM males (48 on glyburide, 45 on insulin)and in 45 controls with normal renal and hepatic function.

	GLYBURIDE (G)	INSULIN (I)	CONTROLS(C)
25OH-D (ng/ml):	7.1 ± 5.1 a	22.5 ± 9.1	21.9 ± 10.3
1,25OH2-D(pg/ml):	29.7 ± 7.6	28.4 ± 4.0	29.4 ± 4.3
PTH (ng/ml):	63.4 ± 22 b,c	52.9 ± 19	50.7 ± 21
BGP (ng/ml):	7.1 ± 1.7 d	6.8 ± 2.6 d	14.1 ± 3.8
D-Pyr (pmol/ml):	17.3 ± 1.9	17.9 ± 2.8	18.1 ± 3.6
BMD (mg/cm2):	395 ± 60 e	397 ± 58 f	441 ± 71

a:p 0.001 vs I,C; b:p 0.01 vs I; c: p 0.006 vs C; d:p 0.001 vs C ; e:p 0.01 vs C; f: p 0.02 vs C.

In conclusion,treatment with glyburide can only be associated with an acclerated vitamin D metabolism.It is possible that the reduction of 25OH-D levels depends on a high conversion rate to the active form,as a consequence of PTH-stimulated increase of 1-alpha-hydroxylase activity,but BMD values are similar in patients taking glyburide or insulin. On the other hand,the occrrence of extremely low BGP levels in all diabetic patients reflects a decrease in osteoblastic activity,which can explain the osteopenia in these patients.

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INTERLEUKIN-6 PRODUCTION BY OSTEOBLAST-LIKE CELLS IS INCREASED BY ADVANCED GLYCATION ENDPRODUCTS.

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Advanced glycation endproducts (AGEs), which result from nonenzymatic reactions of glucose with tissue proteins, have been shown to accumulate on collagen derived from cortical bones of diabetic or aged-animals. In the present study, we examined the effects of AGEs on the production of bone-resorption cytokines by human bone-derived cells and mouse osteoblastic MC3T3-E1 cells. AGEs stimulated the release of IL-6 but not of IL-11 in the culture supernatants from human bone-derived cells. The IL-6 release was inhibited by sodium salicylate, TLCK, TPCK and probucol, known as the inhibitors of the transcription nuclear factor-κB (NF-κB) activation. Electrophoretic mobility-shift assays revealed that NF-κB was activated in the nuclear extracts from the bone-derived cells treated with AGEs. The protein kinase A inhibitor Rp-8-Br-cAMP suppressed the AGEs-stimulated IL-6 production and NF-κB activation. Enhanced IL-6 production and NF-κB activation by AGEs were also observed in MC3T3-E1 cells. These results suggest that AGEs modulate bone remodeling by stimulating the production of IL-6 in osteoblast-like cells, probably *via* activation of NF-κB. Protein kinase A may be involved in AGEs-stimulated NF-κB activation.

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INFLUENCE OF METABOLIC CONTROL ON PTH SECRETION IN PATIENTS WITH DIABETES MELLITUS. F.J.A. Paula, C.M.M. Lanna, G.M.G.F. Paccola and M.C. Foss. School of Medicine of Ribeirão Preto - São Paulo University, Ribeirão Preto, SP, Brazil

Previous studies have shown that increased urinary calcium, phosphorus and magnesium excretion occurs in diabetes mellitus (DM) and have suggested that patients with DM have lower bone mineral density. The objective of the present study was to determine PTH secretion under basal conditions and stimulated during EDTA infusion (30 mg/kg/h) in patients with good and regular metabolic control (Group GR-HbA_1 < 8.5%) and in poorly controlled patients (Group P-HbA_1 > 10%). Blood samples were collected at 10 min intervals during the basal period (30 min) and for 2 h during EDTA infusion for the determination of ionic calcium, PTH (intact molecule), glycemia and Mg. The study was conducted on 8 normal subjects (HbA_1 group M = 4.2 ± 0.2%), 8 GR diabetics (HbA_1 = 7.3 ± 0.4%) and 8 P diabetics (HbA_1 = 13 ± 1%). Glycemia was significantly higher in group P at all times studied (range: 13.2-15.8 mmol/l vs. 6,1-7,9 mmol/l for the GR group). Basal Ca^{++} levels were lower in group P (1.19 ± 0.01 mmol/l) both compared to the normal group (1.22 ± 0.01 mmol/l) and to group GR (1.24 ± 0.02 mmol/l). During EDTA infusion PTH levels were slightly higher in group GR than in the normal group and significantly higher than those of group P (e.g.: + 40 -normal group = 86.9 ± 20 vs GR = 110.2 ± 12.8 vs P = 49.3 ± 5 ng/l). Mg levels for the control group were slightly higher than those for the GR group and significantly higher than those for the P group. We conclude that DM patients with poor metabolic control present disorders of PTH secretion, a change that is particularly important if we consider that calcium ion levels are lower in these patients. This alteration may be related to a higher frequency of osteopenia among diabetics.

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Complications – Experimental and Pathogenesis

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OXIDATIVE DAMAGE TO MITOCHONDRIAL DNA AND ITS RELATIONSHIP TO DIABETIC COMPLICATIONS.

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In order to explore role of oxidative stress-induced DNA damages on the pathogenesis of diabetic complications, we investigated mitochondrial DNA (mtDNA) mutations and 8-hydroxydeoxyguanosine (8-OHdG) contents in the muscle of 16 NIDDM patients and 7 normal subjects. mtDNA 5778bp (8214-13991) deletion was identified in the muscle of the diabetics and the normal subjects. The percentage of the deleted mtDNA [ΔmtDNA (%)] was much higher in the muscle of the diabetic patients (12.2±9.5) than the normal subjects (1.71±1.68, $p<0.01$) . 8-OHdG/dG (10^{-5}) was also much higher in the muscle of the diabetic patients (38.8±20.8) than in the normal subjects (8.6±7.8, $p<0.005$) . ΔmtDNA (%) was highly correlated to 8-OHdG content (r=0735, $p<0.0001$) . 8-OHdG/dG was significantly proportional to duration of diabetes and hemoglobin A1c. ΔmtDNA and 8-OHdG/dG in the muscles of the diabetic patients with nephropathy and chronic renal failure, were significantly higher than those in the diabetics with macroalbuminuria, microalbuminuria or normoalbuminuria. ΔmtDNA and 8-OHdG/dG in the muscles of the diabetics with proliferative retinopathy were significantly higher than those in the diabetics with simple retinopathy or without retinopathy. Oxidative stress causes conversion from dG to 8-OHdG. Because the mtDNA5778bp deletion covered areas coding ND3-ND5 in complex I, the deletion might cause a reduction in mitochondrial oxidative phosphorylation capacity and contribute to the pathogenesis of diabetic complications.

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EFFECT OF DIABETES MELLITUS ON ADENOSINE AND ADENOSINE DEAMINASE (ADA) ISOENZYMES: ADA_1 AND ADA_2 IN BLOOD

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The aim of the study was to evaluate energetic metabolism by assessment of concentration of adenosine (the most important product of ATP degradation). Also the activities of isoenzymes of adenosine deaminase (ADA): ADA_1 and ADA_2 were assessed- both of them are responsible for degradation adenosine to inosine. The subjects were 38 people suffering from diabetes and being treated with insulin. As a control was group of 19 patients without previously recognized IGT or DM. The levels of adenosine were estimated using photometric method and the activities of isoenzymes due to Giusti & Galanti method. It was found that the concentration of adenosine was significantly higher in the group suffering from diabetes than in control group ($p=0.01$). The same was found estimating activities of extracellular ADA_1 ($p=0.02$) and ADA_2 ($p=0.002$). There was not significant difference in the activities of intracellular ADA_1 ($p=0.15$). There was a significant correlation between concentration of adenosine and HbA1c among diabetic subjects ($p=0.02$). Almost the same correlation was found between activity of ADA_2 and concentration of adenosine ($p=0.03$)

Conclusion: It can be postulated that the increased degradation of high energetic phosphates observed among patients suffering from diabetes can be responsible (among other factors) for the development of long-term complications.

2295

ABNORMAL MARKERS OF FREE RADICAL ACTIVITY IN CHILDREN & ADOLESCENTS WITH INSULIN DEPENDENT DIABETES MELLITUS

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Oxygen free radicals are highly reactive substances that is capable of inflicting injury to cell membranes if not adequately scavenged by antioxidants. The metabolic disturbances in diabetes may favour generation of excess free radicals and this might be associated with reduced anti-oxidants activity. Recently the injurious effect of free radicals has been implicated in pathogenesis of diabetic angiopathy, however this has not been well studied in young people with diabetes. Low levels of reduced glutathione (GSH), and plasma thiol (PSH) may reflect excess free radical generation and a depleted anti-oxidant reserve. We have found reduced levels of plasma thiol (PSH) and red cell glutathione (GSH) in 51 children, adolescents and young adults with IDDM (22 males & 29 females), mean age ± SD 14.8 ± 3.4 years, duration of diabetes 6.8 ± 4.5 years, and HbA1c of 8.7 ± 1.5%; (none had any clinical evidence of complications), compared with 21 healthy normal controls matched for age and sex (11 females & 10 males). In the diabetic cohort the GSH and PSH levels were 1266.73 ± 201.89 µmol/l & 458.14 ± 38.34 µmol/l vs 1403.26 ± 277.81 µmol/l & 486.75 ± 69.73 µmol/l in the control group, reaching statistical significance of $p< 0.02$ and $p<0.03$ respectively, Student t-test. We have previously shown significant abnormalities of vascular reactivity in the same study group and this result suggests a possible role of free radicals in pathogenesis of diabetic angiopathy which might start very early in the course of childhood diabetes.

2296

THE DETERMINATION OF ERYTHEOCYTE ALDOSE REDUCTASE LEVEL WITH ELISA IN PATIENTS WITH DIABETES MELLITUS

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Using a modification of the procedure ofsalting out and DE− 52, hydroxylapatite, sephadex− G100 chromatography, aldose reductase was purified to homogeneity from bovine testis. Female New Zealand white rabbits were subcutaneously injected with purified enzyme mixed 1:1 with complete Freunds adjuvant to prepare antiserum. The antibody was purified from serum by a 33% ammonium sulphate precipitation and DEAE− cellulose column chromatography, antibody was linked with Horseradish Peroxidase by sodium periodate oxidation method to prepare antibody− HRP. Approximately 1 ml of blood was hemolyzed immunoassay. In the antibody− sandwich ELISA, antibody was first immobilized on plastic plate to capture antigen, following a second antibody conjugated to HRP. The level of aldose reductase in 22 patients and 10 nondiabetic subjects was determined with the assay method , the results showed that: 1. The level of enzyme was significantly increased in diabetic patients compared with normal subjects (8.37±3.46 vs 3.23±1.16 ng/g Hb, $P<0.01$). 2. The level of enzyme varied approximately 5− fold in diabetic patients and 3− fold in nondiabetic controls, respectively. 3. In patients, the level of enzyme was apparently correlation with fasting plasma glucose level ($r=0.67$, $P<0.05$), but there were not relationship in normal subjects ($r=0.11$, $p>0.05$). 4. There were not statistically correlation between the level of aldose reductase and sex, age, duration of diabetes. Inclusion , quantiative determination of erythrocyte aldose reductase level with ELISA provided an useful mothod in the evaluation on the efficacy of aldose reductase inhibitors, and in the study on the relationship between the polyol pathway and diabetic chronic complications.

2297

TYPE 1 (IDDM) DIABETES MELLITUS AND SERUM TOTAL ANTIOXIDANT CAPACITY (TAC)

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In diabetes mellitus, a sharp reduction of antioxidant defences and an increased production of serum free radical seem to coexist *(Cross CE et al, Ann Intern Med 107: 526-45, 1987)*. For this reason there is increasing interest in the study of mechanisms of antioxidant protection against free radical-induced injury and in the identification of suitable biochemical parameters for the measurement of the Total Antioxidant Capacity (TAC) in body fluids. Several methods have been proposed for the assessment of TAC, including that based on enhanced chemiluminescence *(Whitehead TP et al, Anal Chim Acta 266:265-77, 1992)*. This technique is calibrated with TROLOX and assay results are expressed as µmol L^{-1} of Trolox. This procedure implies the determination of TAC both on whole serum (WTAC) and on its deproteinated fraction (DPTAC), obtained by ultrafiltration, using a Centrifree MS-1 micropartition system (Amicon Massachusetts). We have measured WTAC and DPTAC in two subject groups: i) 15 young Type 1 (IDDM) diabetic subjects (Mean±SD= 30±3.5 yr), affected by medium-term Diabetes Mellitus, in a good metabolic control, evaluated on the basis of HbA_{1c}% (Mean±SD=5.9±1.3%) and of fasting glycemia (Mean±SD=146±34 mg/dL); ii) 16 age- and sex-matched healthy control subjects (HS). Results (Mean±SD) in IDDM were: WTAC =204±137 µmol/L^{-1} and DPTAC=82±30 µmol/L^{-1}; in HS were: WTAC=366±145 µmol/L^{-1} and DPTAC=204±116 µmol/L^{-1}. Statistical analysis showed a significant difference ($p<0.01$) between IDDM and control subjects both in WTAC and DPTAC values. The significanly reduced WTAC and DPTAC in IDDM subjects may be due to an increased free-radicals production. It may be due, at least in part, to the reduction of circulating vitamin E induced by insulin *(Quinones-Galvan A et al, Metabolism 1996, in press)*. As the good metabolic control of our IDDM patients resulted unable to prevent the serum TAC reduction, it remains to verify the role that a supplementary antioxidant therapy may have in the treatment of Type 1 (IDDM) Diabetes Mellitus.

2298

HGH AND IGF-I IN DIABETES MELLITUS.

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Introduction: The role of human growth hormone (HGH) and insulin-like growth factor-I (IGF-I) in the pathogenesis of diabetic late complications is under discussion for long. Therefore we investigated these paramemeters in IDDM- and NIDDM-patients at different stages of the disease.
Subjects and methods: HGH and IGF were measured in 11 IDDM patients without complications (group A: 8m,3f;age:27 +/-6 years; mean diabetes duration:4.9 years), in 9 NIDDM patients with diabetic microangiopathy (B:6m,3f;39+/-16; 19.8),in 47 IDDM adulescents (C:29m,18f;13+/-3;6.9)and 113 age adapted controls (D:44m,69f;44+/-18) as well as in 21 NIDDM patients with peripheral arterial occlusive disease (PAOD) (E:14m,7f;72+/-6;17.4), in 10 NIDDM with microangiopathy (F:6m,4f; 67+/-8;10.2),in 9 NIDDM without complications (G:5m,4f;57+/-14;2.4),in 9 non-diabetics with PAOD stage IIb-IV (H:7m,2f;62+/-7) and in 62 ageadapted controls (I:20m,42f;62+/-8) applying a radioimmunoassay.
Results: The levels of HGH(ug/ml) / IGF-I(ng/ml) were: A:3.0/234 (HbAlc:10.6);B:3.1/162 (8.7);C:2.7/295 (8.6), D: 3.1/212 (5.2);E:1.3/119 (7.9);F:2.3/127 (8.8); G:1.5/ 142 (8.2);H:2.3/127 (5.3);I:2.6/175 (5.4). IGF-I was significantly ($p<0.05$) reduced in all diabetic groups and in the PAOD-controls. HGH levels did not differ significantly. There was no correlation between IGF-I and HbAlc.
Conclusion: IGF-I is reduced in all diabetics more pronounced in the presence of late complications.

2299

HYDROXYL RADICAL MEDIATES N^{ε}-(carboxymethyl) lysine (CML) FORMATION FROM AMADORI PRODUCT

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Free radical generation has been postulated to play a role in atherogenesis. Recent immunological studies demonstrated the presence of N^{ε}-(carboxymethyl) lysine (CML)-modified proteins in several tissues and their increase in aging as well as age-enhanced diseases such as diabetic complications and atherosclerosis. Since CML formation from glucose-protein adducts or fatty acids needs oxidation, it is thought as a biomarker of glycoxidation or lipid oxidation product in vivo. However, the mechanism of CML formation is not well known. In the present study, to examine effects of radical oxygen species on CML formation from an Amadori product, CML generated from glycated HSA was measured by the immunochemical method using anti-CML antibody. Our glycated HSA prepared by 7 day's incubation with 1.6 M glucose and DETAPAC contained undetectable level of CML. Incubation of the glycated HSA with 0.4 mM $FeCl_2$ for 1 hour lead to CML formation at a detectable level. The CML formation was enhanced dose-dependently by addition of hydrogen peroxide, but significantly inhibited by catalase or mannitol. Superoxide anion generated by the xanthine/xanthine oxidase system or hydrogen peroxide itself failed to produce CML from glycated HSA. In addition, superoxide dismutase had no effect on this process. These data indicate that hydroxyl radical generated by Fenton reaction between Fe^{2+} and hydrogen peroxide might play a major role in CML formation.

2300

SERUM LEVELS AND "IN VITRO" PRODUCTION OF CYTOKINES IN INSULIN-DEPENDENT DIABETIC PATIENTS (IDDM). N.T. Foss, R. Nahas, C. Melli, C.L. Silva and M.C. Foss. School of Medicine of Ribeirão Preto - São Paulo University, Ribeirão Preto (SP), Brazil.

The aim of this study was to evaluated the capacity of immunocellular response in IDDM patients by the determination of cytokines (IL1, IL2, IL4, IL6 and αTNF) levels in the serum and in PMNBC cultures supernatant. PMNBC from 37 IDDM patients were cultured in the presence of LPS (10 μg/ml) and IFNγ (50 U/ml) during 24 hours. Lymphoproliferation assay was developed in the presence of PHA (20 μg/ml) during 72 hours, and was measured by 3H-thymidine incorporation. The serum and supernatant cytokines (IL1, IL2, IL4, IL6 and αTNF) were measured by Elisa assay. The data of the IDDM group were compared with those of a control group of 10 normal individuals. The IL1 and αTNF levels were elevated in serum of IDDM patients (6.0 ± 0.3 and 139.1 ± 4.7 ng/ml) when compared with the normal subjects (0.7 ± 0.02 and 38.9 ± 2.9 ng/ml, respectively). However, the IL1 and αTNF levels in the cultures supernatants (67.0 ± 6.6 and 61 ± 4.2 ng/ml) of normal subjects were significantly higher than those in IDDM patients (21.2 ± 2.3 and 25.0 ± 1.2 ng/ml). Serum and supernatant levels of IL2 from the control group were markedly higher (30.2 ± 0.3 and 45.2 ± 4.0 ng/ml) than those from IDDM patients (16.7 ± 0.7 and 17.9 ± 1.8 ng/ml). In contrast, IL4 levels in the serum (27.7 ± 1.0 ng/ml) and in the supernatant (41.6 ± 2.1 ng/ml) of IDDM patients, were elevated compared to the control group values (3.2 ± 0.1 and 14.3 ± 2.6 ng/ml). The elevated serum levels of IL1, αTNF and IL4 observed in IDDM patients suggest that mechanisms of macrophage activation are developed following the course of the diabetes, but the macrophage response can be negatively controlled by the IL4 action. The lower production of cytokines in PMNBC cultures of IDDM patients compared to normal response may suggest a impaired macrophage capacity of defense against infection.

2301

LIPOIC ACID TREATMENT DECREASES NEURAL TUBE DEFECTS IN FETUSES OF DIABETIC RATS.

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Increased oxidant stress is suggested to play a pathogenic role in diabetic embryopathy. Supplementation of vitamin E to pregnant diabetic rats has been shown to prevent fetal malformations. In this study we evaluated the effect of α-lipoic acid (LA), a potent antioxidant, shown to exert therapeutic effects in various conditions associated with increased oxidative stress, including diabetic complications. Female Sprague-Dawly rats (n=57) recieved a daily i.p. injection of LA (30 mg/kg body weight), beginning at first day after matting, for 17 consecutive days. Diabetes was induced by streptozotocin (65 mg/kg body weight i.p) on the 6th day after matting (n=43). Mean maternal blood glucose on the 17th day of gestation was 496 ± 56 and 525 ± 30 mg% in vehicle and LA treated diabetic rats, respectively, $P>0.1$. The prevalence of neural tube defects (NTD) was reduced by LA treatment, from 26% in fetuses of vehicle treated diabetic rats (n=201) to 12% in LA treated group (n=177) ($p<0.05$). Crown rump length (CRL) was reduced in fetuses of streptozotocin-induced diabetic rats (1.69 ± 0.35 cm Vs. 2.07 ± 0.17 cm in control), but was unafected by LA treatment. We conclude that LA has the ability to reduce fetal dysmorphogenesis in diabetic rats, probably by its anti-oxidant capacity.

2302

SIALIC ACID IS DIMINISHED ON THE SURFACE OF CIRCULATING IMMUNO-COMPETENT CELLS IN TYPE II DIABETES

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Sialic (N-acetylneuraminic) acid NANA, plays an important role in cell-to-cell recognition; studies done on red blood cells in diabetic patients give contradictory results for NANA concentration on their membrane. The aim of the present study is to evaluate NANA on the membrane surface of circulating immuno-competent cells of type II diabetic patients. 30 patients are compared to 28 non-diabetic patients(consecutive recruitment). Circulating mononuclear cells are isolated by centrifugation. NANA is studied by two different methods. First, surface negative charges are globally estimated by agglutinability after action of polybrene, a positive charged molecule and expressed as % agglutinated cells; secondly, CD_3 (T- lymphocytes), CD_{19} (B- lymphocytes) and CD_{14}(monocytes) subpopulations are marked with limulus polyphemus (horse-shoe crab) agglutinin - a lectin from the sialic group - coupled to flurorescein-iso-thio-cyanate - and studied by flow cytometry; the fluoroscence is expressed as the median channel of the studied subpopulation. Wheat germ agglutinin (triticum vulgare) a lectin from the N-acetyl-glucosamine group - is used as internal control ; parametric tests are used. Agglutinability (47.3 % versus 39 % - $p = 0.0009$), CD_3 limulus fluoroscence (LF) (22.3 versus 12.6 - $p < 0.0001$), CD_{19} LF (35.8 versus 17.2 $p < 0.0001$) but not CD_{14} LF (48.8 versus 44.9 NS) are significantly diminished in diabetic patients. Results are not significant for triticum vulgare. Agglutinability is significantly predicted by CD_3LF, CD_{19}LF and CD_{14} LF (multiple linear regression, $R^2 = 0.356$, $p < 0.0001$). Diabetes and acute phase reaction (APR) (identified by serum CRP> 1 mg/dl) are independent and significant determinants, with interaction, for CD_3 LF, CD_{19} LF but not CD_{14} LF, while diabetes interacting with APR is the only significant determinant for agglutinability - $p < 0.001$ - (two-way ANOVA) We conclude that sialic acid is diminished on the surface of both T and B lymphocytes in type II diabetic patients. This fact must be interpreted in the context of defence problem encountered in diabetes.

2303

EFFECTS OF EPALRESTAT ON THE GLYCATION OF ERYTHROCYTE MEMBRANE PROTEINS

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It has been reported that fructose promotes the Maillard reaction, causing the late reaction product to be accumulated. We administered the aldose reductase inhibitor, epalrestat, to streptozotocin (STZ)-induced diabetic rats to determine its inhibitory effect on the glycation of erythrocyte membrane proteins. The glycation of erythrocyte membrane proteins was determined according to the method of Miller et al. by measuring the radioactivity of each [^{3}H]- labeled protein band on SDS-PAGE. The results obtained in our experiments showed that the sorbitol content of red blood cells in the epalrestat-treated diabetic rats (STZ+E) was reduced ($p<0.05$) compared with that of untreated diabetic rats (STZ), and that the glycation of erythrocyte membrane proteins was significantly inhibited in the epalrestat-treated group. The inhibition was evident in erythrocyte spectrin (bands 1, 2) after 4 wk-long administration ($p<0.05$, STZ+E vs. STZ) and in all bands after 6 wks ($p<0.001$, STZ+E vs. STZ). These findings suggest that aldose reductase inhibitor tends to suppress the accumulated levels of sorbitol in diabetic animals and the elevation of the Maillard reaction.

2304

IN VITRO PRODUCTION OF GRO-α IN NON-INSULIN DEPENDENT DIABETES MELLITUS (NIDDM) RATS.

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It is widely assumed that susceptibility of diabetic patients to bacterial infection is caused by dysfunction of neutrophils in them. Not only neutrophil function, but also chemotactic factors for leucocytes, are important in defense against infections. Therefore we focused in this study on Gro-α, an intrinsic chemotactic cytokine for neutrophils produced by rat mononuclear cells. We studied *in vitro* production of Gro-α in the OLETF (Otsuka Long Evans Tokushima Fatty) rats, a model of NIDDM, in comparison with that in control rats. Materials and Methods: Peripheral blood and spleens were obtained from the OLETF rats and LETO (Long Evans Tokushima Otsuka) rats, a non-diabetic control strain. Peripheral mononuclear cells (PMC) and spleen cells (SC) were cultured with or without stimulants (10 μg/ml of lipopolysaccharide, LPS or 10 ng/ml of interleukin-1, IL-1) for 5 days. Culture supernatants were collected at 1, 3 and 5 days after incubation. Gro-α concentration in the supernatants was measured using ELISA kits. Results: 1. Gro-α concentrations in IL-1-stimulated PMC and SC were as low as those in unstimulated cultures. There was no significant difference in IL-1-stimulated Gro-α production between the OLETF and LETO rats. 2. Gro-α levels were significantly higher in LPS-stimulated cultures than in unstimulated or IL-1-stimulated cultures of PMC and SC obtained from both the OLETF and LETO rats. LPS-induced Gro-α production by PMC and SC was lower in the OLETF rats than in the LETO rats at 1, 3 and 5 days after incubation. Conclusion: Production of Gro-α, an intrinsic chemotactic factor for neutrophils, by spleen cells of diabetic rats was decreased significantly compared with that in non-diabetic control rats. Reduction of Gro-α production may be one of the causes of susceptibility to infections in diabetics.

2305

CARBOXYMETHYLLYSINE (CML) IS INCREASED IN SERUM OF CHILDREN AND ADOLESCENTS WITH IDDM

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Advanced glycation endproducts (AGEs) are heterogenous protein modifications probably involved in the pathogenesis of diabetic late complications. The relative importance of the different AGE structures is not known. The glycoxidation product CML can be formed from early glycation products. CML was recently shown to be the dominant AGE in vitro and in human lens proteins. CML is also increased in skin collagen of patients with diabetes.

Aim: In the present study we investigated whether the serum levels of CML in adolescent diabetic patients were different from normal subjects. **Methods:** We also compared the serum levels of CML as measured by monoclonal anti-CML antibodies, to the serum levels of AGEs (pAGEs) measured by polyclonal antibodies made from rabbit immunized with AGE-RNase. 19 female and 19 male IDDM patients aged 14±3.2 (mean±SD) years, mean duration 5 (range 0.5-15) years, were compared with 13 normal female and 13 normal male subjects aged 16±1.7 years. HbA1c was 9.6±2.2 in the diabetic group. **Results:** The serum levels of CML and pAGEs were significantly elevated in the diabetic group when compared to controls; CML: 1,08 (0.82-1.37) (median, 95% CI) CML-BSA units vs. 0.70 (0.56-0.97), $p<0.03$, and pAGEs: 6.6 (5.8-7.3) AGE-BSA units vs. 5.5 (4.6-6.4), $p<0.01$, respectively. A significant correlation ($r=0.76$, $p<0.001$) was found between the serum levels of CML and pAGEs in the diabetic group but not in controls ($r=0.17$). No association was found between the Amadori product HbA1c and CML ($r=-0.02$) or pAGE ($r=0.35$, NS). Neither CML nor pAGEs were associated with age, sex or diabetes duration.

Conclusion: The serum levels of CML are increased by 50% in young patients with diabetes. As suggested by in vitro studies, the source of CML does not seem to be an Amadori product.

2306

EFFECTS OF PIOGLITAZONE ON Ca^{2+} HANDLING IN CARDIOMYOCYTES OF DIABETIC RATS

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Introduction: We have shown that contractile function impaired in diabetic myocardial cells at the 15th IDF Congress. The aim was to demonstrate intracellular mechanisms of diabetic myocardial cell dysfunction and effects of pioglitazone (PGZ). **Method:** Seven male Sprague-Dawley rats aged eight weeks were injected with 60 mg/kg of streptozotocine intraperitoneally. Three of them were treated with PGZ (30 mg/kg/day). At 14 days after streptozotocine injection, both PGZ treated (group P) and untreated (group D) rats were sacrificed and the heart was quickly excised. Myocardial cells were isolated using collagenase, then loaded with acetoxymethyl ester of Indo-1 at a concentration of 5 μM for 15 min at 37 ° C. Control myocardial cells were prepared from six normal rats (group N). Cells stimulated with electrical pulses (0.25 Hz, 350 mV, 6 msec duration) were monitored continuously in high resolution video system. Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured at 33 msec interval using ARGUS-50/CA system (Hamamatsu Photonics, Ltd.). $[Ca^{2+}]_i$ was evaluated as fluorescence intensity ratio of 405/480 nm of whole cell fluorescence images, which were obtained simultaneously from a cell exposed by a exciting light of wavelength at 350 nm, visualized by an intensified CCD camera, and stored in an imaging processor. We analyzed velocity of increase (VCa) and time constant of decrease (τ) of $[Ca^{2+}]_i$ changes during myocardial cell contraction and relaxation. **Result:** VCa was reduced in group D (5.80 ± 0.48 sec^{-1}) compared from group N (6.82 ± 0.56 sec^{-1}). Impaired VCa was recovered in group P (7.60 ± 1.30 sec^{-1}). τ in group D was larger than that in group N (584 ± 30.4 vs 475 ± 34.4 msec). In group P, τ became small to 559 ± 12.5 msec. **Conclusion:** These findings suggest that reduced contraction and delayed relaxation observed in diabetic myocardial cells could depend on the impairment of $[Ca^{2+}]_i$ handling, and PGZ may improve dysfunction by smoothing the movement of $[Ca^{2+}]_i$.

2307

THE 1,5-ANHYDRO-D-GLUCITOL PLASMA LEVEL AND HbA_1c CONCENTRATION IN HEALTHY SUBJECTS.

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1,5-Anhydro-D-glucitol (AG) - glucose analogue was reported in human plasma by Pitkanen first in 1972.AG level of diabetic patients is lower than in healthy humans and is probably associated with hyperglycemia and glucosuria,reflects metabolic compensation in 1-2 days before examination.From these properties,AG is proposed to be an useful marker of glycemic control in diabetic patients.The aim of our study was to establish the mean plasma AG level of normal subjects and to analyze the link between AG and HbA_1c in these subjects.32 healthy subjects (fasting plasma glucose 4,7 ± 0,6 mmol/l,creatinine concentration in plasma 91,1 ± 8,8 µmol/l):12 woman and 20 men in the mean age of 46,9 ± 16,2 years,range:16-76 were investigated.Plasma AG concentration was determined using enzymatic method based on pyranose oxidase (pyranose:oxygen 2-oxidoreductase) and HbA_1c was assayed according to the immunoenzymatic method (DAKO kits).The mean level of AG was 168 ± 39 µmol/l,range: 129-206 µmol/l.Any correlation between AG level and HbA_1c was observed.Summarizing AG level could be an undependent on HbA_1c values,index of glycemic control.

2308

VENOUS ERYTHROCYTE COLUMN WIDTH IS INCREASED IN TOE CAPILLARIES OF DIABETIC PATIENTS

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A maldistribution of blood from skin capillaries to subpapillary vessels through arteriovenous shunts has been demonstrated in the toes of diabetic patients. This may lead to an increased postcapillary venous pressure causing dilation on the venous side of the capillary. The aim of the present study was to investigate if the capillary diameter (erythrocyte column) is wider in diabetic patients than in healthy subjects. Fiveteen patients with type 1 diabetes (age: 30±8 years), and 15 age and sex matched controls were investigated. Nine of the patients had short diabetes duration (3±2 years) and no complications, while 6 patients had diabetes since 14±12 years and microangiopathy. Skin temperature and toe blood pressure were similar in patients and controls. Single skin capillaries of the big toe were investigated by videophotometric capillaroscopy and the erythrocyte column width was determined at 6 standardized levels (3 arterial and 3 venous). Ten measurements were performed at each level and mean±SD values for the arterial and venous parts were calculated. A small but insignificant increase was seen on the arterial side in the patients (9.3±1.6 µm; controls: 9.0±1.8 µm). The venous part was wider (p=0.02) in the patients (11.9±1.4 µm) than in controls (10.9±1.8 µm), mainly due to an increase in the patients with microangiopathy (12.4±1.4 µm). The results of the present study show that the venous part of skin capillaries is dilated in the diabetic foot, which further supports the hypothesis of blood passing through arteriovenous shunts resulting in a decreased ratio between pre- and post capillary pressures, leading to a reduced blood flow through nutritive capillaries. These functional disturbances may be of importance for the development of neuropathy and chronic foot ulcers in the diabetic foot.

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DETERMINATION OF GLYCATED ALBUMIN BY ENZYME-LINKED BORONATE-IMMUNOASSAY (ELBIA)

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A new affinity method for quantification of glycated albumin by an enzyme-linked boronate-immunoassay (ELBIA) was established. ELBIA was based on the interaction between boronic acids and cis-diols of glycated human serum albumin (HSA) trapped by anti-HSA antibody coated on a microtiter plate well. We first examined the feasibility of the conventional boronate affinity method. In the conventional method, 8.1 to 18.9% of nonglycated standard albumin nonspecifically binds to the boronate affinity column whose values are regarded as column-blank. Therefore, in the present modified affinity chromatographic method, this column-blank was subtracted from the apparent glycated albumin values to obtain the correct one. The accuracy of ELBIA was evaluated by comparison with this modified affinity chromatographic method. Correlation coefficients of 0.995 (n = 22) and 0.991 (n = 6) were obtained for ELBIA versus the modified affinity chromatographic method for measurement of glycated standard albumin and albumin fractions purified from human sera, respectively. The correlation line for all measurement was $y = 0.996x + 0.846$ (n = 28, r = 0.993). Thus, glycated albumin values determined by ELBIA exactly correspond to those determined by the modified affinity chromatographic method, suggesting that ELBIA reflects the in vivo situation of albumin glycation. Furthermore, we developed a fully automated ELISA system for ELBIA, which allows the multiple, rapid and precise measurement of glycated albumin. In this fully automated system, the intra-assay CV was 3.7% (n = 92) and the inter-assay CV was 4.2% (n = 5). The glycated albumin value of normal subjects was 5.26 ± 0.96% (n = 110, mean ± SD). Further clinical study using ELBIA showed that the correlation line of glycated albumin versus stable-HbA1c was $y = 2.904x - 9.783$ (n = 470, r = 0.876).

2310

CAPILLARY PRESSURE IN SUBJECTS WITH FASTING HYPERGLYCAEMIA

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In support of the haemodynamic hypothesis which proposes that raised capillary pressure and flow are instrumental in the pathogenesis of microangiopathy, capillary pressure is elevated in IDDM patients with microalbuminuria but not in those of equivalent disease duration without evidence of microvascular disease. In contrast capillary pressure is not raised in NIDDM. The prolonged period of metabolic disturbance preceding NIDDM is known to be associated with abnormalities of microvascular function. This study investigated whether this prediabetic phase was also accompanied by abnormalities of capillary pressure. Subjects with fasting hyperglycaemia (FH), defined as individuals with fasting plasma glucose in the range 5.5 to 7.7 mmol/l on two separate consecutive occasions, were recruited from the general population and groups at risk of diabetes e.g. gestational diabetes, family history of diabetes. 21 subjects with FH and 21 age sex and menstrual status matched healthy volunteers (13 male) were investigated. Capillary pressure was measured by direct cannulation of finger nailfold capillaries using a resistance based servo-controlled system. The two groups did not differ in systolic or diastolic blood pressure or skin temperature. Capillary pressure was not different between the two groups (16.9±3.5 controls v 18.0±4.4 mmHg FH, p=0.46). Thus capillary pressure does not appear to be elevated in the prediabetic state.

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ANTIBODIES AGAINST IN VITRO GENERATED AGE: SPECIFICITY AND APPLICABILITY TO ASSAYS FOR *IN VIVO* AGE.

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Most immunoassays for advanced glycation endproducts (AGE) are based on antibodies prepared against *in vitro* generated AGE. Also the majority of assays are of the competitive type, using *in vitro* AGE as tracer/competing antigen. This means, that the final specificity of these assays depends solely on *in vitro* AGE different from the *in vivo* material, that one would analyse. Often, *in vitro* AGE has been prepared by letting protein and sugar react under conditions, that due to differences in eg. the concentration of trace amounts of metal ions, pH, oxygen tension and temperature result in variable products. The aim of our investigation was to compare poly- and monoclonal antibodies prepared against a panel of *in vitro* AGE's. The *in vitro* AGE's included preparations made by incubating proteins (KLH, BSA, lysozyme, RNAse and PPD) with various sugars (glucose, glucose-6-P, fructose and ribose) and by defined chemical modifications of proteins (methylglyoxal-, carboxymethyl-, glyoxal-, GOLD, MOLD and pentosidine). Each AGE was characterised by aminoacid analysis to determine the degree of modification and, where possible, the chemical nature of the modified aminoacids. Ribose BSA containing per mole 48 modified lysines of which one was CML, served as reference material. The detection limit for this compound was in ELISA about 1ug/ml. Our results show, that despite the fact that conditions during protein/sugar incubations vary, carboxymethylated lysine (CML) is by far the most abundant epitope recognised by the raised antibodies. Nevertheless, competitive ELISA's using these antibodies with various *in vitro* AGE's as coating antigen, show differences in the relative response for the different AGE's. The conclusion is, that although the specificity of most AGE antibodies is CML, the final assays using these antibodies may differ in their capability to recognise *in vivo* AGE. Thus thereis an urgent need for standardisation of AGE immunoassays.

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SERUM 8-HYDROXY-GUANINE LEVELS ARE INCREASED IN DIABETIC PATIENTS

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Production of reactive oxygen species are increased in diabetic patients and the oxidative damage may contribute to the development of diabetic microangiopathic and macroangiopathic complications. This study was performed to investigate whether the serum levels of 8-hydroxyguanine (8-OHG), as an indicator of oxidative damage to DNA, was elevated in diabetic patients. Forty-four diabetic patients (41 NIDDM and 3 IDDM) were matched by age for 33 healthy controls. Serum 8-OHG levels were measured by high-pressure liquid chromatography. Diabetic patients had significantly higher mean concentrations of 8-OHG than controls (5.23 ± 4.25 vs. 0.98 ± 0.84 pmol/ml, $p<0.01$, t-test). Among diabetic patients, those with advanced nephropathy (n=11, serum creatinine levels >1.2 mg/dl) had significantly higher 8-OHG levels than the other non-azotemic patients (7.26 ± 4.09 vs. 4.11 ± 4.02 pmol/ml, $p<0.05$, t-test). The 8-OHG levels tended to be higher as the retinopathy progressed in diabetic patients. However, there was no significant correlation between the level of 8-OHG and age, HbA1c, duration of diabetes and creatinine clearance. In conclusion, the diabetic patients showed greater oxidative damage to DNA and such changes might contribute to the vascular complications of diabetes.

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LENS LIPID PEROXIDATION AND HEAVY METAL ACCUMULATION IN DIABETIC CATARACTOUS AND CLEAR LENSES

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Relationship between lens lipid peroxidation and heavy metal accumulation was investigated in 15 diabetic cataractous lenses and 6 clear lenses. Lens malondialdehyde (MDA) concentration which is the product of lipid peroxidation was measured by colorimetric method. Lens Cu, Fe, Cr, Ni, Pb, Mn, Sb and Zn concentrations were measured by electrothermal atomic absorbtion spectrometer. In diabetics, lens MDA concentrations were higher about three times than control group. Accumulation of Cu, Fe, Cr, Ni about six times, Pb, Mn about four times, Sb and Zn about twice times were higher in diabetic cataractous lenses than clear lenses. Significant correlation between Zn, Cr, Mn, Pb and MDA concentrations was determined ($p=0.001$ $r=0.66$, $p=0.005$ $r=0.59$, $p=0.05$ $r=0.44$, $p=0.05$ $r=0.42$ respectively). No significant correlation between Cu, Fe, Ni, Sb and MDA concentrations. Very high concentrations of latter group elements in diabetic lens may suggest that accumulation of heavy metals in diabetic lens is prior process than lens oxidation, and it may be accelerate lens lipid peroxidation.

2314

THE EFFECT OF HYPERGLYCEMIA AND DIABETES ON METHYLGLYOXAL AND ITS DETOXIFICATION PATHWAYS

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The reactive α dicarbonyl, methylglyoxal (MG) has been postulated to produce diabetic complications as a direct toxin and as a precursor for advanced glycation end-products (AGEs). Levels of MG are regulated by rates of production from glucose and the detoxification pathways to D-lactate (glyoxalase) and acetol (aldose reductase). To address factors that determine tissue levels of MG and the activity of degradative pathways we have measured MG, D-lactate and acetol in plasma by HPLC. We measured these compounds in 25 IDDM subjects with minimal complications (<15 yrs duration), 40 NIDDM subjects with normal renal function, and 20 non-diabetic controls. Glycemic control was determined by HbA1c and glucose determination. MG and D-lactate levels were significantly elevated in diabetic subjects relative to controls. (see table below)

mean± SD	MG (nM)	D-lactate (μM)
NIDDM (n=40)	221.8±43.6	12.3±5.9
IDDM (n=25)	215.3±61.9	13.3±6.0
Control (n=20)	154.6±30.6*	8.7±4.7#

* p=<0.0001
\# p=0.03

We also found a highly significant association between plasma MG concentrations and glucose levels in NIDDM ($r=0.74$, $p=0.0003$) and IDDM ($r=0.35$, $p=0.04$) indicating that glycemic control is an important determinant of MG levels. D-lactate and acetol showed no relation to glycemic control. We found no correlation of MG and its degradation product D-lactate in NIDDM ($R=0.12$, $p=0.62$) or IDDM ($R=0.24$, $p=0.17$) when compared with non-diabetic controls who showed a significant relationship ($R=0.49$, $p=0.07$), while acetol showed a significant inverse relationship with MG ($r=0.56$, $p=0.001$). These studies indicate elevated MG in diabetes and that levels of MG, but not its degradation products D-lactate and acetol, are directly related to the level of glycemic control. Acetol and MG demonstrate a reciprocal relationship suggesting that different factors regulate their levels in diabetes.We also show that MG undergoes predictable detoxification to D-lactate in non-diabetics but degrades to a variable degree in diabetes, suggesting that acquired or genetic factors may modify MG detoxification. Understanding the activity of this pathway in diabetic individuals may help to predict their degree of risk for diabetic complications.

2315

DEPRESSING EFFECT OF EXOGENIC AND ENDOGENIC COMPOUNDS ON SERUM GLYCATION IN DIABETES

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The assessment of few substunces impact on nonenzymatic glycation process in diabetes was undertaken in vitro and in vivo experiments. Substrate was obtained by incubation of human serum albumin in 5% water solution (HSA) with 44 mM/l glucose for 20 days, t=+1-+2°C. Fructosamine concentration (FA) by tetrasolium nitroblue method was 273,3±6.6 mkM/l. Addition of serum of healthy persons and IDDM patients in amounts of 50% of the total volume decreased FA to 19,0±1,3 mkM/l and 22,6±0,57 mkM/l (p<0,001) respectively. Serum heating didn·t alter it properties. Physiological concentrations of thermostable substunces of uric acid 600 mkM/l, arginin 170 mkM/l, creatinine 150 mkM/l added to solution of HSA and glucose 17mM/l decreased FA from 38,8±5,8 mkM/l to 12,8±5,2 mkM/l (p<0,01). We proposed, that aminogroup compounds could join with glucose, precluding its binding to body proteins, decreasing glycation. To prove this preposition Bucarban (0,075 g/kg, n=9, gr.1) and Glibutid (0,01 g/kg, n=9, gr.2) were administrated to alloxane diabetes rats for 1 month. FA fell from 7,7±0,2 to 5,7±0,2 mkM/l (p<0,001) in gr.1 and from 7,6±0,1 to 5,4±0,1 mkM/l (p<0,001) in gr.2, showing effects of this common drugs on glycation

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GLYCOXIDATION AND LIPOXIDATION PRODUCTS IN RED BLOOD CELL MEMBRANES IN POORLY CONTROLLED DIABETES.

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Background: Increased blood glucose levels and oxidative stress result in the formation of glycoxidation and lipoxidation products in proteins and phospholipids. These products may be implicated in the pathogenesis of the chronic complications of diabetes. Red blood cell (RBC) membranes are readily available, have a relatively long life span and contain both lipids and protein, hence may be a useful source of markers of both glycoxidation and lipoxidation.

Aims: To establish if markers of glycation (fructose-lysine (FL)), glycoxidation (carboxymethyllysine (CML)) and lipoxidation (carboxymethylethanolamine (CME)) are detectable in RBC membranes, and if levels of these markers differ between subjects with poorly controlled diabetes and non-diabetic controls.

Methods: Thirteen subjects with diabetes (HbA1c >8%, normal renal function) and matched non-diabetic control subjects were studied. Levels of FL, CML, and CME were measured in RBC membranes by gas chromatography/mass spectroscopy.

Results: mean (standard deviation)

	diabetes	controls	t-test
fasting glucose mg/dl	232(114)	78(9)	p=0.0004
HbA1c %	10.6(2.3)	5.7(0.6)	p=0.0001
FL/lysine mmol/mol	12.2 (8.0)	4.3 (2.7)	p= 0.008
CML/lysine mmol/mol	0.20(0.07)	0.21(0.06)	p>0.05
CME/ethanolamine mmol/mol	0.31(0.54)	0.18(0.16)	p>0.05

RBC membrane FL and HbA1c correlated , r=0.57, p<0.01.

Conclusions: FL, CML, and CME are detectable in RBC membranes. FL levels are increased in diabetic vs non-diabetic subjects, and correlate with HbA1c. Levels of CML and CME do not differ between diabetic and non-diabetic subjects. This study provides no evidence of increased oxidative stress in diabetes.

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EFFECT OF DIABETES MELLITUS AND OXIDIZING AGENTS ON HUMAN RED BLOOD CELL ENZYMES ACTIVITY.

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The aim of the study was to measure the parameters of enzymatic activities of membrane acetylcholinesterase (AChE) and cytosolic glutathione peroxidase (GSHP) from red blood cells of diabetic patients and upon the influence of oxidative stress. The membrane AChE activity was measured by the spectrophotometric method of Ellman et al, the GSHP activity in cell haemolysates as the rate of exogenous GSH oxidation according to the method of Romos-Martinez. The maximal enzyme reaction rate of AChE was (17.2 ± 0.8) μmol acetylthiocholine/ml packed cells/min (n=11) for diabetic cells vs. (13.1 ± 0.8) μmol acetylthiocholine/ml packed cells/min (n=14) (p<0.02) for control erythrocytes. For GSHP the enzymatic reaction rate was (11.9 ± 3.0) μmol GSH/ml packed cells/min in diabetic cells in comparison to (13.9 ± 3.9) μmol GSH/ml packed cells/min in controls (p<0.05). The cell exposure to the oxidative agent t-butyl hydroperoxide (tBHP) significantly changed the parameters of enzyme activities. The maximal AChE reaction rate increased but substrate affinity decreased up to tBHP concentration of 0.1 mM for diabetic cells and 0.4 mM for control ones. For higher oxidant concentrations both the maximal AChE enzymatic reaction rate and Michaelis-Menten constant decreased. The GSHP maximal reaction rate increased and substrate affinity decreased up to tBHP concentration of 1.5 mM. The susceptibility of red blood cell membranes from diabetic patients to oxidative stress was much higher than of control erythrocyte membranes. It can be postulated that the alteration of erythrocyte enzymes activity in diabetes is a result of the constant oxidative stress in this disease which can be responsible for the development of long-term complications.

2318

NEW STRATEGY FOR TREATING THE DIABETIC HYPOXIA : PREVENTION OF HEMOGLOBIN-AGE FORMATION

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In the pathologic mechanisms underlying diabetic neuropathy, a role of endoneurial hypoxia, as well as impaired polyol pathways, is recently stressed. However, in the model of streptozotocin-induced diabetes, the endoneurial blood flow (eBF) , which plays a pivotal role in tissue oxygen supply (tOx), remains at the range enough to preserve the anaerobic glycolysis of the sciatic nerve, although the nerve conduction is already disturbed 3 weeks after the diabetic operation. We thus postulated that the dysfunction, not the mass (flow), of the oxygen carrier protein, hemoglobin is problematic at such earlier time points. A positive correlation between eBF and tOx, as seen in non-diabetic rats, was disturbed in the diabetic conditions. This was backgrounded by an impaired capacity of oxygen release from diabetic erythrocytes. The decreased oxygen half partition pressure (P50) was likely to be a crucial factor for limited oxygen supply to the peripheral nerves. The P50 values are generally influenced by the level of the 2,3-DPG in erythrocyte, however, in the diabetes, this may not be the case. The raised level of 2,3-DPG does not necessarily function to elevating P50 values. This is caused by a dysfunction of the diabetic hemoglobin. In the present study, we demonstrated that the dysfunction of the oxygen carrier protein is attributed to the advanced glycation end products (AGE) of hemoglobin, Hb-AGE, rather than the glycation of the carrier protein, HbA1C, and that a novel aldose reductase inhibitor, SG-210, if orally administered for 2 weeks, alleviates the formation of Hb-AGE for improved the tissue oxygenation. A clue may thus be provided for medication of the diabetic neuropathy.

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THE ERYTHROCYTE SORBITOL LEVELS CORRELATE WITH BLOOD GLUCOSE LEVELS AND DIABETIC COMPLICATIONS

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Abnormal polyol metabolism may contribute to the development of diabetic complications. Previous reports on the roles of sorbitol in diabetic patients provided contradictory results probably due to the poor reproducibility of sorbitol assay methods. We devised an improved sorbitol assay and investigated the relationship between sorbitol levels in erythrocytes and glycemic control indices and diabetic complications, and also evaluated the effect of aldose reductase inhibitors on the sorbitol level. Erythrocyte sorbitol concentrations were measured as follows: The washed erythrocytes were mixed with NaOH and ZuSO4, followed by centrifugation. Two ml of the supernatant was added to 2.0ml Tris-HCl buffer containing 10mM EDTA, 2U/ml sorbitol dehydrogenase and 12.5mg NAD, and incubated at 37℃ for 30 min. The NADH thus formed was measured at an excitation wavelength of 366 nm and an emission wavelength of 452 nm.

Fasting sorbitol levels were 67.1±36.3nmol/gHb in NIDDM patients, 95.0±60.8nmol/gHb in IDDM patients, and 38.5± 8.1nmol/gHb in normal individuals. Positive correlations were noted between the fasting blood glucose and sorbitol levels, and between the HbA1c and sorbitol levels. Sorbitol levels did not correlate with the 24h Ccr, microalbuminuria, tibial nerve MCV or ECG R-R100 values. Treatment with aldose reductase inhibitors for one month induced a decrease in the sorbitol levels. Thus, this assay method makes it possible to measure erythrocyte sorbitol with good reproducibility and to elucidate the relation between the diabetic condition and sorbitol levels.

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RELATION BETWEEN EARLY GLYCATION PRODUCT AND ADVANCED GLYCATION END PRODUCT ON ERYTHROCYTE MEMBRANE PROTEIN AND HEMOGLOBIN.

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Protein glycation mainly consists of two sets of reaction in vivo, early reversible and advanced irreversible glycation. Few reports are available concerning comparisons of levels of early and advanced glycation products in vivo. We made use of a radioimmunoassay to detect early glycation and an ELISA for advanced glycation, we assessed the amount of early glycation product (EGP) and advanced glycation end product (AGE) in erythrocyte membrane protein (EMP) and Hb in diabetic (DM) and non-diabetic control subjects (C). The levels of early EGP in EMP were 48.4±2.5 (nmole glucitollysine/mg proteins) in C, and 79.4±13.1 in DM (P<0.001) while levels of EGP in Hb were 4.1±1.4 (nmole glucitollysine/mg Hb) in C and 7.5±2.1 in DM (P<0.001). The levels of AGE in EMP by ELISA were 22.5±7.7 (AGE Units/mg proteins) in C, and 70.7±51.8 in DM (P<0.001), and the levels of AGE in Hb were 3.8±1.2 (AGE Units/mg Hb) in C, and 12.3±2.1 in DM (P<0.001). To investigate the large differences in levels of AGE in EMP and Hb between DM and C compared to EGP, we estimated the the levels of AGE in samples with several proteins (EMP, Hb, albumin) incubated with glucose, fructose, fructose-3-phosphate (F-3-P) or intermediate substance 3 deoxyglucosone (3-DG). The formation of AGE was dramatically increased by incubation with F-3-P and its decomposition products 3-DG, compare to the incubation with glucose or fructose. The AGE inhibitor aminoguanidine effectively inhibited AGE formation by 3-DG. These findings demonstrate that even under the same metabolic conditions the extent of EGP and AGE differs depends on proteins and the extent of AGE dramatically increases in DM patients, although EGP in DM patients remain increases little.

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GLICATED γ-GLOBULINS: A NEW PARAMETER FOR METABOLIC CONTROL OF DIABETIC SUBJECTS?

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The knowledge of the glycation level of γ-globulins in diabetic subjects can be relevant either for the disease control or for its possible relationship with immunological impairment. Consequently the development of specific methods for the determination of such levels is of high interest. Stimulating results have been achieved by matrix assisted laser desorption/ionization mass spectrometry (MALDI/MS), which allows to determine the molecular weight of intact proteins with an accuracy in the range 0.1-0.01%. Fourteen badly controlled diabetic patients [**A**] (mean age ± SD 63 ± 7 years, mean disease duration 12 ± 8 years, mean fasting plasma glucose (FPG) 20.8 ± 4.5 mmol/L, mean furosine 0.47 ± 0.07 μg Fur/mg prot, mean HbA_{1c} 10.9 ± 2.0%), eight well controlled diabetic patients [**B**] (mean age 60 ± 12 years, mean disease duration 13 ± 9 years , mean FPG 8.0 ± 1.2 mmol/L, mean furosine 0.33 ± 0.03 μg Fur/mg prot, mean HbA_{1c} 7.2 ± 0.5%) and eight normal controls [**C**] (mean age 57 ± 9 years, mean FPG 5.5 ± 0.3 mmol/L, mean furosine 0.22 ± 0.02 μg Fur/mg prot, mean HbA_{1c} 5.5 ± 0.4%) were examined. For these subjects the molecular weight of human serum albumin (MW_{HSA}) and γ-globulin (MW_γ) were determined by MALDI and compared with those of genuine, non-glycated proteins. HSA shows mean mass increase (ΔM_{HSA}) of 1491 ± 624 Da for **A**, 321 ± 82 Da for **B** and 76 ± 48 Da for **C**. In the case of γ-globulins ΔM_γ values of 2516 ± 1120 Da, 1128 ± 339 Da and 581 ± 347 Da have been found for **A**, **B** and **C** respectively. The observed mass increase (ΔM_{HSA} and ΔM_γ) must be related to a number of glucose molecules condensed on the proteins. The plots ΔM_γ *vs* FPG, furosine, HbA_{1c} and ΔM_{HSA} result linear, with correlation indexes of 0.754, 0.641, 0.832 and 0.869 respectively. These results show that by MALDI an easy determination of γ-globulins glycation levels can be achieved. Their relationship with other metabolic control parameters suggests its use in the same field and suggests to undertake investigations on its possible relationship with immunological impairment.

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QUANTIFICATION OF CROSSLINE™ IN SERUM AND ERYTHROCYTE MEMBRANE PROTEIN IN DIABETIC SUBJECTS

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Recent studies have suggested that advanced glycation endproducts (AGEs) play an important role in the pathogenesis of diabetic complications. Crossline is a novel AGE-product which has both crosslink and fluorescence similar to AGE-proteins *in vivo*. Although the accumulation of AGEs in specific tissue might explain the role of this compounds for the deterioration of diabetic complications, we need more pragmatical strategy for the evaluation of glycation, the most important events in the development of diabetic complications. Now we developed high performance competitive ELISA system for the determination of crossline. The aim of this study was the cilinical evaluation of this crossline assay system in diabetic patient; the relationship between serum level <S> or values in erythrocytes membrane proteins <M> of crossline and glycemic control or diabetic complications. Serum samples or erythrocyte membrane fractions were collected from 69 NIDDM patients and 21 normal subjects as control. Glycemic control was evaluated by measuring HbA1c and diabetic retinopathy was assessed by ophthalmologist. <S> and <M> of crossline were positively correlated with HbA1c, r=0.312 (p<0.05) and r=0.427 (p<0.01), respectively. In patients with retinopathy, however, <M> of crossline significantly increased (p<0.01) independently to HbA1c levels, although no relation was observed between HbA1c levels or <S> of crossline and diabetic retinopathy. These results suggested that 1) serum levels of crossline reflect diabetes controls, 2) crossline levels in membrane also correlated to glycemic control and would be possible indicator or predictor of diabetic complications such as retinopathy.

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Complications – Clinical Problems

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HYPEROSMOLAR HYPERGLUCEMIC NONKETOTIC COMA, CLINICAL EXPERIENCE IN HOSPITAL 25 IMSS MONTERREY, N.L. MEXICO.

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We analized the cases of patients with hyperosmolar hyperglucemic coma in Hospital 25 IMSS, Monterrey N.L. México; in the period January 1993 - December 1995. They were 406 hospitalized patients with diabetes type II, 20 out of all with hyperosmolar coma: 3 cases associated with ketoacidosis, 17 hyperosmolar hyperglucemic nonketotic coma (HHNC): 1 case in 1993, 7 in 1994 and 9 in 1995. 7 Out of 17 patients were women (41%) and 10 men (59%). The mean age was 67 years (44 - 87), with mean duration of diabetes: 12 years (2 - 30 years), only 3 cases with recent onset of diabetes. 9 patients in treatment with oral hypoglucemiants agents (54%), 2 with insulin (11%), 1 with diet alone (6%) and 5 without treatment (29%). Precipitating causes: 6 cases due to pneumonia, 2 urinary infection and 2 gastroenteritis, one case due to pancreatitis (6.6%), 1 due to stroke (6.6%), 1 due to surgery stress (gastrectomy) (6.6%), and 1 due to chemotherapy (6.6%) and 7 cases with unknown etiology. The mean initial glycemia was 1043 mg/dl (+/- 443). The mean corrected serum sodium was 163 mEq/lt. (+/- 24). the mean osmolarity was 378 mOsm/lt (+/- 52). 9 patients died (53%), mostly due to complications: sepsis (3 cases), ARDS (2 cases), pulmonary thromboembolism (2 cases) and acute myocardial infarction (2 cases). In conclusion, HHNC is a infrequent complication but severe in the diabetic patient type II, mostly in that patient with inadecuate control of diabetes and delay in medical assistance, precipitating by infection or acute stress. Poor prognosis is seen in advanced age, severe hyperglycemia, hypernatremia, hyperosmolality, severe acidosis and developing some complication. Th adequate treatment with insulin, potassium and liquids improve the prognosis.

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TREATMENT OF CHRONIC ERECTILE DYSFUNCTION WITH TRANSURETHRAL ALPROSTADIL IN DIABETIC AND NONDIABETIC MEN.

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Erectile dysfunction is a common problem in diabetic men. Previous short-term studies have shown that alprostadil delivered by a transurethral system produces erections and restores sexual intercourse. We have now studied the clinical safety and efficacy of MUSE® (alprostadil) delivered by this system over a 3-month period in diabetic and nondiabetic men. 1511 patients (age: 30-84; mean 62) with organic erectile dysfunction (mean duration: 4 yrs) were enrolled in a prospective, double-blind, placebo-controlled trial. Patients self-administered transurethral alprostadil (125, 250, 500, and 1000 µg) in the clinic; those achieving an erection sufficient for intercourse (996 patients) were randomized to home therapy with either active drug or placebo. Diabetes was present in 240 patients (39 insulin-dependent; 196 non-insulin-dependent; 5 unspecified). Key results are shown in the table below. In patients who responded at home, approximately 7 of 10 active drug systems resulted in sexual intercourse. Adverse effects, other than penile pain, were uncommon in the group as a whole. 88% of patients completed the 3 months of home treatment. Transurethral alprostadil is well tolerated and can restore erections and sexual intercourse in both diabetic and nondiabetic men with chronic erectile dysfunction.

% Reporting Intercourse on:	Active	Placebo	p-value
Overall	64.9	18.6	<0.001
Diabetic	64.3	21.1	<0.001
Nondiabetic	65.0	17.7	<0.001

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ENDOTHELIN-1 AND ATRIAL NATRIURETIC PEPTIDE IN DIABETIC PATIENTS WITH HYPEROSMOLAR HYPERGLYCEMIC SYNDROME

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To investigate whether endothelin-1 (ET-1) and atrial natriuretic peptide (ANP), potent vasoregulatory peptides with diverse actions, are enrolled in the pathophysiology of patients with severe decompensated hyperglycemia and dehydration. Plasma and urinary ET-1 and ANP immunoreactive substances were measured in 16 diabetic patients with hyperosmolar hyperglycemic syndrome (HHS) by specific radioimmunoassays. Plasma and urinary ET-1 levels (7.0 ± 4.1 pg/ml and 890 ± 130 ng/g Cr, respectively) were significantly higher in HHS patients in the acute phase, and after recovery, plasma ET-1 (3.6 ± 0 7 pg/ml) returned to normal range (3.4 ± 1.3 pg/ml), while urinary ET-1 level (2,360 ± 680 ng/g Cr) was still increased in HHS patients than that of the control group (260 ± 30 ng/g Cr). Plasma ANP level of patients with HHS was not significantly different during acute (22.0 ± 5.2 pg/ml) or recovery stages (22.1 ± 3.6 pg/ml) from that of control subjects (18.4 ± 2.5 pg/ml). In contrast, urinary ANP excretion was significantly higher in acute stage of patients with HHS (323 ± 100 ng/g Cr) than that of control subjects (48 ± 3 ng/g Cr), and after recovery, urinary ANP level (86 ± 10 ng/g Cr) was not significantly different from that of controls. Serials follow up of plasma and urinary vasoactive peptides and clinical manifestations showed that plasma ET-1 level was significantly correlated to the plasma angiotesin I, blood urea nitrogen, and creatinine concentrations. Our results demonstrated that plasma ET-1 may be involved in the blood pressure homeostasis and renal synthesized ET-1 and ANP contributed to the maintenance of the tubular functions in state of dehydration induced by severe hyperglycemia.

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Diabetes mellitus and hormone replacement therapy: risk-benefit situation

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Clinical experience has shown that hormone replacement therapy (HRT) is effective in relieving climacteric symptoms as well as in preventing osteoporosis and cardiovascular disease. These diseases are known as the main causes of morbidity and mortality among women with diabetes mellitus. The purpose of this study was to evaluate the possible risk factors of application HRT in women with non-insulin dependent diabetes. We examined carbohydrate and insulin change, lipid metabolism and blood coagulation in women receiving Divina for a year. Metabolic changes indicate that Divina acted beneficially not deteriorating the course of diabetes mellitus (P<0,001).

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DECOMPENSATION IN 633 ECUADORIAN DIABETICS

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We wanted to know the frequency of ketosic hyperosmolar decompensation (basal blood glucose 300 mg/dL + cetonuria), non ketosic hyperosmolar decompensation (basal blood glucose > 600 mg/dL without cetonuria) and hyperglyemic decompensation (basal blood glucose > 150 mg/dL or > 200 mg/dL two hours post-prandial) in patients with diabetes of five cities of Ecuador. It was a descriptive study that used an univariate/bivariate analysis, Data Base and Quattro softwares. We found 14.6% of hyperosmolar decompensation (ketosic and non ketosic) in insulin dependent diabetes mellitus patients in contrast of 53.6% of hyperglycemic decompensation ($p < 0.01$). In non insulin dependent diabetes mellitus patients we observed 6% of non ketosic hyperosmolar decompensation in comparation with 47.8% of hyperglycemic decompensation ($p < 0.01$). Oriental area of Ecuador had 80% of total decompensation, coast area 51.5% and mountain area 48.5%. We conclude that hyperglycemic decompensation is more frequent than ketosic/non ketosic decompensation. Finally, we thought that percenteges obtained in this study were alarming.

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QUANTITATIVE EVALUATION OF SCLEREDEMA DIABETICULUM

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Diabetic scleredema is diffuse edema with sclerotic changes of the posterior neck in patients with diabetes mellitus. We applied a new apparatus equipped with a suction device (Cutometer SEM575) to measurement of skin elasticity for quantitative evaluation of the skin lesions. Thirty-nine diabetic patients were measured using this device. The value of skin elasticity (VSE) in patients over ten years in diabetic duration was lower than that under ten years in diabetic duration. VSE in patients with diabetic retinopathy and diabetic neuropathy was lower than that in patients without these complications. VSE ($71.3 \pm 11.1\%$) in patients with scleredema diagnosed clinically was lower than that ($83.3 \pm 11.1\%$) in patients without scleredema, therefore sclerotic changes in the skin were indicated quantitatively. Histopathological examination confirmed the diagnosis of diabetic scleredema. Skin elasticity measurement using a suction device was useful for quantitative evaluation of diabetic scleredema.

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HEMODIALYSIS(HD)-INDUCED HYPERKETONEMIA IN DIABETIC PATIENTS

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Aim: Recentry, we have found that hyperketonemia(HK) occurred in hemodialysed diabetic patients. Thus the aim of this study was to address the mechanism(s) by which HK is caused in those patients.

Methods: We studied 31 diabetics and 15 non-diabetic patients(N). Of diabetic patients 15 were on good control(G), and 16 on poorly controlled(P). HD was performed using glucose-added bicarbonate dialysate, and either heparin or nafamostat mesilate(FUT) was used as an anticoagulant during the dialysis. Plasma glucose(PG), 3-hydroxybutyrate(3OHBA) and free fatty acid(FFA) were measured before and after HD.

Results: Although 3OHBA remained unchanged after HD in groups N and G, it increased significantly in group P($p<0.01$), irrespectively of use of heparin or FUT. Plasma FFA also elevated only in group P($p<0.01$). In group P the percent change in plasma FFA was significantly smaller in the patients performed HD with FUT than that in those with heparin($p<0.05$). In addition, the percent change in plasma 3OHBA was significantly correlated with the percent change in plasma FFA in group P performed heparin-used HD($r=0.58$, $p<0.01$). Such relationship was not found in either group N or G.

Conclusions: These findings strongly suggest that glucose-added bicarbonate HD using either heparin or FUT as an anticoagulant is able to evoke hyperketonemia in poorly controlled diabetic patients on HD.

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IRON DEFICIENCY ANEMIA IS ASSOCIATED WITH GASTRIC AUTOIMMUNITY IN INSULINDEPENDENT DIABETIC PATIENTS (IDDM)

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Previous studies have shown a high prevalence of anti-parietal cell-antibodies (PCA) in IDDM. This condition can induce chronic atrophic gastritis and elevated levels of gastrine accompanied with iron malabsorption, and hypochromic anemia. In a series of 29 IDDM patients (11 male, 18 women) with increased titers of PCA (> 1/20 dilution) 41% had a gastrine level > 100 ng/l and 45% showed significantly lower circulating iron levels (<50 µg/dl). Twelve patients (4 m, 8 w) with high gastrine levels had a peripheral hematogram typical for hypochromic, microcytic anemia (41%) that could be normalized with parenteral iron injections. This group (A) was compared to a second group (B) consisting of 121 PCA negative IDDM patients (71 m, 50 W) with the same age distribution. In group B only 6.6% showed elevated gastrine ($p<0.0001$) and 19% had low iron levels ($p<0.01$). Anemia was present in 16% of the patients but only 14 had hematologic evidence of iron deficiency. As compared to group A, a significant higher incidence (Fisher exact test: $p=0.0085$, Odds ratio: 4.023, 95% CI: 1.560-10.371) of iron deficiency anemia looks to be present when PCA titers are elevated. All the patients with anemia were explored in order to find other potential etiologies. For this reason 2 cases of Biermer anemia and 1 case with auto-immune hypothyroidism were excluded from group A. In group B, 1 folium deficient anemia and 4 normochromic anemias of various origin were excluded. Since there is some doubt about the influence of the level of metabolic control on iron levels, 103 serum iron levels were correlated with the corresponding HBA1c taken at the same moment. The correlation coefficient ratio was 0.057 (p value of 0.56) indicating a complete lack of correlation. In conclusion it looks important in practice to keep gastric auto-immunity in mind as a frequent cause of iron deficiency in the presence of hypochromic anemia in IDDM. The ionic form of iron and the way of administration can be important to maintain a normal hematopoiesis in this kind of patients.

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THIS ABSTRACT HAS BEEN WITHDRAWN BY THE AUTHOR.

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Anemia in Non-Uremic Diabetic Patients

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Anemia is a frequently noted disorder in diabetic patients. So, we evaluate the causes and characteristics of anemia in diabetics without uremia(creatinine clearance rate $\geq$ 30 ml/min/m^2). Total 192 patients (M:F=73:119, 14 IDDM and 178 NIDDM) with disease duration of mean 10.5 $\pm$ 7.4 years, were investigated. In order to analyze the causes of anemia, serum iron, total iron binding capacity, ferritin, vitamin B_{12}, and folate levels were measured and if necessary, serum erythropoietin(EPO), Coombs' test, serum haptoglobin, bilirubin, peripheral blood smear, and bone marrow studies were taken in 69 cases. Serum EPO levels were measured in 20 non-diabetic anemic patients as a control.

Serum hemoglobin(Hb levels were mean 11.1 $\pm$ 1.1g/dl with 180 mild anemic(Hb 9-12 g/dl) and 12 moderate anemic (Hb 6-9 g/dl) patients. Erythrocyte indices were normocytic in 134 (69.8%) out of 178 patients, macrocytic in 42 (21.8%), and microcytic in 16 (8.3%). The Hb levels were negatively correlated with diabetic duration (r=-0.21, p=0.03) and the amount of albuminuria(r=-0.39, p=0.01). The evaluable causes of 69 anemic patients were iron deficiency(21.7%), chronic disorders(18.8%), folate deficiency(4.3%), vitamin B_{12} deficiency(1.4%), hemolytic anemia(1.4%), aplastic anemia(1.4%), however causes were not explained clearly in 35 (51.0%) patients. Serum EPO levels of patients with unexplained anemia were mean 17.1 $\pm$ 8.0 mU/ml, which were significantly lower than that of non-diabetic anemic patients(144.9 $\pm$ 108.0 mU/ml, p<0.01). Blunted EPO response to anemia was noted in patients with diabetes showing unexplained anemia.

In conclusion, causes of anemia were not interpreted clearly in many diabetics patients. Insufficient EPO response may play some roles to unexplained anemia in non-uremic diabetic patients.

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ANALYSIS OF DIABETIC KETOACIDOSIS AND HYPEROSMOLAR NONKETOTIC COMA CASES

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In this retrospective study, diabetic ketosis, ketoacidosis (DKA) and hyperosmolar nonketotic coma (HNKC) cases were analysed in a 15 year period (1980-1995). Of 82 cases evaluated, 47 of them fulfilled the criteria for DKA, 23 cases for diabetic ketosis and the remaining 12 cases for HNKC. Of 47 cases with DKA, 66% had juvenile onset (<30 years) type I diabetes, 14.9% had adult onset (≥30 years) type I diabetes and the remaining 19.1% had NIDDM (Non insulin dependent diabetes mellitus). Of patients with diabetic ketosis, 78% had NIDDM, 13 % had juvenile onset, 4.3% had adult onset type I and 4.3% had secondary diabetes. All cases with HNKC were diagnosed as having NIDDM. Ketoacidosis was found to as initial clinical presentation in 13.4% of cases. In the whole group, the most common precipitating factor was infection (32.9%). Omission of insulin therapy (9.8%), trauma (4.9%), myocardial infarction (2%) and cerebrovascular accident (1%) were other precipitating factors. In 25.6% of patients, the cause of ketoacidosis was unknown. As to DKA cases, urinary tract infection in two patients (4.3 %), hypoglycemia in five patients (2.1%), thrombophlebitis in one patient (2.1%) , acute renal failure in two patients (4.3%) and for diabetic ketosis cases, hypoglycemia in 4 patients(17.3%) and sepsis in 2 patients(8.7%) developed as a complication during the treatment. In HNKC cases, sepsis, brain edema, thrombophlebitis and mesenteric vascular disease were seen in one patient (8.3%) for each. Mortality rates for DKA, diabetic ketosis and HNKC cases were 6.4%, 8.7% and 33.3% respectively. The data suggest that DKA and HNKC are still major problems in diabetic patients despite the advance in diagnosis and treatment.

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Limited Joint Mobility and Respiratory Function Tests in Children with Insulin Dependent Diabetes Mellitus

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Limited joint mobility (LJM) is an early complication of insulin dependent diabetes mellitus (IDDM) and it is also associated with the increase in the frequencies of complications observed in long term IDDM.Published data regarding the respiratory function tests performed in patients with IDDM having LJM were conflicting . 60 patients with IDDM were included into the study to evaluate the relationship between LJM and respiratory function tests.These patients were separated into two groups as LJM (+) (n:32) and LJM (-) (n:28) .The weight, height and HbA1c values did not differ significantly between these two groups.The age of the patients and the duration of diabetes were significantly higher in LJM (+) group (p<0.01).

Respiratory function tests were performed with spirometer for all patients in both of the groups. Despite the significantly low levels of FVC observed in LJM(+) group

(LJM(+) mean±SD: 88.3±18.01, LJM(-) mean±SD: 104.3±9.07) (p<0.05), no difference was found considering FEV1 for both of the groups (LJM(+) mean±SD: 77.25±19.6, LJM(-) mean±SD: 87.25±19.02) (p>0.05).Results of the function tests in LJM(+) patients was correlating well with a restrictive type of respiratory disorder.

It is well known that diabetes can cause changes in collagen metabolism .LJM is shown to be related to changes in collagen metabolism. Collagen is also important for the architecture and the functions of lung . Our data support the view that LJM and restrictive type of respiratory dysfunction occur together.

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EVALUATING SEVERITY OF ACUTE COMPLICATION OF DIABETES IN KOREA BY USING APACHE III SCORING SYSTEM

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As an acute complication of diabetes, diabetic ketoacidosis(DKA) and hyperglycemic hyperosmolar state(HHNS) need intensive care in an early stage of disease, because of relatively high mortalities. To evaluate the expected mortality of acute complication of diabetes, we enrolled 154 patients(87 male, 68 female, mean age 50±20 yr) who need intensive care unit from March, 1986 to March, 1995. We used APACHE III as an objective grading methods of disease severity and mortality and ADA criteria as a diagnostic tool for DKA and HHNS. The overall mortality of 154 patients was 20.1%(31/154). Ther were significant difference in BUN(46.6 ± 28.0 vs 32.0 ± 18.7 mg/dl), age(59.3±20.0 vs 43.3 ± 18.2 yr), duration of diabetes(7.4±7.2 vs 4.6 ±5.1 yr) and APACH III score(69.9 ± 24.9 vs 44.8 ±11.7) between death group(n=31) and survival group(n=123)($p<0.05$). The mortality of DKA(n=106) was 20.7%, while that that of HHNS(n=48) was 18.8%. The difference of APACH III score between both groups was not significant($p>0.05$). Logistic regression analysis about the relationship between APACH III score and risk of death revealed the equation: $\log_n R/(1-R) = 0.04314 \times (\text{APACH III}) - 3.488$. According to this equation, the estimated risk of death was over 50% at the point range 80-85 and over 95% at 140-145. APACH III scoreing system could be a useful guideline to predict and to evaluate the outcome and it would be valuable in clinical decision making and proper management of acute diabetic complication.

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FAILURE TO DETECT THE MALIGNANCY IN POOR-CONTROLLED DIABETICS WITH HIGH LEVEL OF TUMOR MARKERS

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To elucidate the reliability of the tumor markers (TMs) for detection of the malignancy in diabetics, serum CEA(EIA), CA19-9(RIA) and DUPAN-2(EIA) were determined in 74 patients(M:42,54.8 y.o.,F:32,53.0 y.o.) with 62 NIDDM and 12 IDDM. In 17(23 %) of patients, TMs were in high value. Mean of HbAlc level was 11.7 %. Mean of serum CEA was 41.7 ng/ml, CA19-9 was 74.7 U/ml, and DUPAN-2 was 910 U/ml. The frequencies of high level of TMs were 16/17(94 %) in CEA,14/17(82 %) in CA19-9 and 3/17(18 %) in DUPAN-2. Both high CEA and CA19-9 was 11/17 (65 %), and all of high TMs was 3/17(18 %). By various studies: gastrofibersopy,colonofiberscopy,ERCP,US and CT, 3 of all diabetics(5 %) beared some malignancies were diagnosed.They had progressive gastric cancer(GC),sigmoid cancer and both GC and multiple colon cancers. All of the patients suffered from these cancers had both high CEA(153 ng/ml) and CA19-9(99 U/ml). But none of patients had high level of DUPAN-2 and no liver and billiary tract metastasis. Whereas,in diabetics beared no malignancies, all of TMs changed in lower values accompanied with the improvement of BS control(HbAlc:12.2 - 9.7 %,CEA:10.4 - 3.4 ng/ml,CA19-9:67.4 - 35.4 IU/ml,DUPAN-2:910 - 413 U/ml)

These results suggest that in poor BS controlled diabetics, serum CEA,CA19-9 and DUPAN-2 have a tendency to be in high value and variability with BS level. Then, these TMs should not be reliable to detect malignancies.

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INVESTIGATION 230 CASES OF KETOACIDOSIS DURING A 6 YEAR PERIOD

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The aim of this study was to gather data about diabetic ketoacidosis morbidity and mortality and to investigate the diagnosis and the treatment. 230 adults were treated with diabetic ketoacidosis (DKA) during a 6 year period from 1989 to 1995 from an area with a population of 350 000. All these diabetics were treated in the same department and their medical records were reviewed retrospectively. The average age of the patients was 48 year (18-78) with a 1,52:1 ratio of men and women. 69 DKA repeatedly occuring episodes were treated in 16 patients (11men, 5 women). The following reasons for DKA were found: infection was proved in 56 cases (24,3 %), newly diagnosed diabetes was in 20 cases (8,6 %), pancreatitis was in 8 cases (3,5 %), myocardial infarction and stroke was in 4-4 cases (1,8-1,8 %)- other reasons in 31 cases and in 107 cases (24,3%) it was not possible to identify the reason. Of the 230 DKA cases 12 patients died which represent a 6,08 % morbidity. Their avarage age was 61 year (41-78). Most patients were admitted to the department from an emergency ward.The diagnosis of DKA was often made too late. These diabetics spent an avarage 3,7 hour in the emergency ward. During this time they received an average 1,3 l infusion and often unnecessary bicarbonate treatment.During the first 24 hours the patients received an average 5,7l infusion and 56 U insulin in the department.DKA still have a high morbidity and mortality . It is important to educate on the first first symptomes of DKA especially to those who had recurring DKA. The patients with recurring DKA often need psychological support as well. The use of glucometers and urine test strips could help to diagnose DKA at an earlier stage.In conclusion the results suggest that, better education of the patients and the medical staff could reduce morbidity and mortality of DKA.

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DIABETES MELLITUS AND MITRAL VALVE PROLAPSE

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Mitral valve prolapse (MVP) is the most common cardiac valvular anomaly ethiopathogenesis of which is not known. Mitral valve prolapse can occur as a primary anomaly, and it can also be detected during the course of some systemic diseases. It is suggested that autoimmune mechanisms may play a role in the ethiopathogenesis of mitral valve prolapse, but it is not known whether diabetes mellitus plays a role in MVP. For this reason, we planned this study to determine the prevalence of MVP in both type I and type II diabetic patients. One hundred three patients (35 male, 68 female) whose mean age is 47 years are taken in the study. Seventy six of the patients had type II diabetes, 27 had type I diabetes. Patients who had autoimmune, metabolic and heart diseases that can influence MVP were excluded. Sixty volunteering normal subjects without any diseases were taken as control group. Two-dimensional M-mode colour Doppler echocardiography was applied to all patients after physical examination and routine laboratory analysis were made. Mitral valve prolapse was diagnosed when one or both of the mitral valves deplaced back in to the left atrium more than 3 mm in systole. Mitral valve prolapse was detected in 3 (5%) of control group and 13 (12.6%) of 103 diabetic patients. Prevalence of MVP was higher in diabetic patients but the difference was not statistically significant ($p>0.05$).

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DIABETIC KETOACIDOSIS IN ENDOCRINOLOGY DEPARTMENT, HOSPITAL 25 IMSS, MONTERREY, N.L. MEXICO.

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We analized the cases of Diabetic Ketoacidosis (DKA) in the period January 1993 - December 1995. There were 496 hospitalized diabetic patients in that period of time in the Departament of Endocrinology, 121 with diabetes type I and 375 with type II; 72 patients with DKA, 29 cases in 1993, 24 cases in 1994 and 18 cases in 1995. We found only 26 charts, the remainder were not included in our study, because they were not found. Out of 26 patients, 11 were men (42%) and 15 were women (58%), mean age was 36 years (13 - 59), 22 patients with diabetes type I (85%), 4 patients with diabetes type II (15%). The evolution of diabetes, was about 10 years in average. Only 1 patient with recent diagnosis. In relation to previous treatment before the admission to hospital:12 patients required insulin intermediate twice a day (46%), 4 patients with insulin intermediate once a day in morning (15%), 5 patients with combined insulin (rapid and intermediate) twice a day (19%) and 3 patients taking only oral hypoglucemiants (12%). Precipitating factors like infections were the most frequent, were 11 cases (42%): 6 urinary infection and 4 respiratory infection, 1 periodontal abscess. Digestive etiology 2 cases (8%): 1 secundary to digestive bleeding and 1 due to acid-peptic syndrome; 2 cases (8%) due to inadecuate treatment for diabetes and suspension of insulin, 3 cases (11%) due to inadecuate diet; 1 case (4%) due to myocardial infarction and 7 cases (27%) with unknown precipitating factors. 11 patients developed complications: 5 cases (45%) due to hypokalemia, 2 cases (18%) with pneumonia, 1 case (9%) with myocardial infarction, 1 case (9%) pulmonary thromboembolism, 1 case (9%) acute renal failure, 1 case (9%) hypoglycemia. There were 3 died patients (12%), 2 due to myocadial infarction and 1 case due to metabolic encephalopathy and pneumonia. In conclusion the education of diabetic patients about the disease and complications, is the keystone for preventing DKA.

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FREQUENCY OF THYROID DYSFUNCTION IN TYPE 2 DIABETIC PATIENTS.

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To assess the prevalence of thyroid disease in type 2 diabetes (NIDDM) we used the sensitive TSH assay. The study population consisted of 319 patients, 189 females(F) and 130 Males(M), aged 37-83, with HbA_1C less than 10%, normal kidney and liver function and no clinical signs of thyroid disease. The patients with abnormal TSH were assayed for FT_4, TT_3, thyroid antibodies. Thyroid dysfunction was diagnosed in 15.7% (50 patients) with a much higher prevalence in females: 24.9%F, 2.3%M. The commonest diagnosis was subclinical hypothyroidism (11.6%F, 0.8%M), followed by hypothyrodism (6.9%F, no males), subclinical hyperthyroidism (4.2%F, 0.8%M) and hyperthyroidism (1.6%F, 1.5%M). All 5 hyperthyroid patients had T_3 toxicosis. Thyroid antibodies were detected in 11/31 patients. Conclusions: 1)Thyroid function should be screened routinely in NIDDM patients (especially in women) to detect the frequent asymptomatic thyroid dysfunction. 2)Further study is currently performed to confirm the association between NIDDM and T_3 toxicosis.

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KETOACIDOSIS IN NATIVE AMERICAN APACHES WITH NON-INSULIN DEPENDENT DIABETES MELLITUS

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Background: The Native American Apache as with other Native American groups have high rates of non-insulin dependent diabetes mellitus while insulin dependent diabetes mellitus is rare. Diabetic ketoacidosis in this population would be expected to be unusual but when present has been attributed to acute alcohol use. A retrospective chart review was undertaken to look at the incidence and characteristics of ketoacidosis in this population. Methods: Cases of diabetic ketoacidosis were identified at the Mescalero and White Mountain Apache reservations. Retrospective chart reviews were used to confirm the acidosis and determine the influence of alcohol. Alcohol use was considered recent if included in the history of present illness as a contributing factor; a lifetime history of alcohol use was considered present if recorded as a problem in the past medical history. Results: We found seventeen patients out of 727 diagnosed diabetics who had episodes of diabetic ketoacidosis. All were at least three-quarters or greater Native American heritage. The mean age was 41+/-14 (mean +/-SD). The patients were predominantly male (88%), with a body mass index of 24.9kg/m2+/-4.4. Recent alcohol use was noted in only 27% of presentations while lifetime use of alcohol was noted in 94% of cases. Conclusion: Diabetic ketoacidosis is not unusual occurring in 2.3% of known diabetics in this Native American population of Apaches. Recent alcohol use was less common than expected despite high rates of lifetime alcohol use. Diabetic ketoacidosis is a significant complication this NIDDM population and the role of alcohol needs to be further delineated.

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THE IMPORTANCE OF MONITORING THYROID AUTOANTIBODIES IN ADULT IDDM

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To evaluate a clinical revalence of thyroid autoantibodies(Th-AAb) we assessed antibodies to thyroglobulin(TgA,RIA) and to microsomes (MsA,ELISA) in 50 patients at diagnosis(Dg) of IDDM (25years) and repeteadly during 8 years follow-up.
Results: At Dg incidence of TgA and MsA(0%,8%)in men(M) was in normal range, while in female(F) both TgA(8%) and MsA(15%) were increased. During follow-up Th-AAb prevalence signif.increased: in F we found TgA in 33% and MsA in 67% as compared to 31% and 56% in M. Th-AAb first detected in the first 5 years had a marked tendency to persist in contrast to those first detected after 5 years. Depending on the nature of Th-AAb the patients were divided.

	M:F%	TSH mIU/l	Pathological US
TGA++,MsA++	7:33	5.4+1.2 x	100% x
TGA-,MsA++	21:28	3.2+0.8	86%
TgA+,MsA+	43: 6	2.5+0.7	28%
TgA-,MsA-	29:33	2.5+0.7	12%

++persisting +sporadic -negative US ultrasonography x $p < 0.001$ TSH 1:2,3,4 US 1:3,4
Conclusions:Th-AAb monitoring revealed:1)an unexpected high prevalence of autoimmune thyroid disease(AITD) in adult IDDM early after Dg. 2)high prediction of the clinical relevant forms of AITD was associated with Th-AAb detected within the first 5 years and which persisted. 3)Neither a sporadic Th-AAb appereance observed later nor negative findings were associated with AITD.

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INTENSIVE CARE OF PATIENTS WITH DIABETES KETOACIDOSIS M.Dudás, I.Fazekas and I.Iványi. Department of Internal Medicine,Gyula,Hungary

The authors working at a department of internal medicine specializing in diabetology surveyed the data of the patients with hyperglycaemic ketoacidosis treated between 1991-1995.74 patients'94 episodes justified hospitalization during that period.The average age was 51,7(8-84)yrs.The patients had had diabetes for 7,8(9 months-36yrs)yrs on average.25 relapses occured in 10 cases.The most frequent reasons precipitating them were enteral infection (25,5%) and respiratory infection(23,4%).In the case of 29 patients(30,8) along with other factors,the failures of dieting also contributed to acute metabolic disorders.On admission the level of consciousness was closely related to the increase in bloodsugar and urea nitrogen level as well as the decrease in the ph level of capillary blood.The first specific step taken was administering salt solution(1191 ml on average in the first hour) parenterally.In the majority of the cases fast-affecting insulin (Lilly Humulin R,Novo Actrapid HM) was administered by means of infusion pumps or,in the other cases,low-dosage intravenous and intramuscular infections.The authors emphasized the importance of potassium supply.They tried to avoid agressive and quick acidosis correction during the treatment.Despite the application of the latest therapies,relatively high mortality (15,9%)was brought about by chronic statuses lasting for several days then.

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MELAS BROKED OUT 24-YEARS LATER AFTER ONCET OF IDDM -AN UNUSUAL AUTOPSY CASE-

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A typical mitochondrial(mt) myopathy, encephalopathy, lactic acidosis and stroke-like episodes(MELAS) syndrome is sometimes accompanied by insulin dependent diabetes mellitus(IDDM). The objective of this presentation is to disclose a relationship between both diseases. **Case Report**: A 55-year-old female died of pneumonia followed by unconsciousness due to high blood sugar levels(831 mg/dl) and a severe stroke-like episode. Family history was unequivocal. After the birth of the patient's second baby at age 27, she began suffering from IDDM. As a result insulin therapy was needed, but the DM control was extremely poor because of fluctuating marked increases in blood sugar. From age 51, she started to suffer from hearing loss which finally led to deafness. At age 53, her husband noticed changes in her personality, and she complained of visual disturbances(cortical blindness). At this time, a limb muscle biopsy demonstrating abundant ragged-red fibers and an mtDNA analysis revealing a heteroplasmic A-3243-G transition of tRNA confirmed the mt disease. **Autopsy Finding**: The macroscopic findings of the brain showed marked ventricular enlargement (dominant to the occipital portion) with a softening of the cortex. The pancreas showed remarkable atrophy and amyloid depositions among islets of Langerhans. The microscopic findings comfirmed the mt disorder, scar stage of IDDM and diabetic nephropathy. In conclusion, this case indicates that age-related accumulation of the mutant mitochondrias between each tissue over a period of 24 years is clinically important to the onset of the symptoms and occurrence of MELAS and seems related to long-termed IDDM.

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DIABETIC KETOACIDOSIS IN TRIPOLI. THE CAUSES AND OUTCOME OF 100 CONSECUTIVE CASES

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To describe the characteristics, causes and outcome of patients with diabetic ketoacidosis we collected the following data on 100 consecutive admissions. Male/female ratio was 51/49, their mean age 30.6 ± 15.5 years, duration of diabetes 8.7 ±7.7 years, age at diagnosis 23.7 ±14.7 years, body mass index 22.6 ± 5.4, insulin treated 73%, tablet treated 5% and 22% were new cases of diabetes presenting with diabetic ketoacidosis. Arterial pH 7.12 ± .14 and serum bicarbonate 7.8 ± 4.0. Causes of diabetic ketoacidosis were insulin omission 41%, inappropriate medical advice 3%, infection 13%, including one case presented with diabetic ketoacidosis and pulmonary tuberculosis, non-infection illness 3% and undetermined 39%. Mortality was 2%. BMI was significantly higher among females than males (24.5 ± 6.3 vs 20.9 ± 3.4). $P < 0.001$. The number of underweight subjects was significantly higher among males than females (20/51 vs 6/49). $P < .01$. There was a trend among males to omit insulin more than females (25/51 vs 16/49) NS. The low mortality rate is noted. The data suggests many of the cases of diabetic ketoacidosis are avoidable, especially those caused by insulin omission. Measures required for prevention include a considerable need for education for patients, health professionals and the general public.

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BREATHING PATTERN AND RESPIRATORY MECHANICS IN INSULIN-DEPENDENT DIABETES MELLITUS.

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The interaction between the presence of insulin-dependent diabetes mellitus (IDDM) and the performance of inspiratory muscles has, so far, been scarcely studied. To better focus this topic we evaluated 6 IDDM patients with a stable and good metabolic control (HbA1c repeatedly < 7.2% in the previous 12 months), without smoking history, or without microvascular complications (somatic or autonomic neuropathy, retinopathy or nephropathy), as compared to a sex-and-age matched group of 6 non diabetic individuals. In each subject we measured pulmonary volumes, the arterial blood gases, time and volume components of ventilation such as tidal volume (VT), respiratory frequency (Rf), and mean inspiratory flow (VT/Ti), pleural pressure swings (Pplsw), dynamic compliance (Cldyn), and the transdiaphragmatic pressure (Pdi). We also evaluated the maximal inspiratory pressure (Pplsn) during the sniff manoeuvre. No difference was measured in pulmonary volumes, as well as the arterial blood gases, Pplsn, Pdi and the breathing pattern, between diabetic patients and controls. In contrast IDDM patients were characterised by a lower Cldyn, a higher Ppslw%Pplsn (a measure of respiratory effort), a higher (Pplsw%Pplsn)/VT (a measure of neuroventilatory coupling of the respiratory pump), and a greater tension time index [(Pplsw%Pplsn)(Ti/TTot)]; ($p<.05$ for all). Finally Cldyn tended to be inversely related to (Pplsw%Pplsn)/VT ($r=-0.62$). In conclusion in IDDM patients, even if in good and stable metabolic control and exempt from clinical evidence of microvascular complications, despite a normal respiratory muscle strength and gas exchange, we observed an increase in both respiratory effort and neuroventilatory coupling, due to a significantly increased elastic load

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EFFECTS OF SNK-860 AND AMINOGUANIDINE ON GLUCOSE-INDUCED IMPAIREMENT OF HUMAN NEUTROPHIL KILLING

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The aim was to elucidate the mechanism for glucose-induced impairment of neutrophil killing and to examine effects of SNK-860 (SNK), an aldose reductase inhibitor (ARI) and aminoguanidine (AG). Lucigenin-enhanced chemiluminescence (CL) to fMLP as respiratory burst and metabolites were measured in neutrophils after incubation with glucose (G), SNK (10μM) and AG (10μM). (1) Changes in CL: Both D- and L-G inhibited CL in a dose dependent fashion (40mM D-G: 65±3%, 40mM L-G: 72±3% of 5mM D-G, mean±SE, n=7). SNK and AG improved CL decreased by 40 mM D-G (40mM D-G: 66±2%, +AG: 72±3%, +SNK: 70±2%, $p<0.05$ vs 40mM D-G, +AG+SNK: 80±2%, $p<0.05$ vs +AG, $p<0.01$ vs +SNK, n=12), while SNK and AG had no effects on CL decreased by 40mM L-G. (2) Changes in metabolites: Sorbitol levels increased by 40mM D-G (0.246±0.023 n=8, $p<0.001$ vs 5mM D-G 0.141±0.017 nmol/10^7cell) was normalized by SNK (0.155±0.021), but not AG (0.252±0.029). However, fructose production (1.04±0.20 nmol/10^7cell/h) increased by 40mM D-G was decreased by SNK (0.70±0.12) and AG (0.71±0.14), respectively. Both AG and SNK had no effects on glycolytic intermediates.

Our results indicated that SNK improved decreased CL by blocking increased polyol pathway activity, while AG worked through different metabolic mechanisms from the inhibition of glycation. It seems likely that AG and ARI may be effective for the prevention of susceptibility to infection as well as the prevention of microangiopathy in diabetic patients.

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DIABETES MELLITUS AND DEEP VEIN THROMBOSIS

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The main aim of this study was to research what is the morphological changings at early stage of deep vein (D.V.) on experimental diabetic rats, and the relationship between deep vein thrombosis (DVT) in hind limbs and diabetes, as well as whether the plasma level of PAI-1 is the risk factor to the DVT with diabetes. Induced male Sprague-Dawley diabetic rats were randomized into three groups: diabetic, diabetic with insulin and controls, and maintained for twelve weeks. Blood samples were taken for t-PA, PAI-1 determination. D.V. together with muscles were isolated from hind limbs to study the vascular lesions and the development of thrombosis. The results of this study showed (1) PAI-1 activity was elevated in diabetic rats and daily insulin treatment restored it to control level. (2) Endothelial lesions and smooth muscles proliferation of deep vein significantly enhanced in diabetic rats ($p<0.01$). (3) A negative correlation was found between plasma levels of PAI-1 and t-PA ($r=-0.69$, $p<0.001$). From this study, could we also pay more-attention to DVT with diabetes in clinical future?

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SELECTED MARKERS OF INFLAMMATORY RESPONSE IN PATIENTS WITH TYPE I DIABETES AND LATENT FOCUSES OF INFECTION.

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Disturbances in inflammatory response play an important role in the pathogenesis of diabetes and its late complications. The aim of the study was to assess selected parameters of acute phase reaction in patients with type I diabetes and present focuses of inflammation. The study was performed in a group of 15 patients, 8 male and 7 female, aged 21-48 years, mean diabetes duration 5.2±3.6 years, who suffered from chronic purulent tonsillitis and/or peridental abscesses. Mean HbA_{1c} in the study group was 9.84±1.04 %. 15 type I diabetic patients matched for age, sex and metabolic control were used as controls. We evaluated: serum concentration of alpha-1 acid glycoprotein (AGP) and alpha-1 antychymotrypsin (ACT) (rocket immunoelecrophoresis according to Laurence) and the profile of their glycosylation (affinity immunoelectrophoresis with concanavalin A according to Bog-Hansen). The results of glycosylation profile were expressed as reactivity coefficients. We noticed markedly higher concentration of AGP and ACT in patients with chronic focuses of inflammation in comparison with control subjects (AGP: 1.38±0.02 vs 0.94±0.05 g/l, $p<0.05$, ACT: 0.55±0.18 vs 0.37±0.02 g/l, $p<0.05$). We also observed significant differences in glycosylation profile in these patients. AGP-RC and ACT-RC values were much higher in patients with focuses of inflammation when compared with the control group (AGP-RC: 1.19±0.14 vs 1.08±0.06, $p<0.05$, and ACT-RC: 3.44±0.53 vs 2.83±0.23, $p<0.05$). The results obtained in this study might indicate, that the presence of latent focuses of infection exacerbates a chronic inflammatory process in patients with diabetes. AGP and ACT and their glycosylation profile seem to serve as good laboratory indicators which confirm the presence of infection.

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THE PREVALENCE AND TREATMENT OF DEPRESSION IN ADULTS WITH DIABETES. M.Sheikman,M.D.,University of Massachusetts,Worcester,MA,U.S.A.

According to current controlled studies,the prevalence rate of major depressive disorder in patients with diabetes varies from 8.5% to 27.3%. Depression was found to have a more malevolent course in diabetic patients who have a greater relapse rate than in depressed,but physically healthy, population. The severity of depression correlates strongly with many symptoms of diabetes. In our previous studies depression has been found to be associated with an increased rate of complications of diabetes. In many patients depression significantly impaired diabetic management. In the group of 78 patients with serious chronic macrovascular and microvascular complications of IDDM and NIDDM 11 patients presented symptoms of depressive disorder,such as depressed mood,loss of energy,sleep disturbance,diminished abilities to concentrate,feelings of worthlessness and inappropriate guilt,2 patients had recurrent thoughts of death and one had suicidal ideations. Depression is a common accompaniment of chronic painful diabetic neuropathy. Treatment with antidepressants is indicated in these patients and is found to be successful in many cases. Some of the tricyclic antidepressants,despite showing effective antidepressant effect,were associated with worsening of diabetes producing hyperglycemia. The monoamine oxidase inhibitors were found to have a tendency to exaggerate hypoglycemia and lead to weight gain. Serotonin-selective reuptake inhibitors were found to reduce blood glucose level and could be considered as a treatment of choice for diabetic patients with depression. This group of antiderressants may lead to improvement in both depression and glucose control not causing significant impairment in alertness. Serotonin-specific reuptake inhibitors (fluoxetine,sertraline and paroxetine) cause fewer troublesome side effects,such as anticholinergic side effects and sedation,than the tricyclic and tetracyclic drugs,and are also safer when taken in overdose as compared to the tricyclic drugs. Sertraline has the least effect on cytochrome CYP2D6 activity in human liver microsomes and less effect in increasing the blood level of other medications with which combined and may be safer to use in diabetics than either fluoxetine or paroxetine.

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SERUM INTERLEUKIN-8 LEVEL IS INCREASED IN TYPE I DIABETIC PATIENTS

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Endothelial derived Interleukin-8 (IL-8) is a strong stimulator of polymorphonuclear neutrophils (PMN). Previously we have shown, that in diabetes unstimulated PMN are activated and their response to stimuli is decreased. The explanation of this might be very interesting for pathogenesis of late diabetic complications. The aim of the study was to evaluate serum IL-8 concentration in type I diabetic patients. The study was performed in a group of 42 patients, aged 19-43 years, 25 female and 17 male with mean duration of disease 10.8±8.2 years and HbA_{1c} 7.6±0.8 %. IL-8 level was estimated with the use of the ELISA test. We noticed significantly higher serum IL-8 level in diabetic patients in comparison with healthy subjects (160.29±34.81 and 39.93±4.96 pg/ml, respectively, $p<0.0001$). The results might indicate, that active inflammatory process plays an important role in the pathomechanism of late diabetic complications.

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RELATIONSHIP OF PERIODONTAL POCKET DEPTH WITH VIT-C LEVEL AND GLYCAEMIC STATUS.

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To assess the relationship of glycaemic status (HbA_1C) and serum Vit-C(mg/dl) level with periodontal pocket depth (PPD), 54 NIDDM (duration 1-5 yr) and 54 non-diabetic (ND) patients were studied. Among the NIDDM subjects, 40 were on OHA+diet and 14 were on insulin. The relationship of PPD with Vit-C and HbA1C is shown in the table.

			Periodontal Pocket depth		
			<3 mm	3-5 mm	>5 mm
Vit-C	<0.6	NIDDM	3 (5.55%)	23 (42.59%)	1 (1.85%)
		ND	5 (9.25%)	15 (27.77%)	---
	0.6-2.0	NIDDM	7 (12.96%)	20 (37.03%)	---
		ND	15 (27.77)	19 (35.18%)	---
HbA_1C	<6%		1 (1.85%)	8 (14.81%)	---
	6-10%		8 (14.81%)	25 (46.29%)	1 (1.85%)
	>10%		1 (1.85%)	10 (18.51%)	---

The results showed the number of patients is higher in NIDDM than ND with PPD >3mm when the Vit-C level is <0.6mg/dl. Among NIDDM subjects greater PPD is observed when the Vit-C level is low and HbA1C level is higher. Follow-up of larger group of patients is going on to confirm this result.

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MYCOTIC COMPLICATIONS IN PATIENTS WITH INSULIN DEPENDENT DIADETES MELLITUS.

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We examined 200 patients with IDDM (51 men & 149 women aged 18-68),the duration of disease 2-17 years.Mycological,serological,immunological examination of patients was carried out. 28 patients (14.3%) with IDDM had candidiasis:3 patients - candidosis-chronic mucocutaneus candidosis (CMC),10 -with oral candidosis,8-with esophageal candidosis,1 -with intestinal candisosis, 1 -with gastric candidosis,12-with vaginal candidosis,1-with candidal balanoposthitis & 2 - with candida sepsis.In patients with different forms of candidosis the T-supressor count was low,T-helper/T-supressor index was increased.Good control of IDDM after insulin treatment & short-term courses of antifungal therapy led to quick remission of candidosis. These data show the dependence between decompensated IDDM and different forms of candidosis / exept CMC/.

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ORAL HEALTH IN INSULIN DEPENDENT DIABETES MELLITUS (IDDM)

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The aim of our study was to evaluate the oral health status of IDDM patients (pts). For this purpose, 55 pts (26 male) aged 21.4 ± 7.5 years, with diabetes duration of 7.5 ± 6.3 years, were submitted to oral soft tissue inspection and palpation, dental probing, complete radiographic dental examination (periapical and byte-wing), salivary flow (SF) measure, and determination of DMFT (decayed-missed-filled teeth) index, plaque index, gingival index, periodontal pocket depth, loss of attachment and alveolar bone loss. By means of those periodontal parameteres, it was determined the presence of gingivitis (GV) or periodontitis (PO) in each of 4 sites per tooth and the pts were classified as having gingivitis and/or periodontitis levels I to IV respectively when they had 1-25%, 26-50%, 51-75% or 76-100% of sites affected. Statistical analysis was done by Mann-Whitney and Kruskal-Wallis (one way Anova), and univariate regression. Data were expressed in mean (SD) or median (range). The most frequent alterations of oral soft tissues were enlargement of sub-mandibular salivary gland in 12 pts (21.81%) and labial dryness in 4 pts (7.27%). The DMFT index was 10 (0 - 27). The correlation coefficient between DMFT and age was $r = 0.63$ $p < 0.001$. Salivary flow was 1.1 ± 0.6 and there was xerostomy (SF <1.0 ml/min) in 20 pts (36,4%), having no correlation with blood glucose. The most frequent periodontal alterations were GV level IV in 18 pts (32.7%) and GV level I in 12 pts (21.8%). PO associated with GV was noted in 16 pts (29%). We observed 64.7 ± 37.6 and 6.7 ± 18.8 sites affected by GV and PO respectively. No correlation was found between diabetes duration and % of sites affected by GV or PO. Age > 25 years was associated with % of PO affected sites ($p < 0.0007$), but not with the % of GV sites. In conclusion, our study has observed a great percentage of sites affected by periodontal disease in IDDM patients, and a significant correlation between age and the prevalence of caries and of periodontitis.

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PATTERN OF MUCO-CUTANEOUS YEAST INFECTION IN DIABETES - A STUDY ON 460 CASES AT BIRDEM

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It is recognized that patients with diabetes have increased risk of muco-cutaneous yeast infections. To characterise the yeast infections in diabetes according to their anatomical structure invaded, sex, age, duration of diabetes and glycaemic status, we have investigated 460 diabetic patients at dermatology department of BIRDEM. Based on microscopy and culture, Candidial intertrigo 47.8%, C. paronychia 23.5%, C. nail 9.6%, C. vulvovaginitis 6.7%, Pityriasis versicolor 6.7%, C. balanitis 2.6%, Oral candidiasis 2.2%, Perianal candidiasis 0.7% was diagnosed. Pityriasis versicolor ($p<0.5$) and oral candidiasis ($p<0.01$) were significantly higher in male whereas candidial paronychia was significantly higher ($p<0.001$) in female. Vulvovaginal candidiasis was very high below 40 yrs of age ($p<0.001$) whereas C. paronychia ($p<0.02$) and C. intertrigo ($p<0.05$) was high above 40 years of age group. Candidial vulvovaginitis clearly showed higher frequency ($p<0.001$) in newly detected diabetes (<1 month duration) than old diabetic group (>5 years duration). Accepted range of glycaemic control (<10 mmoll/L 2 HBG) showed lower frequency of C. vulvovaginitis and C. nail than who had hyperglycaemia ($p<0.01$). The rest perianal candidiasis and C. balanitis failed to show any relation with sex, age, duration of diabetes and glycaemic status.

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DENTAL PATHOLOGY IN DIABETIC PATIENTS: ABSENCE OR LOSS OF TEETH

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The objective of this work was to investigate possible differences in the number and topography of tooth loss in diabetic patients (DPs) relative to patients that were nondiabetic (NonDPs). We studied DPs voluntarily presenting themselves at the general odontologic services of the public subsector plus, for each one of them, two NonDPs who were selected at random among those seeking services on the same date of registration. Our sampling thus consisted of 44 DPs and 89 NonDPs; each patient was evaluated by both an odontologist and a diabetologist through the use of an *ad hoc* protocol designed by them, with the sex distribution being similar between the two groups (male DPs and NonDPs, 32% and 34%, respectively). The median age was 44.5 years for the DPs and 49 years for the NonDPs. The distribution according to the type of diabetes among the DPs was the following: IDDM, 18%; NIDDM, 59%; insulin-requiring, 16%; gestational diabetes, 2%; and diabetes of as-yet-unidentified nosology, 5% of the cases. Some 61% of the PDs had had their conditions diagnosed fewer than 10 years earlier. With respect to dental status, the DPs exhibited a significantly higher incidence of tooth loss than the NonPDs, with 44% of the former having missing teeth as opposed to only 26% of the latter ($X^2 < 0.000001$). The affected teeth showing a difference in representation among the two groups that was significant at a level of $p < 0.0005$ were as follows: (a) upper jaw: incisors, left canine and secondary premolar, and right lateral incisor and primary premolar; (b) lower jaw: left premolar and secondary molar and right canine, primary molar, and secondary molar. Whereas the greater tooth loss in the DPs would be a consequence of the higher frequency of vascular lesions in those patients, the difference in the topography of their dental pathology relative to that of the NonDPs could be attributed to the specific characteristics of the blood supply between the two sectors of the jaw.

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FATAL GASTRIC MUCORMYCOSIS IN A YOUNG IDDM PATIENT

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Gastric Mucormycosis is a rare complication of diabetic ketoacidosis. We present a case of a 17 years old patient with IDDM since age 11. Five days before admission she experienced epigastric pain, dyspnea and fatigue. On admission, she had diffuse abdominal pain, drowsiness, dehydration, pallor, tachycardia and tachypnea. Blood glucose was 450 mg/dl, BUN 21 mg/dl, creatinine 0.8 mg/dl, hematocrit 27%, Wbc 29,600/mm3. Arterial blood pH was 7.2, p02 92 mmHg, pC02 12.7 mmHg, HC03 3.2 mEq/l. Urinalysis disclosed glucose ++++ and ketones +++. Treatment with insulin, IV fluids and eletrolytes was started, with marked improvement of the ketoacidosis that, however, recurred on the 8th day. At that time, she had severe epigastric pain worsened by foods, fever (38.5 C) and leukocytosis. Three days later, the pain improved, but a 5 cm tender epigastric mass was noticed. An abdominal ultrasound scan revealed gastric wall thickening. A gastroscopy showed a 10 cm ulcer on the greater curve, with infiltrated margins and covered with fibrin. The bottom of the ulcer was irregular and blood was oozing. Biopsies revealed Zygomycete fungi. Treatment with amphotericin B was started up to a cumulative dose of 325 mg. However, on the 32nd day, she had a massive upper gastrointestinal bleeding which ultimately caused her death. This case suggests that gastric Mucomycosis should be included in the differential diagnosis of upper gastrointestinal hemorrhage complicating diabetic ketoacidosis.

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CLINICO-BIOCHEMICAL AND RADIOLOGICAL FEATURES OF TROPICAL CALCIFIC PANCREATITIS AND EARLY FIBROCALCULUS PANCREATIC DIABITES PATIENTS

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The exact relationship between tropical calcific pancreatitis (TCP) and fibrocalculous pancreatic diabetes (FCPD) is still unclear. Earlier data on late FCPD cases suggest that exocrine pancreatic damage *per se* may not be the only factor leading to diabetes in FCPD patients. As a part of an attempt to clarify the issue the serum(S) levels of fasting and postprandial glucose, C-peptide and insulin (by ELISA) and lipids were estimated in 25 TCP and 10 early FCPD cases (age in yrs: 22±6 in TCP vs 25±6 in FCPD, M±SD). ERCP was done in 15 TCP and 9 FCPD cases. FCPD subjects showed normal to near normal BMI (19.58±2.73; M±SD) which was almost similar to TCP subjects (18.23±2.73). Compared to TCP, the FCPD patients had about 1.4 times higher fasting S glucose (TCP: 4.51±1.02, FCPD: 6.19±2.00, mmol/l, M±SD), 2 times higher postprandial S glucose (TCP: 6.85±2.25, FCPD: 15.14±2.85), about 2 times less fasting S C-peptide (TCP: 0.51±0.17, FCPD: 0.29±0.18, nM, M±SD), 1.5 times less postprandial C-peptide (TCP: 1.15±0.52, FCPD: 0.77±0.41), about 1.3 times less fasting S insulin (TCP: 60±21, FCPD: 46±24, pM, M±SD), 1.6 times less postprandial S insulin (TCP: 212±109, FCPD; 130±75) levels. Analysis of C-peptide-glucose and insulin-glucose ratios revealed the marked differences between the two groups (C-peptide-Glucose, M±SD: Fasting - 0.12±0.05 in TCP vs 0.05±0.03 in FCPD, t=4.91, $p<0.001$ and 2 h postprandial - 0.20±0.13 in TCP vs 0.06±0.04 in FCPD, t=3.30, $p<0.002$; Insulin-Glucose, M±SD: Fasting - 14.46±7.10 in TCP vs 8.33±5.27 in FCPD, t=2.80, $p<0.01$ and 2 h postprandial - 35.68±23.00 in TCP vs 9.35±6.74 in FCPD, t=3.46, $p<0.001$). Although the absolute levels of C-peptide and insulin in FCPD showed insulin secretory capacity, the ratiosl reveal that their capacity of increasing insulin secretion to combat hyperglycemia is almost nil. Subjects showing mild, moderate and severe changes of chronic pancreatitis in ERCP were 0, 1(7%) and 14 (93%) respectively in TCP and 0, 0 and 9 (100%) respectively in FCPD Groups. Conclusions: a) Malnutrition is not the cause but probably a consequence of diabetes in FCPD and b) Neither the onset nor the severity of diabetes in FCPD follow a straightline relationship with the degree of ductal damage.

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STUDY OF PLASMA INTERCELLULAR ADHESION MOLECULE-1(ICAM-1) LEVELS IN PATIENTS WITH NIDDM

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Intercellular adhesion molecule-1(ICAM-1) is an adhesion molecule belonging to the immunoglobulin superfamily group which can bind lymphocyte function associated antigen-1(LFA-1). ICAM-1 has a role of lymphocyte activation and immunological modulation, and plasma ICAM-1 levels have been reported to increase in autoimmune diseases. On the other hand, ICAM-1 expression is increased on the surface of endothelial cells in human atherosclerotic lesions and supposed to have a role in the development of atherosclerosis. In this study, we examined plasma ICAM-1 levels in patients with NIDDM, and compared with those in normal subjects. We also studied the difference of ICAM-1 levels between the patients with or without diabetic microangio-pathic complications. Plasma ICAM-1 levels were measured using commercial ELISA kits. Plasma ICAM-1 levels were significantly higher in NIDDM patients than in normal subjects. In NIDDM patients, plasma ICAM-1 levels were significantly correlated with fasting plasma glucose and HbA1 levels but not with any of serum total cholesterol levels, serum triglyceride levels, and ages. Plasma ICAM-1 levels were significantly higher in NIDDM patients with microangiopathy than in NIDDM patients without microangiopathy. In NIDDM patients with diabetic nephropathy, plasma ICAM-1 levels were significantly correlated with the amount of urinary microalbumin but not with 24 hour creatinine clearance. In summary, plasma ICAM-1 levels may be related to the progression of diabetic microangiopathy in NIDDM patients.

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SERUM LEVELS OF ZINC AND COPPER IN NEWLY DIAGNOSED MALNUTRITION RELATED DIABETES MELLITUS SUBJECTS

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Changes in serum (s) levels of Zn and Cu have been reported in diabetes mellitus and the elements have also been implicated in the etiopathogenesis of various types of diabetes and its complications. In the present study the serum levels of Zn and Cu were measured in 21 newly diagnosed malnutrition related diabetes mellitus (MRDM) subjects under 30 years of age [fibrocalculus pancreatic diabetes (FCPD) 13 and protein deficient diabetes mellitus (PDDM) 14] along with 29 NIDDM patients in the same age group. Fifteen nondiabetic subjects with BMI <19 were included, which served as a matched Control for PDDM cases. Fasting serum values Zn and Cu were measured by atomic absorption spectrometry. Serum values of Zn and Cu [Median (Range), μmol/l] in Control subjects were as follows: Zn 15.9 (13.14-55.34) and Cu, 19.19 (10.85-86.08). As compared to Control the NIDDM subjects showed similar S Zn [24.01 (9.93-88.7)], but both PDDM and FCPD groups showed significantly higher values of SZn [35.02 (6.30-69.41), $p<0.05$ and 31.75 (22.17-41.75), $p<0.01$ for PDDM and FCPD respectively). In cases of S Cu all the 3 diabetic groups showed significantly higher values compared to Control [NIDDM 31.51(16.83-57.3), $p<0.001$; PDDM 28.79 (11.01-43.12), $p<0.04$; and FCPD 29.26 (20.14-58.07), $p<001$]. The Zn-Cu Raios were significantly lower in NIDDM and PDDM subjects (Median value; 0.776 and 0.961 respectively) compared to Control (1.025) and FCPD (1.145) patients. The results suggest that Zn and Cu may have a role in the etiopathogenesis and progression of diabetes in MRDM as well as in NIDDM subjects. The details of this role remain to be elucidated.

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ANTI-HEV (HEPATITIS E VIRUS) SEROPREVALANCE IN DIABETIC PATIENTS

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Hepatitis E virus (HEV) causes epidemic outbreaks of enteral hepatitis in Asia and Africa. It is responsible of acute non-A, non-B viral hepatitis (NANBVH) in the underdeveloped countries, and in general population, anti-HEV is very high. Socioeconomic and educational status of the country are very important risk factors for HEV infection. Although it is not known which systemic disease constitutes a risk factor, anti-HEV seroprevalance has been investigated in different patient groups, except in diabetics. Therefore, we designed this study to evaluate the prevalence of HEV infection in our diabetic patients. Ninety six diabetic patients (40 men, 56 women) were included in this study protocol. Liver function tests were determined and chronic complications of diabetes were examined. Seventy four healty persons (38 women, 36 men) without any diseases were taken as a control group. Serum samples of both groups were investigated for IgG antibody to HEV with ABBOTT ELISA kit. Anti-HEV (IgG) seropositivity is found in 7 (7.3%) diabetic, 4 (5.4%) nondiabetic patients. There was no difference between patient and control groups.

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Aim : TRACE ELEMENTS AND DIABETIC COMPLICATION

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Method : Controlled comparative study. 106 patients were recruited. 68 are diabetes and 50 are normal health control. Hair samples from the occiput were cut and the hair ashes were prepared for trace elements analysis by inductivley-coupled plasma (ICP) emission spectrometer model : Shimadz ICPQ-1012. 27 from the study group and 28 from the control group also had their blood and RBC Zn and Cu analyzed.

Result : Table I

	Study group (58)	Control group (48)	P value
Age	38.38 ± 10.06	34.12 ± 9.07	0.283
Hair Mg++	83.38 ± 76.70	86.49 ± 9.26	0.255
Hair Zn++	136.86 ± 49.53	139.94 ± 31.93	0.01
Hair Ca++	1101.16 ± 930.68	1070.55 ± 72.43	0.006
Hair Fe++	22.44 ± 11.39	27.58 ± 12.33	0.311
Hair Cu++	6.05 ± 2.43	8.60 ± 5.49	0.04

Table II - RBC Zn / RBC Cu

	Study (27)	Control (28)	P
RBC Zn	167.77 ± 23.21	168.06 ± 22.63	0.565
RBC Cu	9.97 ± 2.48	10.75 ± 1.93	0.772
Serum Zn	12.02 ± 1.89	11.47 ± 1.23	0.008
Serum Cu	18.14 ± 3.18	16.75 ± 2.87	0.254

Conclusion : There were statistical significant differences in Zn++, Cu++ and Ca++ in hair of diabetes, as compared with normal control. There was also statistically difference in serum Zn++ in diabetes as compared with normal. No statistical difference was found in Mg++ both in hair and in red blood cell (RBC) in the study and control group. In conclusion, we proved that hair analysis study with ICP spectrometry was useful in trace elements analysis of hair, and it confirmed our previous study with X-ray spectrometry of low Cu++ in diabetes and its application. However, we cannot find any association between diabetic complications and Mg^{2+} or $Zinc^{2+}$.

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DIABETES MELLITUS AND HEPATITIS C VIRUS INFECTION

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Serum transaminase levels of patients with diabetes mellitus are frequently evelated. This is often atributed to fatty infiltration of the liver without further investigation. Indeed, hepatitis C virus (HCV), as well as the other hepatotrophic viruses, may be responsible for the increase in serum transaminase levels. Therefore, we designed this study in order to determine whether the HCV seroprevalance is increased among our diabetic patients. Two hundred thirty two diabetic patients (100 male 132 female) were investigated. The mean age of patients was 51 years (range 17 to 80 years). Liver function tests were performed for each patient. HCV antibodies (anti-HCV) were investigated with third generation enzyme-linked immunosorbant assay (ELISA) (Organon Teknika). Twenty seven patients (11.6%) were found to be anti-HCV positive. In this group, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were 131.96±161.86 U/L and 97.33±80.55 U/L, respectively. ALT and AST levels of the anti-HCV negative patients were 41.05±58.11 U/L and 40.91±46.86 U/L. Serum transaminase levels (ALT, AST) of the anti-HCV positive diabetic patients were higher than those with anti-HCV negative ones, and this differences were statistically significant ($p<0.001$). There was no difference of being anti-HCV positivity among type-1 vs. type-2 diabetics and insulin users vs. nonusers ($p>0.05$). Similarly, it was also found that age and body mass index didn't affect anti-HCV positivity ($p>0.05$). There was correlation between anti-HCV positivity and previous operations and/or blood transfusions ($p<0.05$). Anti-HCV prevalance varies between 0.3% and 1.8% among healthy population in Turkey. The prevalance we have found among our diabetic patients (11.6%) is higher than average values for Turkey. For this reason, if serum transaminase levels of diabetic patient are elevated different reasons other than hepatosteatosis must be considered.

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SERUM TNF α LEVELS AND DIABETIC COMPLICATIONS IN NIDDM PATIENTS

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TNF α has a wide range of biological activities and it is important to clarify the role of TNF α in diabetic complications. In animal model we already reported that in vivo TNF α productivity was significantly enhanced in long-term hyperglycemia and N-acetylcysteine suppressed the increased TNF α production, and inhibited the development of peripheral neuropathy. In this study, we examined serum TNF α levels in NIDDM patients and analyzed relationships between the TNF α levels and diabetic complications. Subjects were 150 NIDDM patients (age 62.1 ±12.0, range 19-81) and sex- and age-matched 150 non-diabetic healthy controls who were not associated with malignancy, infection, allergy and autoimmune diseases and not taking steroid and other anti-inflammatory drugs. Serum TNF α was measured by the sensitive ELISA kit. Serum TNF α levels were significantly higher in NIDDM patients (3.15±1.62 pg/ml) than in controls (1.76±1.61 pg/ml) ($P<0.001$). In the NIDDM patients, the TNF α levels were significantly correlated with age ($r=0.19$, $P<0.05$), duration of diabetes ($r=0.23$, $P<0.005$), serum Cr levels ($r=0.47$, $P<0.001$), urinary protein levels ($r=0.23$, $P<0.01$), severity of retinopathy ($r=0.25$, $P<0.01$), and severity of hypertension ($r=0.25$, $P<0.01$) and slightly with motor nerve conduction velocity, but not with sex, BMI, fasting blood glucose levels, HbA1c, total cholesterol, HDL-cholesterol and triglyceride levels. The results indicate that secondary increased TNF α under chronic hyperglycemic state may participate in the development of the diabetic complications.

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HCV INFECTION PREVALENCE IN TURKISH DIABETIC POPULATION

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In the present study, we investigated the effect of several epidemiological and clinical factors on prevalence of the hepatitis C virus (HCV) infection in patients with diabetes mellitus. The study included 61 Type 1 (36 males/25 females, mean age 17.1±7.4 years and mean diabetes duration 3.2±2.2 years) and 101 Type 2 (61 males/40 females, mean age 57.7±11 years and mean diabetes duration 10±8.4 years), totally 162 diabetic patients. Results were compared with 0.8-1 % prevalence rate in healthy blood donors screened for HCV. Relationships between HCV infection and several parameters, including age, sex, history of blood transfusion and/or alcohol use, type of diabetes, duration of diabetes, modes of therapy (oral antidiabetic drug/insulin), regulation of glycaemia and presence of long-term diabetes complications and liver functions were evaluated. None of the Type 1 diabetes patients showed HCV seropositivity. However, this frequency in Type 2 cases was found to be 5.9 % (6/101). Serum levels of AST, ALT, alkaline phosphatase and gamma-glutamyl transpeptidase increased in all HCV (+) patients. 5 of these were diagnosed as having chronic liver disease (4 with chronic active hepatitis and 1 with decompensated liver cirrhosis) confirmed by liver punction biopsy and/or laparoscopy. Moreover, of the 6, three patients revealed HCV positivity. We could not define any relationship between HCV infection and mode of diabetes therapy, diabetes duration, type of diabetes and history of alcohol use or blood transfusion. These findings have indicated that HCV infection is more prevalent among diabetic patients than in normal population, HCV positive diabetic patients have a higher risk of chronic infections possibly due to immune system defects commonly seen in diabetes, and finally increased liver enzymes might always not indicated hepatosteatosis, in patients with diabetes HCV infection should also be probable.

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MATURITY ONSET DIABETES OF THE YOUNG

Clinical characteristics
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Maturity onset diabetes of the young is genetically determined disorder. We studied 173 individuals in 11 families with MODY . The aim of the study is to find the clinical characteristics of this condition. NIDDM was diagnosed in 129 patients. 20 patients diagnosed before the age of 25 years, and 6 patients before the age 10 years. Two patients presented with Ketoacidosis . 6 patients developed proliferative retinopathy. 4 patients developed nephropathy of whom 2 progressed to chronic renal failure . On conclusion ketoacidosis though rare but it may occur if the diagnosis is delayed , severe microvascular complication occurred in patients with persistent hyperglycaemia but genetic predisposition may have a role in our patients.

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HIGH PREVALENCE OF DIABETES MELLITUS AMONG PATIENTS WITH PNEUMOCOCCAL BACTEREMIA.

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Streptococcus pneumoniae is an important pathogen causing pneumonia, bacteremia and meningitis in adults as well as in children. Infection is a significant cause of morbidity and mortality in patients with diabetes mellitus (DM). In this study we report a retrospective survey of all patients with pneumococcal bacteremia hospitalised in five non-university hospitals in the Netherlands during 1993-1995. The records of patients with DM were analysed. Pneumococcal bacteremia was diagnosed in 177 patients (96 men and 81 women, mean age 60.1 ± 22.5 years). Chronic obstructive pulmonary disease, malignancy and DM were the three most common risk factors (31.3, 21.5 and 19.8% respectively). All except one of the 35 patients with DM (19 men and 16 women, mean age 68.8 ± 12.3 years) had type II diabetes. DM was diagnosed (W.H.O. classification) at admission in 7 of the 35 patients. Eight patients were on insulin therapy, 16 patients used oral hypoglycaemic drugs. Nephropathy (proteinuria) was present in 40%, retinopathy and clinical signs of neuropathy in 11.4% each of the diabetic patients. Length of the hospital stay was 24.0 ± 24.4 days in patients with DM and 21.6 ± 22.4 days in non diabetic patients. Three diabetic patients (8.6%) and thirteen (9.2%) other patients needed artificial ventilation. In-hospital mortality was 28.6% among patients with DM and 23.2% in patients without DM. It is concluded that in this retrospective analysis DM is the third most common risk factor in patients with pneumococcal bacteremia. A discussion covering the issue of pneumococcal vaccination for patients with DM is therefore warranted.

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MICROVASCULAR COMPLICATIONS IN AFRICAN AND INDIAN SUBJECTS WITH DIABETES OF LONG DURATION

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A retrospective study was undertaken to assess the development of microvascular complications (retinopathy and nephropathy) in 219 [132 African (B); 87 Indian (A)] patients with duration of diabetes >10yr, who were attending the diabetes clinic at the King Edward VIII Hospital. Retinopathy was evaluated by findings at annual fundal examination. Markers of nephropathy included persistent dipstix proteinuria, hypertension (HT), abnormal serum creatinine or glomerular filtration rate (GFR). Glycated Hb and blood glucose levels were also examined. Of the 219 patients, 172 were classified as NIDDM(B:A =96:76) and 47 as IDDM(B:A =36:11). The mean age of onset was later in Africans than in Indians, both for IDDM ($p < 0.05$) and NIDDM ($p < 0.01$). For IDDM, the prevalence of retinopathy was 53.2% (B=55.6%; A=45.5%); persistent proteinuria was present in 23.4% (B =25%; A =18.2%). Hypertension was found in 34% and was more prevalent in Africans (41.7%) than in Indians (9.1%) ($p < 0.05$). Onset of retinopathy occurred earlier in African (156 mth) than in Indian (216 mth) patients ($p < 0.05$). For NIDDM, the prevalence of retinopathy was 64.5% (B = 68.8%; A 59.2%) and that of persistent proteinuria 25% (B =30.2%; A =18.4%). Hypertension was found in 68% and was more prevalent in Africans (B vs A; 84.4 vs 47.4%; $p < 0.01$). Onset of retinopathy ($p < 0.05$) and hypertension ($p < 0.01$) was earlier in African than in Indian subjects. Comparison between IDDM and NIDDM showed that the prevalence of HT ($p < 0.01$) and abnormal GFR ($p < 0.01$) was higher in NIDDM patients. Retinopathy (n = 219) was significantly associated with duration of diabetes ($p < 0.001$) plasma glucose ($p < 0.05$) and HbA_1 ($p < 0.01$). This study has shown that as in other population, microvascular complications are common in Indian and African patients with long-standing diabetes.

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METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS CARRIER STATUS IN DIABETIC PATIENTS. M.Calvagno, M.Burgos, J.Alvariñas, A.García and G.Burlando, Servicio de Nutrición, Hospital Tornú, Argentina.

Objective: Knowing the importance of methicillin resistant S.aureus (MRSA) in diabetics, to study the frequence of MRSA carrier status in a sample of diabetic patients and compare it against a non-diabetic subjects sample.

Methods: During one year period (June 1995 to June 1996) we study the presence of MRSA carrier status in 40 IDDM and NIDDM patients (20 out-patients; 20 in-patients); diabetes duration of over 10 years. Diabetic in-patients were matched to a in-patients non-diabetic control group (sex and age). In all of them we performed a nasal test to determine the carrier status. For out-patients was requiered a period of over a year without any hospital internation.We excluded in any case patients receiving anti-MRSA in the month of testing. Statistical analysis: Fisher Exact Test.**Results**:Comparing diabetics against non-diabetics, we found:

	IN-PATIENTS		
	Carriers		Non-carriers
Diabetics (n=20)	6 (30%)		14 (70%)
Non diabetics (n=20)	0 (0%)		20 (100%)

Fisher Exact Test: p=0.02

In Diabetics, dividing in out and in-patients ve found:

	DIABETICS		
	Carriers		Non-carriers
Inpatients	6(30%)		14(70%)
Outpatients	1(5%)		19(95%)

p=0.09.

Conclusion: MRSA carrier status was more frequent in diabetic inpatients than in non-diabetics.In diabetics, frequency maybe higher among inpatients.

2370

EVIDENCE ON COMPLICATIONS AND RISK FACTORS FROM NATIONAL DIABETES MANAGEMENT NETWORK IN POLAND.

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As an integrate part of a newly established national network for managing diabetes care, we assessed the prevalence of late complications and risk factors using the DiabCare BIS. The goal was to establish a long term quality circle and to decrease the prevalence of complications. Data were assessed in a randomly selected population (n = 1271) covering all 12 region in Poland. Data were entered, analysed and published using the latest computer technology, by the central diabetes clinic in Warsaw in close co-operation with the regional participants. The establishment of the baseline description revealed the following findings on the St. Vincent targets (data are presented as %no / %yes / %missing), blindness 95.7 / 3.2 / 1.1, MI/CABG/Angioplasty 97.3 / 1.5 / 1.1, cerebral stroke 98.6 / 0.3 / 1.1, ESRD 97.8 / 0.6 / 1.6, amputation above ankle 97.6 / 0.7 / 1.6. amputation below ankle 98.1 / 0.1 / 1.9.The following were reported on the prevalence of smoking (data are presented as %no / %yes / %missing) 77.8 / 21.2 / 1.1 while the prevalence of alcohol consumption was reported as 94.5 / 4.2 / 1.3. The results proves the ability of the network to monitor and highlight problems in diabetes care in Poland. The data will be further analysed in order to explain dependencies between risk factors and complications in Poland. Secondly while the next phases of the project will secure the monitoring and continuous improvement of diabetes care.

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STAGE-SPECIFIC DEGENERATION OF GERM CELLS IN THE SEMINIFEROUS TUBULES OF NON-OBESE DIABETIC MICE

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The reasons for fertility problems in IDDM are largely unknown. The aim of the present study was to evaluate the role of autoimmunity-associated phenomena in the testis as a possible cause to the derangement in spermatogenesis. **The** stage-specific apoptosis of germ cells in the insulitis-**phase of prediabetes** was quantified in the testis of non-obese diabetic (NOD) mice. **The seminiferous epithelium** of normal BALB/c and NOD mice **contained cells positive in *in-situ*** end labelling (ISEL) of DNA. ISEL-positive **(+) germ cells formed** clusters in the seminiferous epithelium of the NOD mice **in striking difference** to the seminiferous epithelium of the BALB/c mice, **which contained only** individual ISEL+ cells. ISEL+ cells were present in the **basal and luminal compartments** of the epithelium. Ultrastructural analysis **confirmed that the cells were** undergoing apoptosis. The ultrastructurally apoptotic **cells included** spermatogonia, spermatocytes and spermatids. In cytological squash preparations of segments of **seminiferous** tubules of 17 to **20-week-old NOD** mice **the number** of ISEL+ **cells/mm tubule** was lower in **segments representing** stages I-II of the seminiferous epithelial wave but had **increased markedly** in stages III-IV thus exceeding clearly the number of **ISEL+ cells** in the BALB/c mice. The numbers of ISEL+ cells/mm tubule in the **other** stages were similar in the two strains of mice. Analysis of ^{32}P-3'-end **labelled** DNA from the testes showed that the BALB/c mice had relatively more DNA fragmentation than the NOD mice. These data suggest that autoimmune insulitis in the NOD mice is associated with abnormal stage-distribution of apoptosis in the seminiferous epithelium, resulting in derangement of spermatogenesis.

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COMPARISON OF METABOLIC STATUS AND DIABETES COMPLICATIONS IN THREE POPULATIONS

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Some data suggest different patterns and rates of complications in different populations. This study compared the metabolic status and complication rates in 3 different populations - urban non Aboriginal Australians, non urban Aboriginal Australians and Tongans living in Tonga. The assessment protocol included physical examination, measurement of HbA_{1c}, microalbuminuria and lipids, and eye examination through dilated pupils. Data were collected on a total of 971 Non Aboriginal Australians (NAA), 143 Aboriginal Australians (AA) and 195 Tongans (T). The findings were compared to the NAA after separately matching AA and T groups with NAA for age, gender and duration of diabetes. Overall mean (SD) age was 57 (9) y and mean duration of diabetes was 8 (8) y. Results are shown in the Table.

	NAA	AA	Tongans
BMI > 27	66%	74%	81% #
Smoking	41%	62% *	32% #
Hypertension	14%	30% *	47% #
Coronary Disease	23%	31%	17%
Chol (Mean SD)	5.7 (1.4)	5.6 (1.2)	5.3 (1.1)
HbA_{1c} (Mean SD)	8.3 (1.8)	8.2 (1.9)	9.8 (1.9) #
Microalbumin	31%	63% *	72% #
Retinopathy	44%	46%	57% #
Neuropathy	14%	22%	31% #
PVD	10%	11%	9%
Foot Ulcer	1%	5% *	4%
Amputation	0%	1%	8% #

* Significant difference NAA v AA: # Significant difference NAA v Tongans.

This study confirms different rates of metabolic abnormalities and diabetes complications in different populations after matching for age and duration of diabetes. Tongans had the poorest diabetes control and the highest complications rates. Microalbuminuria was more common in both AA and T compared with NAA.

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THE CAUSE AND TREATMENT OF SEXUAL DISFUNCTION IN DIABETIC MEN.

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Serum fasting levels of luteinizing hormone (LH),follicle-stimulating hormone (FSH),prolactin (Prl),testosterone (T) were measured in 230 diabetic men. 51% of patients with IDDM aged 18-50 had sexual disfunction & low basal T level,elevated basal Prl level in serum.After good metabolic contol of diabetes by insulin serum fasting concentration of Prl & T in diabetic men aged 18-36 became normal, in men with IDDM aged 36-50 with good control of diabetes serum T remained lowered & Prl-elevated. In these patients improvement of sexual function & hypothalamic-pituitry-gonadal axis disorders can be maintained by relatively small doses of Parlodel & treatment with Sustanon -250.Diabetic patients showed no increase of pituitary LH & FSH after administration of Gonadotropin-Releasing-Hormone & low increase of T after administration of chorionic gonadotropin.

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RISK FACTORS FOR THE INCIDENCE OF LATE VASCULAR COMPLICATIONS OF NIDDM: 15-YEAR FOLLOW-UP

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In assessing the contribution of cardiovascular risk factors and that of diabetic millieu to vascular complications in NIDDM, their incidence in surviving patients may overcome some problems created by mortality data. From the follow-up examination of 1329 out of 4420 NIDDM patients followed for 15 years (1973/74 - 1988/89), the incidence of micro- and macrovascular complications (proteinuria & nephropathy, symptoms of leg vascular disease, ischaemic heart disease [IHD], and cerebrovascular events) was estimated and related to the levels of base-line risk variables using logistic regression. For new cases of proteinuria & heavy proteinuria, hyperglycemia was the common predictor (alongside diastolic hypertension, smoking and overweight);hyperglycemia and glycosuria were among significant predictors of leg vascular complications (with duration of diabetes, smoking, male sex, diastolic hypertension, and proteinuria). On the other hand, systolic hypertension and male sex prevailed among factors predicting both IHD (with high cholesterol, and overweight), and stroke. The data confirm the higher involvment of diabetic millieu in micro- than macrovascular incidents, with diabetic foot placed in between.

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SEXUAL DYSFUNCTION IN DIABETIC MEN

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The study was carried out to establish a protocol for investigating and treatment of Sexual Dysfunctions in Diabetic Men.20 Diabetic men with Sexual Dysfunction were studied in the last 2 years.They were investigated as per Protocol devised by the team.This involves a structural interview of both partners, Complete Physical Examination, Psychiatric assessment and following Clinical tests were carried out a)Bulbocavernous Reflex b)QST of vibration sense c) Intra Cavernous Injection (ICI) with papaverine d) Nocturnal Penile Tumescence and Rigidity assessment(NPTR) e) Dynamic Infusions Cavernosometry&Cavernosography(DICC) f) Hormonal profile. All had abnormal ICI response, QST Vibration, sensation was abnormal.The NPTR study revealed 4(20%)with Psychogenic impotency and 16(80%)with organic impotency.DICC was done in 6 patients where surgery was contemplated.Findings on DICC includes Dorsal vein,Crural & Urethral leaks. 3 Patients had Penile Implants & 4 took ICI rest were treated conservatively.The study showed that organic impotency is more commoner than psychogenic impotency compare to previous studies.

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Glycemic control ,growth and complications in children with insulin-dependent diabetes mellitus

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The influence of glycemic control on growth and on the development of complication in diabetic children was studied. The subjects of the study were 408 children with insulin-dependent diabetes mellitus (IDDM), who were enrolled in a Summer camp program for diabetic children in Kinki District, Japan from 1972 to 1990. Many of the children had high mean levels of HbA1, regardless of age. The height and weight were below the standards for the respective ages in many children, indicating the retardation of growth. However, S.D. scores for height and weight and other physical indices were not related to the mean levels of HbA1. By contrast, the prevalence of diabetic retinopathy was related to an elevated mean level of HbA1, but that of albuminuria was not. Serum cholesterol levels were higher in children with higher mean levels of HbA1, but serum triglycerides appeared not to be related to glycemic control. The incidence of retinopathy during the observation period closely related to the degree of the mean levels of HbA1, but that of albuminuria did not. During these observation period for five years in average ,their SD score of height and weight, body mass index, Rohrer index and percent desirable weight did not changed; their insulin roquirement, Hb A1, systolic blood pressure, serum cholesterol level and the incidence of relinopathy incrcased year by year. Also intensive insulin therapy and selfmonitoring of blood sugar were used recetly much more.

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MALE SEXUAL DYSFUNCTION IN IDDM
INCIDENCE-DIAGNOSIS AND TREATMENT

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Impotence is a common problem among male diabetics.Demonstrate the exact etiology is of importance to the therapy .102 young IDDM male patients ,with a mean duration of diabetes for 11 years have been evaluated.They have been followed for 10 years .At onset patients were between 19 and 36 years old,15 of them showing impotence (15 %).After 10 years 92 patients were available for follow up,among them 34 (37 %) with impotence.Using arterio,evoked potentials of the Pudendal nerve, cavernosography and Prostin TM intracavernous,the main reasons for impotence were classified as follows: neurogenic n=12(35 %), vascular n=5 (15%) combined vascular/neurogenic n=9(26%),medication n=4(12%) psychogenic n=4(12%).Comparison of those 20 who acquired impotence and those having maintained normal sexual functions (n=58) will show a good correlation with regard to parameters usually considered related to diabetic control.The mean glycosylated hemoglobin during 10 year follow up was 7,4%(4,5-6,1%) in those with normal sexual activities and 9,4 % in the impotence group(n=20).At onset 11 men showed microalbuminuria,1 macroalbuminuria and 5 developed microalbuminuria during the observation period.It was of importance to find 2 impotent diabetics without microalbuminuria after 10 years,classified as psychogenic.The efficacy of treatment in those accepting therapy is good:7 refused,11 men are regularly injecting Prostin TM with good results ,4 patients had vascular repair with good evidence in 2 of them, 6 men took advantage of a penile prosthesis,3 are using a vacuum erection device.Trazodon TM was prescribed to 3 diabetics without organic disease. Sexual disturbances based on organic deficiencies were correlated with diabetic control.It is worth establishing the right cause of the organic disease in order to provide the patient with adeqaute treatment.Severe diabetic control might result in prevention of sexual disturbances.

2378

MICROVASCULAR AND ACUTE COMPLICATIONS IN THAI IDDM PATIENTS

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The incidence of IDDM in Thailand was quite low and the reports of diabetic complications in Thailand were largely from NIDDM. To study the prevalence of microvascular and acute diabetic complications, and their relation to duration of diabetes and glycaemic control, a cross-sectional study was done in diabetic clinic of Rajavithi Hospital, the tertiary governmental hospital in Bangkok. During the year 1995, there were 2,978 diabetic patients who attend the diabetic clinic regularly (more than 2 visits a year). Forty six patients were IDDM (31 females and 15 males). The prevalence of IDDM in our diabetic clinic was 1.5%. The mean ± SD age of 46 IDDM patients was 29.3 ± 7.4 years (range 14-47). Mean ± SD duration of diabetes was 9.8 ± 7.2 years (range 1-26). Onset of diabetes varied from 5 to 34 years (mean ± SD = 19.7 ± 7.0). Prevalence of hypertension was 13.0%. Normal HbAic by enzyme immunoassay (2.6-4.9%) was found in 8.7% of the patients. Mean ± SD of total cholesterol, triglyceride and HDL-cholesterol were 5.4 ± 1.6, 1.3 ± 0.7 and 1.3 ± 0.5 mmol/L respectively. For the regimen of insulin usage, 67.4% used twice daily of insulin injection, 23.9% used one injection of intermediate acting insulin and 8.7% used multiple insulin injection regimen. Mean daily dose of insulin was 43.6 ± 22.9 units per day. An albumin excretion rate of 30-300 mg/24 hr and higher than 300 mg/24 hr were found in 17.4% and 15.2% respectively. The prevalence of all retinopathy was 34.8% (background diabetic retinopathy 19.6% and proliferative diabetic retinopathy 15.2%). Of all patients 23.9% reported one or more severe hypoglycemic attack during the last 12 months and 10.9% reported hospital admission for ketosis over the same peroid. Microvascular and acute complications were clearly related to duration of diabetes and glycemic control. In this study, HbAic above the lowest quatiles (> 6.5% or > mean + 4SD) was significantly increase the risk of microvascular complications.

2379

FACTORS RESPONSIBLE FOR SEXUAL IMPOTENCY IN DIABETES MELLITUS: BIRDEM EXPERIENCE

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Autonomic neuropathy is one of the disgraceful complications of Diabetes Mellitus. Most of the time management is unsatisfactory. Eighty six diabetic subjects complaining of sexual problems were studied in the Endocrine Out-patient clinic, BIRDEM. Their mean age was 48.93±0.99 years. Among them 37 (43%), 36 (42%) and 13 (15%) were found above 50, 41-50, below 40 years of age respectively. Mean duration of diabetes was 6.54±0.66 years. 16 (22%), 21 (24%) and 46 (54%) subjects had diabetes more than 11, 6-10 and less than 5 years respectively. Overall glycemic status by HbA_1c was 7.5%. On clinical examination peripheral vascular disease, peripheral neuropathy were revealed in 10 (12%) and 30 (35%) subjects respectively. Psychiatric component was elicited in 45 (52%) subjects. Mean serum prolactin was 147±10 mmol/ml with above 100 mmol/ml in 59 (67%) and less than 100 mmol/ml in 27 (31%) subjects. Mean testosterone level was 5.03±0.20 ngm/ml. 44 (51%), 17 (20%) and 25 (29%). Subjects were treated with dietary modification, insulin and oral hypoglycemic agent respectively. Not only neurological but also hormonal deficiency, vascular and psychiatric components should be borne in mind while dealing with sexual impotency in diabetes mellitus.

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Glycemic control and complications in paediatric type I diabetes mellitus patients in Russia.

In 1996 296 paediatric patients with type I diabetes mellitus (IDDM) were referred to the National Diabetes Reference Service. 4% were referred from Moscow, 30.7% were referred from Moscow region. Age of patients varied between 2 and 16 years (25% 2-6 y.o.; 32,7% 7-11 y.o.; 42,3% 12-16 y.o.). 5,4% of patients had newly diagnosed IDDM; 72.9% of patients were diagnosed 1-5 years ago; 15.2% were diagnosed 6-10 years ago and 6,5% more than 10 years ago. All patients received human recombinant insulin preparations according intensive treatment regimen.

The average level of HBA1c was 11.06±0.12%. We observed lipodistrophy in 61.5% of patients; retinopathy in 7,1%; neuropathy in 5,7%(detected by examination and electro-myography); Mauriac syndrome in 4,4%; liver problems in 4,7%; limited joint mobility in 8,4%; cataract in 5,7%; nephropaty in 6,7% (detected by increased urine albumin excretion and changes in glomerular filtration rate); hart problems in 0.67%; lipoid necrosis in 1%.

We conclude that poor glycemic control is responsible for high complication rates in our patients.

2381

EPIDEMIOLOGY OF DIABETES MELLITUS IN MOSCOW DISTRICT (CHILDREN REGISTER DATA).

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Recently all high developed countries show a pronounced rise in the number of cases of insulin-dependent diabetes mellitus (IDDM) including children's population. Only limited studies concerning epidemiology of diabetes mellitus have been published in the native scientific literature. For example, there is none of corresponding information about population of Moscow district. The present study reflects the degree of distribution of the disease and its complications among the Moscow district children. Observation of 430 children with IDDM (322 from newborn to 14 years of age, and 108 teenagers from 15 to 17 years old) showed a high percentage of diabetic patients: 25.79 of 100000 children studied, and 42.08 of 100 000 teenagers). Sensory neuropathy (11.64%), retinopathy (7.69%) and cataracts (2.88%) were the main complications in children, while retinopathy (17.66%) and sensory neuropathy (16.89%) prevailed in teenagers including some cases of diabetic foot. In all age groups the above complications were more frequently seen in girls. On our evidence the average level of microalbuminuria (MAU) forms 33.82±7.22 mg/l in children with the disease duration >3 years, while 32.34% of children examined had a microalbuminuric stage of diabetic nephropathy. When evaluating healing IDDM in children of Moscow district 8.0% glycosilated hemoglobin was revealed in 4.64 % of children with IDDM,; 8.0-12.0% in 30.61% of children, and >12.0% - in 64.74 % of children's patients.

2382

EVALUATION OF PENILE VASCULAR SYSTEM IN DIABETIC PATIENTS WITH ERECTILE DYSFUNCTION

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Patients with diabetes have often erectile disorders. The pathogenesis of these disorders are complex and at present the exact mechanisms are not known. 199 of 1242 patients with erectile dysfunction were diagnosis to be diabetes mellitus (DM) and evaluated. 168 had (84.4 %) (Group 1) DM before admittance while the remaining 31 (15.6 %) (Group 2) had been diagnose on routine chemistry profile for erectile dysfunction. Mean duration of type 1 DM (n:146) and type 2 DM (n:22) were 82.1±58 and 80.1±76 months respectively, no statistically significant could be confirmed between duration's of this two groups ($p>0.05$). Among 82 patients evaluated by color Doppler ultrasonography, 33 had (40.2 %) arterial insufficiency, 15 had (18.3 %) caverno-venous incompetence, while the remaining 9 had (14.1 %) normal penile vascular system. The most frequent component of erectile dysfunction was observed to be arterial insufficiency (42.4 %) and mixed type (43.8 %) impotence in group 1 and 2 respectively. In conclusion, detailed evaluation of vascular damage observed in diabetic patients which plays a crucial role in pathogenesis of neurogenic and vascular complications can be successfully imaged.

2383

THE EFFECT OF DISTANCE FROM HEALTH CARE CENTRE ON COMPLICATION RATES IN THE EURODIAB IDDM STUDY

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Access to health care affects the risk of developing IDDM complications and may depend on distance from health care centre. We report findings from EURODIAB, a cross-sectional complications study of 3250 subjects with IDDM from 31 European centres. Participants were grouped according to distance from the clinic (<10km (group 1), 10 -20km (group 2), 20-50km (group 3), 50-100km (group 4) and 100km + (group 5)). There were no significant differences in age, HbA_{1c}, or systolic blood pressure between the distance groups or between the sexes. More people were referred to the clinic with complications from the groups living farthest away (54% and 44% in groups5 and 4 versus 27% in group 3 and 23% in groups 1 and 2 p = 0.00001), a distinct trend which was the same for both men and women. This measure was internally valid when comparing referral rates with other complication variables found at examination. Prevalence of cardiovascular disease showed a similar trend with a higher percentage of events found in participants living farthest away from the clinic (16 % in group 4 and 5 combined, and 7% 11% and 9% in groups 3 2 and 1 p= 0.01) although this was not demonstrated as clearly in men as women (p = 0.01, p=0.007). Proliferative retinopathy showed the same trend (18% in group 5 versus 10 % in group 1 p=0.1) but was significant only in men (19%, 16%, 9%, 7% and 10% p=0.03); as was neuropathy (42% in group 5 versus 31% and 29% in groups 2 and 1 respectively p = 0.02). A similar pattern was seen with macroalbuminuria (albumin excretion rate ≥200 µg/min) (12% and 11% in group 5 and 4 versus 8% in groups 1, 2 and 3) but was not significant for either sex. There was no association between distance and microalbuminuria (17%, group 5,21%,23% 21% and 22% p= 0.7) or background retinopathy (42% group 5, 47%,47%,44%, and 47% p=0.7). In conclusion, high rates of severe complications at presentation suggest that those living farthest away may experience delays in diagnosis, but, when seen by a specialist, they receive the same standard of care as those living near the clinic.

2384

LONG TERM COMPLICATIONS AND SEXUAL DYSFUNCTIONS IN MALNUTRITION RELATED DIABETES MELLITUS.

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Malnutrition related diabetes mellitus(MRDM) comprises 35%(94 cases)of the diabetic population at our centre. Prevalence of peripheral neuropathy is very high (65.9%) and a good number (28%) occurs in the initial 5 years of diabetes. Incidence of retinopathy(23.4%), nephropathy(21.3%) & hypertension(21.3%) increases with duration of diabetes. Retinopathy and nephropathy occur in the initial 5 years, emphasising a need to evaluate it from the begining of diabetic life. A subset of patients (4 cases) did not have any of these complications even after 20 years of diabetes. Impotency was detected amongst 16 male (30%) patients, out of a total of 52, where neurogenic cause was 75% as evidenced by good response to intracavernosal injection of papaverine and presence of cardiac autonomic dysfunction. Secondary amenorrhoea among 20 female(47%) patients out of total 42, had a negative correlation with body mass index. Secondary amenorrhoea due to a functional defect at hypothalamo-pituitary axis, as evidenced by low FSH & LH Level and reversion following improvement in body weight.

2385

METABOLIC CONTROL AND PREVALENCE OF MICROVASCULAR COMPLICATIONS IN YOUNG PATIENTS WITH DIABETES.

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Danish nationwide investigations (1987, 1989) have previously demonstrated unsatisfactory blood glucose control in unselected young diabetic patients. The purpose of the present survey was to study the prevalence of microvascular complications in a cohort of children participating in both previous nation-wide investigations and their association to HbA_{1c}. In 353 patients (50.1% of the inception cohort) we were able to collect urine, blood samples, a standardized questionnaire, fundus photo's (with central reading) and a standardized physical examination. Mean age was 20.6 ± 3.3 years and mean diabetes duration 13.2 ± 3.2 years. HbA_{1c} (normal range 4.3 - 5.8, mean 5.3%) and urine albumin excretion rate (AER) (upper normal limit (95%): 20 µg/min) in at least two timed overnight urine collections were analyzed centrally. Average HbA_{1c} was 9.7 ± 1.7% (mean ± SD). Males had significantly ($P<0.015$) higher values than females. Eighty-eight percent of the children (n= 309) were treated with three or more daily insulin injections. Mean daily insulin dose was 0.92 ± 0,25 ie/kg/24 h. Retinopathy was present in 60% and was associated long diabetes duration, high HbA_{1c}, diastolic blood pressure and AER (all $p<0.01$) while subclinical neuropathy (VPT by biothesiometry >10 V) was found in 30% and was asso-ciated to male gender, age, duration, linear height and retinopathy (all $p<0.01$). Microalbuminuria (>20-150µg/min) and nephropathy (>150µg/min) was found in 9 and 3.7%, respectively and was positively correlated to diastolic blood pressure ($p=0.009$) and retinopathy ($p<0.001$). Microvascular complications are frequent in children and teen-agers with IDDM. Only 38 of the patients had HbA_{1c} below 8%. Despite intensive efforts by health care providers current treatment of diabetes in young people is unsatisfactory.

2386

IMPOTENCE IN ETHIOPIAN DIABETIC MEN. SEYOUM B. ADDIS ABABA UNIVERSITY, ADDIS ABABA, ETHIOPIA.

Prevalnce of impotence was assesed among 292 consecutive diabetic men attending the Tikur Anbessa Hospital diabetic clinic. The mean age was 41.4 years (range 18-86 years). One hundred fortynine (51.6%) were type I and 143 (49%) were type II patients. The mean duration of diabetes was 9.9 years and 37.7% have known long term diabetic complications. The overall prevalence of impotence was 48.7%. The mean duration of impotence was 3.5 years. In the majority impotence startd after the diagnosis of diabetes mellitus however in 3.7% impotence occured before the diagnosis of diabetes mellitus. Many of the patients (79.1%) have never complained to physicians and 59.2% of the patients do not know that impotence Is the complication of diabetes mellitus. All but 10 patients (7.5%) have not lost libido. Impotence is significantly higher in Type II as compared to Type I patients (94/143 versus 40/132 $x^2 = 33$ $P < 0.001$) and in patients with complication than without (76/104 versus 54/159 $x^2 = 34.1$ $P<0.001$). The mean duration of diabetes mellitus is significantly higher in patients with impotence than without impotence (12.3 years versus 8.1 years $P<0.001$). We conclude that impotence is a common and significant problem in our diabetic men and we recommend further study to asses its social and psychiatric inpacts.

2387

DIACOMP: A GLOBAL STUDY OF IDDM COMPLICATIONS

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The WHO sponsored Diamond Study is an international study of childhood onset insulin-dependent-diabetes mellitus, which has fostered the development of a number of sub-projects with particular foci to address important questions about IDDM. The DIAmond COMPlications Study (DIACOMP) is the most recent of these sub-projects. The rationale for this study is based on the observation of diverse cause specific mortality experience in IDDM across the world. Considerable variation by ethnicity and/or geographic location of micro and macrovascular complications is likely to underlie these mortality patterns. DIACOMP is designed to explore variation in IDDM complication rates and risk factors.

The study is being organized on two levels at present. Level I is a survey of all identified cases in the registries. The second level, which will start after completion of level I survey will be a brief physical examination and measurement of proteinuria (Micral II®, Boehringer Mannheim) and HbA_{1c} (DCA 2000 Analyzer®, Bayer Diagnostics) along with a more detailed questionnaire. Survey forms will be translated into the local language and then back translated to English to validate the translation. Both Diamond and Non-Diamond centers are participating. Diamond populations have (by definition) a short duration (5-14 years), while the Non-Diamond centers will provide longer duration experience (15-24 years). Twenty-two centers (14 Diamond centers and 8 Non-Diamond centers) have registered from Asia, Europe, North, South and Central America, and Australia. Three Workshop/Training Sessions have been held in Copenhagen, San Francisco and Vienna in April, June, and August 1996 respectively. Eleven centers have had their local protocols approved and 11 have completed clinical training for level II (7 have completed both steps). Centers will start data collection in early 1997. An updated progress report will be presented.

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PREVALENCE OF SEXUAL DISORDERS IN A SELECTION-FREE DIABETIC POPULATION (JEVIN)

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There is extensive clinical literature on sexual disorders among diabetic patients, but a paucity of studies on their prevalence in selection-free populations. In the present trial (JEVIN) 90% of all insulin-treated diabetic patients (IDDM/NIDDM n=127/117) aged 16-60 years and living in the city of Jena (100,247 inhabitants) were studied: Each subject underwent a structured interview followed by a clinical and laboratory examination. The prevalence of sexual disorders was 32% in IDDM and 46% in NIDDM male patients. Patients with sexual disorders were older (IDDM 47.5±9.8 vs 37.7±11.6, p=0.0004; NIDDM 53.4±4.3 vs 49.4±8.2 ys, p=0.04) and had longer diabetes duration (IDDM 23.1±13.8 vs 13.5±11.1, p=0.001; NIDDM 12.4±7.5 vs 8.4± 5.8 ys, p=0.03), but there were no significant differences ($p<0.05$) between the groups as regards HbA1c, BMI and insulin dose/kg body wt. The prevalence of diabetes long-term complications in men with vs without sexual disorders: IDDM/NIDDM: retinopathy 65/53% vs 50/ 18% (p=0.34/0.03), neuropathy (assessed according to Young et al.) 58/48% vs 9/34% (p=0.001/0.47), nephropathy 65/50% vs 12/36% (p=0.001/0.45). In addition all the patients completed standardized questionnaires according to Bradley et al./Lewis et al. to assess quality of life/treatment satisfaction and one question concerning sexual disorders. The quality of life of IDDM patients with sexual disorders was lower than that of patients without sexual disorders (42.2±11.4 vs 54.2±8.5, p=0.0005), but there were no differences ($p<0.05$) in NIDDM patients. In women the prevalence of sexual disorders was 18/42% in IDDM/NIDDM.

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FAMILIAL CLUSTERING OF IDDM COMPLICATIONS IN THE DCCT.

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Of 1441 DCCT subjects, 372 had 467 first degree relatives with diabetes. Retinopathy (R) was assessed in relatives using ETDRS score and nephropathy (N) using albumin excretion rate (AER). All analyses were adjusted for treatment group of the DCCT proband, gender, age, body weight, duration of diabetes and HbA1c. Complete data was available for 241 relatives (52%) of 217 DCCT subjects. Familial aggregation was evaluated by comparing the prevalence of R and N in relatives of DCCT probands positive (+) vs negative (-) for R or N. In the DCCT secondary intervention cohort there was an increased risk of severe R (ETDRS ≥ 47, or clinically significant macular edema, or laser Rx in either eye) among relatives of R+ probands vs R- probands [odds ratio OR 3.1, 95% confidence interval C.I. 1.2 - 7.8, $p < 0.05$]; and an increased risk of N (AER > 40 mg/24hr) in relatives of N+ vs N- probands (odds ratio OR 5.4, 95% CI 2.2 - 13.7, $p<0.001$). In the DCCT primary intervention cohort, there was no increase in risk of any R (≥ 1 microaneurysm or worse) in relatives of R+ probands and there were too few events to assess the risk of N. Clustering of severity (level) of R or N was measured by determining the correlations of log ETDRS and log AER among probands and relatives, adjusting for number of family members. For R, the parent-child correlations were significant, but the sib-sib were not. For N, no correlations were significant.

Intrafamilial Correlations of Severity

	Retinopathy (R)			Nephropathy (N)		
	INT	CON	Comb	INT	CON	Comb
Parent-child	.311*	.344*	.327*	.090	.188	.138
Sib-sib	.000	.148	.060	.176	.000	.107

*p < 0.01; INT=Intensive Rx, CON=Conventional Rx, Comb=combined cohort adjusted for treatment group

Conclusions: The DCCT provides the first evidence, to our knowledge, for increased risk of severe R in relatives of R+ probands, and for intrafamilial clustering of the severity of R. We confirm the risk of N among relatives of N+ probands.

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Education for People with Diabetes

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EVALUATION OF DIABETIC FOOTCARE EDUCATION.
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Introduction: In reducing the rate of limb amputations in diabetics, which is one of the targets of the St Vincent Declaration (SVD), patient education plays an important role. The aim of this study is to determine the success of our footcare education program as advised in the SVD. **Methods:** Patients (P) who visit the foot clinic for the first time are given information orally by the podiatrist and referred to the patient information-centre for video-instruction (AV) and brochure (B) on footcare. A questionnaire, concerning general information, knowledge and practice questions was sent to all new patients in 1994 (n=179). 140P(78%) were eligible. For every patient a "knowledge-score" (KS) and a "practice-score" (PS) was calculated. The student-*t*-test was used for the statistical analysis. **Results:** 67P(48%) received AV+B, 15P(11%) AV, 25P(18%) B and 33P(23%) no information (N). The KS and PS were: AV+B 72% and 63% resp. vs. N 55% and 61% (KS $p<0.05$); AV 69% and 67%; B 67% and 58%. P from the group AV+B who read diabetic magazines had higher KS and PS scores than non-readers (76% and 66% vs. 62% and 52%; KS and PS, $p<0.05$). P with only elementary education had a lower KS than P with higher schooling (54% vs. 73%, $p<0.001$). P with visual/physical handicap vs. no handicap, PS 57% vs. 64%, $p<0.05$. **Conclusion:** Only 47% of the patients followed a complete education program on footcare. This group scored a significant higher knowledge-score compared to patients without information. Evaluation of education programs is useful, as proposed by the SVD.

2391

EVALUATION OF AN EDUCATIONAL PROGRAM: "HOW TO TREAT MY DIABETES"
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Introduction: "Education of the diabetic patient is not part of the treatment, it is the treatment itself" (E. Joslin 1927). In 1993 the DCCT study showed the value of educational programs to reach a desirable metabolic control. In 1988 began in Germany the educational program: HOW TO TREAT MY DIABETES", we started the same program in 1994.

Objective: To evaluate the educational intervention with a graded test.

Methods: The program consist of four modules: Self monitoring, Diet, Foot Care and Exercise, was taught to a group of no more than ten patients, using a rotafolio, 2 hours a day, two days a week for two weeks. Randomly we took 100 patients who had attended the program and ask them to take a test before and after the educational program, according with the results we grouped them in three categories: **A** from 80 to 100; **B** from 60 to 79; and **C** those bellow 60 points.

Results:

	BEFORE	AFTER
A	13%	56%
B	18%	38%
C	69%	6%

Conclusions: After the educational program patients tend to increase knowledge about their disease. We think this program is easy to teach and permits its objetive evaluation trough a graded test.

2392

VIDISA(means in spanish HEALTHY DIABETIC LIFE)

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Ussually in our health settings the diabetic patient does not participate actively in the management of their condition. They are told about the importance of education, exercises and nutrition but it seems that nobody has the time to tell them how to do it. The main goal of our project was to give the participants an opportunity to do and to learn how to manage their own condition by participating in a structure program that offered exercise, breakfast according to the personal needs monitoring of blood glucose before exercises and the opportunity to know for the first time the results of the HBA1c. The program promoted the adquisition of basic management skills for the daily life. The patients were selected from our pharmacy profiles, evaluated by our medical team and nutritionist. They attended a three month-three day out of the week sessions. All the activities were complemented with an educational experience. Evaluation of the intervention was base on tests on cardiovascular endurance, strengt, flexibility, coordination and a comparison of two glycated hemoglobin. Results of this evaluation showed improvement in all areas, including up to 35% improvement in the HBA1c test. VIDISA is now a support group organized to help the diabetic patient of our community

2393

THE VALUATION OF EFFICIENCY OF THE EDUCATION PROGRAM FOR IDDM PATIENTS

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The aim of our investigation was creation of the education program for patients with IDDM and valuation of its efficiency. During 1995 in endocrinology clinic was educated 41 persons with IDDM. The education was conducted under the 14-day structured program (about 40 hours). The control group included all patients IDDM found in endocrinology clinic in the same period. The increase of a level of knowledge of IDDM patients is revealed as in main, as in control group (from 71.04 till 93.36 %, $p < 0.05$ and from 70.55 till 90.90 %, $p < 0.05$ accordingly). In main group was significant decrease of levels 24h main blood glucose level and main amplitude glucose fluctuation level ($p<0.05$). In control group the similar tendencies are revealed. At comparison control and main groups the significant distinctions are revealed only in quantity of days, conducted by patients in clinic (30.85 and 23.95 days accordingly, $p < 0.05$). Thus, the education permits to reach that effect, as the usual hospitalisation in endocrinology clinic, but in shorter terms, that makes it economically expedient.

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PSYCHOLOGICAL CHARACTER OF POORLY MOTIVATED DIABETICS

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To improve motivation, psychological characteristics of poorly motivated diabetics (PMD) was investigated. Subjects were consecutive 217 diabetics who attended 2 weeks education program. Motivation level was discussed and rated into 10 by the multidisciplinary team members including psychologist. PMD defined as 6 or lower point was 45(21%), and most of them had barriers; psychological (73%), pysiological (58%) or social (33%) problems. Motivation level was significantly predictive to patient's attitudes 2 years later. Psychological aspets of PMD was compared with general diabetics.General health questionaire revealed neurotic in 65% of PMD. The egogram pattern of PMD was obviously deviated, and frustrated, self-centered, superman and poor adaptation type were 3-10 times more frequent than general population. The tree test (a drawing of tree with fruits) of PMD revealed more frequently abnormal as compared with general diabetics;opened top of the trunk (3.5 times) may indicate poor perspective, narrow trunk (4.2 times) may indicate low confidence, cut of trunk line (3.9 times) may indicate non-selfintegration. Evaluation of motivation level and psychological approach to poorly motivated diabetics is necessary for education.

2395

THE EFFECT OF HOME VISITING PROGRAM ON BLOOD GLUCOSE CONTROL IN DIABETIC CHILDREN IN IRAN

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This is a quasi - experimental research carried out in order to evaluate "the effect of home visiting program on blood glucose control in diabetic children " in Tehran (1995). 45 diabetic children and thier families participated in this program in two groups: the visit group (23 patients) and control group (22 patients) . The average age of patients was 9.3 (7-12) years.The parents' Knowledge about diabetes and glycated hemoglobin (G.Hb) was measured in the two groups in pre- test stage . glycated hemogolobin checked every 3 months. this method was followed - up for one years . In addition, the practice of the visit group was measured and compared . Data were gathered by questionnair and check list format . colorimetric technique was employed to measure glycated hemoglobin. Data Analysis showed that parents' Knowledge about diabetes had Increased (P less than 0.05) According to the results of paired T - test , practice in visit group had increased from 35.2 percent to 79.8 percent ($p < 0.0001$). In this research , long - term diabetic control was evaluated by measurement of glycated hemoglobin(non diabetic Range 2.5 - 4%). in visit group, glycated hemoglobin in pre- test was 6.5 ± 1.5 percent and decreased to 5.5 ± 1.1 percent in mid - way test ($p < 0.0001$) The rate of G.Hb was decreased in post-test After one year to 4.6 ± 1 percent (p less than 0.0001) wilcoxon test indicated that the number of Hypoglycemic shock was decreased . ($p< 0.05$) . Based on results obtained in this study, It is recommended that home visit can be used as a basic and essential approach to control and prevent the chronic diseases possibly developed due to diabetes. with appropriate patients' education and follow - up we have been able to obtain a better control and prevention of complications .

2396

ESTIMATION OF STANDARD OF KNOWLEDGE OF PATIENTS WITH DIABETES MELLITUS ON FOOT SELF-CARE.

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The aim of this investigation was to determine the initial standard of knowledge of diabetics on foot self-care. There were 30 patients examined during this investigation using the questionnaire prepared by the group taking up the problem of Diabetic Foot in the Azerbaijan National Committee of SVD.This form contains 15 questions with 4 answers for each.In that way, maximal possible value was 60 points.The average value received by patients was 25+-1,6 points that means that more than half of accomplishments were wrong.The standard of knowledge on foot self-care didn t depend on sex of patients (24,7+-3,29-men;27,8+-1,84-women,p>0,05).The correlation between age and standard of knowledge on foot self-care as well as that between duration of disease and standard of knowledge wasn`t determined.Persons with higher educational index had a higher standard of knowledge on foot care(28,0+-3,21)than that persons with lower educational index(19,9+_2,22,p<0,05). The group having took an amputation of lower limb revealed higher standard of knowledge on foot care(31,9+-1,79,p<0,001).These results show that diabetics began to interest with information on foot self-care **only** after the appearance of. such problem as diabetic foot syndrome.Every patient must take a training course on foot self care as soon as diabetes will be revealed.

2397

LONG TERM EFFECT OF A STRUCTURED OUTPATIENT EDUCATION PROGRAMME IN IDDM - A 6 YEAR FOLLOW-UP

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Aim: We evaluated the long term effect of our structured outpatient diabetes teaching and treatment programme (DTTP) for intensive insulin therapy (IIT) in IDDM patients.

Methods: 3 and 6 years after the DTTP all patients, who participated between 6/1989-6/90 in the programme, were invited for a follow-up visit. Out of 123 patients 13 subjects (12%) could not or did not want to take part in the follow-up. However, patients or their relatives were contacted by telephone to get basic information about their status. 2 (2%) patients died and 2 were lost for follow-up.

Results: 106 patients (86%) (57 female, age: [mean±SD] 44±12 years, diabetes duration: 20±11 years) completed the follow-up after 6 years.

	Baseline	3 years	6 years
HbA1c (%)	8,4±1,9	7,4±1,2***	8,3±1,5 ns
Severe hypoglycaemia (n/year)	0,49±1,33	0,16±0,44***	0,22±0,54***
BMI	23,3±3,0	23,7±3,0**	23,8±3,1***

*ns=not significant, **p<0.01,***p<0.001 vs. Baseline*

Daily insulin dosage remained unchanged (0,57±0,19 IE/kg body weight vs. 0,55±0,17 after 6 years, ns). 98 (93%) of the patients continued with IIT over 6 years. The number of daily insulin injections (2,5±1,0 vs. 4,5±1,2, p<0.001) and use of regular insulin (39±18% vs. 55±18%, p<0.001) increased significantly compared to baseline. A high frequency of daily blood sugar monitoring (3.5/day) was maintained by the patients over 6 years.

Conclusion: 6 years after participation in the structured patient education programme frequency of hypoglycaemia was considerably reduced, whereas the improvement of metabolic control after 3 years could not be maintained. These results clearly indicate the need of a continued patient education with an „expert programme for IIT" in IDDM patients.

2398

PATIENT EDUCATION BASED ON DIABETES EDUCATORS' POINT OF VIEW

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The aim of this study was to clarify the diabetic patient education based on diabetes educators' point of view. The data for this study were collected by a unstructured questionnaire from 56 diabetes nurse in Finland. The data were analysed mainly qualitatively using content analysis. According to the answers the main targets for patient education were self management and support of every day life. Other targets were good diabetes control and good quality of life. About 80 % of participants felt being able to deliver good patient education. Supporting factors for succesfull patient education were adequate time, good co-operation of the treatment team and personalities of educator like experience and enthusiasm. About 20 % felt being insufficient in patients education. Things behind were lack of time, few contacts with other diabetes educators like Medical Doctors and weaknesses in the skills and knowledge of the instructors. The participants wanted to develope their skills in patient education by increasing recources (mainly more time), contacts and co-operation with other diabetes educators and train themselves as well as try new education methods. Further research are needed in evaluating good patient education.

2399

INFLUENCE OF PATIENT EDUCATION ON GLYCEMIC CONTROL IN DIABETIC PATIENTS IN TURKEY

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We evaluated the efficacy of an eight-day structured patient education program in Turkish non-insulin dependent diabetic subjects. Two hundred and fifty NIDDM patients participated the education program in our outpatient diabetes teaching unit. Patients were interacted by a diabetic education team both one - on - one and in classes. Fifty of these patients completed the program. data is covering this group. Mean age was 53 ± 10 years and duration of diabetes was 8.2 ± 4.2 years. All of them were on diet and on drug treatment either with insulin,insulin plus oral agents or only oral agents.The fasting plasma glucose (FPG), postprandial two hour glucose(PPPG),and HbA1c levels were evaluated just before and at least six months after the completion of the education program FPG levels fell from 10 1± 4 mmol/L to 7.1 ± 2.9 mmol/L ($p< 0.01$) and PPPG levels decreased from 13 ± 4.9 mmol/L to 10 ± 3.9mmol/L ($p<0.01$).HbA1c levels also declined (6.4 ± 1 vs 8.7 ± 2%, $p<0.001$). Although 38% of these patients had low education levels (primary school or less), they showed significant improvement in their glycemic controls with the structured education program. As mentioned, only 50 of 250 patients completed the full education program. The reason for this ratio (20% of all participants) was the low socio-economic and cultural status of the patients. Other striking data was that the most of the diabetics who dropped from the program were living in other areas of the country and visitted our outpatient clinics for a short period. In conclusion,these results indicate that under-education of the patients with NIDDM is a major health problem also in Turkey. This teaching experience with an educated diabetes team seems highly satisfactory and may be a helpful model in improving the glycemic control and life quality of diabetics. In order to increase the number of participants to such courses, education programs, including the educators and patients should be organized throughout the whole country.

2400

EDUCATION AND TREATMENT OF DIABETIC PATIENTS WITH CHRONIC ISCHEMIC HEART DISEASE IN GEORGIA.

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The aim of the present study was to assess treatment and education efficacy in NIDDM patients with chronic coronary heart disease (CCHD).32 patients with NIDDM were observed (mean age 59.4yrs; diabetes duration 12.7yrs; body weight -84.5kg; mean heigh 168±8.4cm). 8 patients had MI in the anamnesis, CCHD diagnosis was confirmed by the specific changes on the ECG. 21 patients had arterial hypertension. Most patients had decompensated DM (HbA1c-11,2±0,32%), hyperinsulinism (fasting IRI 23.4±3.4mmol/l), hypertriglyceridaemia (2.85±0.33 mmol/l), elevated VLDL - CH levels (1.34±0.12 mmol/l). All patients took a 4 - day course of education at the Diabetes School, attention was mainly paid to dietary recommendations (low saturated fat content), physical activity and self - monitoring (peripheral vessel palpation, BP - measurement, cardiac angina episode registration).Patients were treated with antidiabetic,antianginal, hypotensive and antihyperlipidaemic drugs. Repeated examination 6 months later revieled decrease in frequency and intensity of anginal attacks, BP normalization. 8 patients stopped smoking. Body weight normalization tendency was observed (mean 77.2kg). HbA1c levels dropped to 8.1±0.2% ($P<0.001$), IRI content decreased insignificantly (22.1±3.1 mmol/l), TG and VLDL- CH levels also dropped 15.2±0.26 mmol/l and 0.7±0.12, respectively,$P<0.01$) The results demonstrate that education improves quality of life and metabolic control of the patients,thus helping them to live with DM and CHIHD.

2401

THE EFFECT OF A LONG-TERM EDUCATIONAL PROGRAMME FOR BLIND DIABETICS ON THE LEVEL OF METABOLIC EQUALIZATION.

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Every year in Poland 400 diabetes sufferers either with poor sight or completely blind is registered. The main problem of patients who turned blind is adaptation to the new situation,problems with independent living,injection of insulin and conducting self-control.**Aim**:evaluating efficiency of the long-term educational programme for blind suffering from diabetes.**Methods**: Two models of complex influence on patients have been introduced.The former is group education conducted in groups of 20 patients and repeated twice a year.It covers basic information concerning diabetes,self-control,interventional actions and using the Novo Pen injector.The latter model is repeated every three months,one-day observation in a room for daily-basis patients,which is combined with an individual educational programme and evaluation of metabolical equalization.Having undergone the programme,patients switched to using acoustic Novo Pen injector.After 6 and 24 months the level of patients' knowledge was checked with a test containing 10 questions and with application of 1-5 point scale.The state of metabolic equalization was also tested,by means of evaluating the average glycemia,concentation of HbA1c and the dosage of insulin.**The results of research**:The level of patients' knowledge has increased after 6 months by 33% and after 12 months by 41%.The average glycemia has lowered from 253 mg% to 168 mg%, after 2 years-down to 126 mg%.The concentration of cholesterol has decreased from 258,7 mg% to 240,8 mg% after 24 months.At the same time there was an increase in the fractions of HDL from 48,7% to51,7%,HbA1c from 10,1% to 8,5%.The daily dose of insulin was reducted from 58,1 units a day to 35,9 units a day.A positive body weight reduction has been observed.BMI has lowered from 30,1 to 27,7.**Suggestions**:In blind patients suffering from diabetes,it is possible to reach metabolic equalization by means of complex education with use of acoustic insulin injectors.In this way patients may become independent,further complications can be hindered and diabetes treatment costs can be lowered.

2402

Surveying The Trained Diabetic Educators in the State of Bahrain

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The main aim of this survey study was to assess the "Trained Diabetic Educators" (Nurses); identify barriers; specific needs and wants.

The method was carried out by sending a circular to all the Trained Diabetic Educators to meet at the College of Health Sciences were surveyed self administered questionnaire in June, 1996.

The results showed that out of the 100 Trained Diabetic Educators only 43 responded from privit and government health sectors all over Bahrain.

The age ranged from 24 to 40 years old. As for sex there were 88% females and 12% males. The duration since completion of the diabetic course ranged 1-4 years and all stated that they benefited from the course. The majority were able to implement what they have studied; develop their teaching/learning package and media. The barriers included no time; shortage of staff, no chance; no support; not accepted by their leaders; and priority to other responsibilities.

However, though 81% perceived that their supervisors are interested in diabetic education, yet they were approached only one time that suggests a thought rather than act from both sides. Around 47% attempted to develop diabetic team in their working area but were suppressed by their supervisors. The view regarding the "Diabetic Course" was positive and suggested to extend the period of the course; the clinical hours and project, work. The plan or specific needs and wants is to establish diabetic educational TV program; establish diabetic team and carry out research projects.

In conclusion the survey pointed out deficits and strengths which gave directions for further planning diabetic education and improving communications between the Trained Diabetic educators and their supervisors.

2403

EFFECT OF EXERCISE THERAPY ON AEROBIC THRESHOLD IN PATIENTS WITH NIDDM

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The main aim of the study is to evaluate effects of exercise therapy and improvement of carbohydrate metabolism, such as HbAlc and FPG, on aerobic threshold (AT).

Methods

285 patients with NIDDM are subjected. At the beginning of their educational hospitalization, the patients were subjected to an exercise load test on a bicycle ergometer, and AT was determined by analyzing breath samples. They underwent more than two weeks (mean 3.4 weeks) of exercise therapy one hour each day under the direct supervision of the exercise physiologists, then AT was again determined at the time of discharge from the hospital. The results were compared with those of a control group without exercise therapy.

Results and Conclusion

Oxygen uptake ($\dot{V}O_2$) at AT was significantly increased in male patients in 30's, 40's and 50's. The values increased from 14.7 ml/min/kg before exercise therapy to 16.3 ml/min/kg posttherapy, 15.1 to 16.1, and 14.0 to 14.8, respectively, in each group. No statistically significant increase was noted in 60's, while the value increased from 13.0 to 13.7. There was also a significant increase in power (work rate; WR) at AT after the therapy. The same tendency was observed in female patients. No changes were noted in $\dot{V}O_2$ and WR at AT in the control group. There was a significant improvement in HbAlc in both groups. However, the improvement rate of HbAlc was not statistically correlated with that of $\dot{V}O_2$, nor that of WR at AT. The term of exercise therapy was significantly correlated the improvement rate of $\dot{V}O_2$ at AT ($r=0.229$), and that of WR ($r=0.314$)($P<0.001$).

It was demonstrated that three to four week exercise therapy during educational hospitalization improved aerobic capacity and QOL of patients.

2404

EVALUATION OF KNOWLEDGE FOR DIABETES EDUCATION PROGRAM IN DIABETES NUTRITION CENTER, SURABAYA, INDONESIA

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ABSTRACT: Education program was regularly done every month in Diabetes Nutrition Center, Dr.Sutomo Hospital, Surabaya Indonesia. The goal of the program is to help patients with diabetes gain the knowledge and skills that enable them to care for themselves and to develop the attitudes that will enable them to make behavioral changes. The educators form the basis of the team approach to diabetes education. The team is consisted of Internists, nutritionist, psychiatrist, and nurses. The topics of education program are To understand Diabetes, Foot Care, Diabetes and Nutrition, and Psychological aspect of Diabetes. The education group was maximally 15 patients and the program was given one topic every week until four weeks. The education on 1996 has already conducted every month started on February to August 1996 with total coverage of 77 persons. Pre-test evaluation revealed 55.05 ± 20.77 and the post-test was 74.92 ± 21.11 with ($p < 0.05$). The evaluation proves that the increment of knowledge among participant is successfully achieved. This program is supported by Diabetes and Nutrition Center, Surabaya, Indonesia and also funded by IDF Eli Lily Fund 1995-1996.

2405

CURRENT STATUS AND PROBLEMS IN INITIAL INSTRUCTION FOR INJECTION TECHNIQUE WITH INSULIN PEN-INJECTOR

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The main aim of the study is to investigate instruction items and duration required for the initial instruction for the use of insulin pen-injector.

Subjects and Methods

Subjects were 62 patients (M/F=41/21) with NIDDM aged 55.1 ± 12.0 years (M ± SD). After the first injection, subjects injected insulin 9 times by themselves with careful attention to the following 7 skills: 1) being able to identify the name and unit of insulin, 2) shaking an NPH preparation more than 10 times, 3) sterilization of cartridge rubber cap, 4) void injection, 5) sterilization of the injection site, 6) correct injection, and 7) removing a needle 5 seconds after injection. Then, nonperformance rate of each skill was calculated.

Results and Conclusion

The nonperformance rate in each skill increased with increasing the age of the subjects; 4.9%, 8.0% and 13.8% in groups (40's, 50's and 60's), respectively. It decreased with increasing the number of injections, 15.5%, 6.7% and 4.0% in the 1st to 3rd, 4th to 6th, and 7th to 9th injections, respectively. In fifteen patients (M/F=7/8, 58.5 ± 12.3 years of age) who had have a tendency to forget to shake the NPH preparation more than 10 times, a sticker "Shake more than 10 times" was used as a reminder. The nonperformance rate decreased to 6.0% after using the sticker, in comparison with 82.7% before using it.

As conclusion, it is necessary to give a practical training to elderly patients for a long period. In addition, since the nonperformance rate decreased with increasing the number of injections, it is essential for medical staff to assist the patients for a long duration until they master the technique satisfactorily. Since there are many patients who do not remember the name and unit of insulin and who forget to shake the NPH preparation, it is important to use stickers to instruct them properly.

2406

DIABETES PATIENT AND COMMUNITY HEALTH EDUCATION/ TRAINING PROGRAMS IN A DEVELOPING COUNTRY

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Beginning 1993, we have designed and implemented a variety of structured group health education (ED)/ training (TR) programs in various parts of Karnataka State, tailored to the specific needs [diabetic patients (PT) and families (FM) vs. general public (PU); ED/TR only vs. ED/TR plus medical screening (MS) and treatment (MT)] and capabilities [literary and motivation levels; English vs. regional languages; urban vs. rural] of the recipient population. Thus far we have conducted 48 programs, each providing for 50-850 people. Some important features of the programs include (a) 100% voluntary free professional service (specialist doctors, counselors, dietitians, social workers); (b) Multiple educational techniques and audiovisual aids (lectures, discussions, nutrition demonstrations); (c) Structured syllabi and course content (Diabetes teaching guide-100 pages; Diabetes introductory booklet - 14 pages]; (d) Efficient adaptation of local resources (under a tree, village school, health center, marriage hall, industrial sites, hotels etc.); (e) Vigorous anti-smoking, anti-alcohol, prudent diet-predominantly vegetarian and physical fitness campaign; (f) Medical screening (Height, body weight, spot blood glucose, BP check) and medical treatment (drug adjustment, follow up) to the extent possible in rural/poor situations. Conclusion: India projected to bear world's largest burden of diabetes by 2010, needs to urgently and systematically implement the positive health care experiences of volunteer organizations, like ours.

PROGRAM	RECIPIENTS	DURATION	ACTIVITY
Diabetes Self Care Intensive (12):	PT, FM	8 hrs	ED+TR+/-MS+/-MT
Diabetes Self Care Basic (28):	PT, FM	2-4 hrs.	ED+TR
Diabetes Awareness (5):	PU, PT	2-4 hrs.	ED+TR+/-MS
Diabetes & Heart Awareness (3):	PU, PT	2-4 hrs.	ED +TR +/-MS

2407

KNOWLEDGE OF RISKS/BENEFITS OF HORMONE REPLACEMENT THERAPY IN PERIMENOPAUSAL DIABETIC WOMEN

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200 consecutive female patients in our diabetic clinic completed a questionnaire concerning their own knowledge and beliefs about post-menopausal hormone replacement therapy (HRT). 185 questionnaires were returned (91%). 75 of these women were likely to be perimenopausal (defined as aged between 45-65 years). 69 patients (92%) said they were menopausal/approaching the menopause and 21 (28%) were taking HRT. 27% had never heard of HRT. Of those that had heard of HRT, 47% had received information about HRT from a doctor, 36% from media sources and 33% from friends or relatives. Of these, only 36% thought they had been given adequate information. Only 55% felt able to answer any questions of possible risks and benefits of HRT. Of those who answered, response rates agreeing with the following statements were as follows: "HRT is safe in diabetes":- 37%; "HRT improves diabetes":- 15%; "HRT improves blood cholesterol levels":- 20%; "HRT reduces risk of brittle bones (osteoporosis)":- 83%; "HRT worsens blood pressure":- 17%; "HRT increases the chance of heart disease":-12%; "HRT increases the risk of cancer":- 37%. Our survey, in a patient group who regularly receive health promotion advice and in whom the impetus for HRT may be greater than the general population, reveals a poor awareness of HRT, a perception of inadequate information and a perception that HRT is not safe in diabetic women.

2408

"HOLISTIC" VERSUS "BLOOD SUGAR" COUNSELLING IN DIABETES

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Complex behavioral factors are responsible for translation of diabetes self care knowledge into positive self care practices, which are crucial for better metabolic control and improved health in diabetes (and other interactive chronic non-communicable disorders). In order to evaluate the clinical effectiveness of the comprehensive health education and training program being provided at our diabetes center for the last two years, we have evaluated selected health behavior of 121 subjects, (structured questionnaire, and personal interview , 76 no insulin, 45 on insulin; male 63, female 58, diabetes duration 10 y). Medical management, introduction to and increased compliance with UGM, SBGM and exercise, were associated with improved BP and glycemic control. 70 % of the obese people lost an average of 4 kg, where as the remaining could not lose or gained weight. About half of the counseled subjects discontinued consumption of deleterious food and addictive substances. Conclusion: Multifactorial nature of diabetes and associated degenerative disorders, make comprehensive health counseling mandatory; further innovations in counseling strategies and patient empowerment are crucial.

TIME	SBP	DBP	FBG	PPBG	SELFINJ	UGM	SBGM	EXERCISE
PRE	143	87	177	252	66%	34%	39%	45%
POST	130	82	102	186	73%	64%	73%	79%

TIME	EGGYOLK	REDMEAT	UNSKIMMILK	ALCOHOL	SMOKING
PRE	37%	36%	43%	16%	13%
POST	12%	19%	23%	10%	7 %

[SBP/DBP= Systolic/ Diastolic BP; FBG/ PPBG= Fasting/ Post Prandial Blood Glucose; UGM/ SBGM= Urine/ Self Blood Glucose Monitoring; SELFINJ= Self Injections]

2409

EVALUATION OF A FIVE-DAY STRUCTURED TEACHING PROGRAM FOR DIABETIC PATIENTS - A ONE YEAR EXPERIENCE

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The aim of the present study was to evaluate the effect of a five-day structured teaching program for diabetic patients one year afterwards. 101 insulin-treated diabetic patients,of mean age 35.4±11.1 years and mean duration of the disease 10.0±7.9 years, who had passed the program were followed up at reeducation one-day sessions 6 months and one year later. There was a significant increase in the overall quality of life (score 52 after six months and 51 after one year, resp., vs 41 before education, $p<0.01$), due to reduction in depression (4.0 after one year vs 5.1 before education, $p<0.01$) and anxiety (4.97 vs 7.63, $p<0.001$), and increase in well-being (13.8 vs 12.1, $p<0.05$) and energy (9.63 vs 8.78, $p>0.1$).The metabolic control improved significantly, judging by HbA1 level (from 9.1% to 8.0% after six months and 7.8% after one year, $p<0.05$). There was a significant increase in diabetes-related knowledge at the end of the five-day program (81% vs 52%, $p<0.01$), being maintained unchanged 6 months (80%) and one year (79.5%) later.The patients carrying sugar with them increased dramatically 6 months after education (from 4% to 89%), this percentage being slightly reduced a year aftewards (82%). There was a significant rise in the number of patients keeping a log book - from 16% to 72% after six months and 66% a year later. The rate of severe hypoglycaemia decreased from 0.15 to 0.06cas/pat/yr, ($p<0.01$) after one year and the rate of diabetic ketoacidosis fell from 0.30 to 0.14 cas/pat/yr ($p<0.01$). These results demonstrate that structured patient education improves the quality of life of diabetic patients and their metabolic control and significantly reduces the rate of acute complications, and we consider reeducation as absolutely necessary to maintain this beneficial effect of education.

2410

ASSESSMENT OF EDUCATIONAL EFFECTS ON NIDDM PATIENTS.

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Lifestyle modification consisting of diet combined with aerobic exercise can be effctive in diabetes control and reduction of macrovascular complications.

An experimental design was conducted to evaluate the effects of diet and exercise education. We studied 8 NIDDM patients at our metabolic clinic. They were assigned into the experimental (N=4) or control (N=4) group at random. Our 2-month intervention included 2 times of educational sessions with instruction on nutrition, exercise, and behavioural modification strategies to the experimental group. They were encouraged to adhere the protocal and record their diet and exercise.

There was statistically significant different in the change of daily calories between 2 groups (-150±127 vs +388±116 Kcal/day, $p<0.05$). Improvement was observed in diabetes knowledge, attitude, behavior, glycosylated hemoglobin levels, and serum lipid levels in experimetal group. In conclusion, diet and exercise education are effective in diabetes control and reduction of macrovascular risk factors.

2411

DEVELOPMENT OF PATIENT SATISFACTION WITH DIABETES EDUCATION QUESTIONNAIRE

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The assessment of patient satisfaction has become an important concern in the evaluation of health services and diabetes education is no exception. This study describes the process and outcome of the development of an instrument, ***Patient Satisfaction with Diabetes Education Questionnaire (PSDEQ)***. The PSDEQ aims to measure patients' satisfaction with diabetes education provided by diabetes nurses in the local setting. The process of item generation and content validity testing will be reported. Pascoe (1983)'s definition was used as the working definition of patient satisfaction. Patient satisfaction is defined as a health recipient's reaction to salient aspects of the context, process and result of his service experience. Generation of domains and items from the empirical and conceptual sources was then completed. 100 diabetic patients were interviewed upon their completion of the education sessions to identify all characteristics they regarded as important to the rating of satisfaction. Satisfaction statements were generated from the data. Conceptually, review of the definitions and standards of quality diabetes education was conducted and items were also generated on this focus. Together with those generated from the patient interviews, 38 items assessing 5 domains were generated. The domains covered communication content, nurse's attributes & behavior, relationship, convenience & accessibility and perceived outcome. A seven point Likert Scale was adopted. Content validity was assessed by 5 experienced diabetes nurses and diabetologists and 5 patients. 25 items were finally retained after modification and elimination by criteria of irrelevance, ambiguity, and undesirable similarity. Further testing on validity and reliability of the instrument will be done by administering the instrument to 150 subject samples from two local Diabetes Centers. Item-scale correlation, with an acceptable Cronbach alpha coefficient set at 0.70, will be done to examine the internal consistency. Factor analysis will be used to confirm the expected factor structure and how these factors underlie the set of items. Evaluating service using the PSDEQ will help to identify specifically the instances satisfying or dissatisfying to the patients. The findings will enable the planning of service more focused to the patients' needs and improving patient satisfaction.

2412

THE EFFECTS OF DIABETIC EDUCATION PROGRAM ON DIABETIC CONTROL.

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Education for diabetic patients is important for their glycemic control. To evaluate the effects of diabetes education, we studied 57 inpatients who were admitted to our diabetic unit during Jan-Feb,1995. Fifteen items were assessed which included knowledge on diabetes and its complications, diet, exercise, medications and foot care. Of each item, score 1, 2, 3 and 4 represented poor, partial, complete understanding without action, and complete understanding with action. Patients were assessed before and after 1-2 weeks of educational program. Their mean score improved from 16.2±3.3 to 39.4±4.4 ($p=0.0001$) after education. The mean glycohemoglobin (HbA1c) decreased from 10.6 ±2.5 % to 8.7 ± 1.8 % ($p=0.0001$) at outpatient clinic follow-up. The improvement in education scores was correlated with the decrease of HbA1c levels ($r=0.32$, $p=0.02$). In conclusion, our inpatient education program improves not only patients knowledge but also their glycemic control.

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IMPACT OF EDUCATION ON GINGIVAL INDEX IN DIABETIC AND NON-DIABETIC SUBJECTS. Z.Stolarza, S.Goldstein and C.González, Servicios de Nutrición y Odontología, Hospital Francés, Buenos Aires, Argentina.

Objective: To determine the impact of an odontologic educative plan on the oral clinical status in diabetics, and to compare this effect vs a non-diabetic sample.**Methods**: We studied 15 diabetic patients (group I) and 16 non diabetic subjects (group II); each patient in a group was randomly alocated in one of two subgroups; in the first one (subgroup A), they received programmed information on the appropiate bucal care methods, reinforced in a weekly contact, during three months. In the second subgroup (subgroup B), just a general information was brought, without a particular reinforcement.The oral clinical status was qualified through gingival index (Löe and Silness, modified) at the beginning and at the end of the study (month 3) for each patient. Metabolic control was evaluated by HbA1c. Statistical analysis: One tailed Fisher Exact Test; X^2 (Yates correction) and non-parametric ANOVA. **Results**: In both groups, gingival index improvement was more frequent and stronger in subgroup A (in Group I -diabetics-, $p=0.028$; in group B -non-diabetics- $p=0.029$). No between groups difference was found. HbA1c improved significantly in subgroup A diabetics.**Conclusions**: In patients under this educative plan (both diabetics and non diabetics) gingival index was significantly reduced (improved) in comparison with non educate subjects. May be of great interest to persist in this kind of educational work to prevent further bucal complications in diabetic patients.

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KNOWLEDGE AND COMPLIANCE OF HYPOGLYCEMIC AGENTS IN DIABETIC PATIENTS IN TAIWAN.

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To know patient's knowledge and compliance of hypoglycemic agents (HA), we studied 104 diabetics (46 males and 58 females) by questionnaire. Eighty-seven patients were non-insulin-dependent (NIDD) with mean (±SD) age of 58±14 years and 17 patients were insulin-dependent (IDD) with age of 20±9 years. Fifty-four (52%) patients had additional medications other than HA. Most (90%) patients knew the action of HA they were using. However, only 53% of patients completely followed doctor's prescription. The most common cause of poor compliance was forgetfulness. There were 25% of patients experienced side effect(s) of HA. The most common side effect was hypoglycemia. Patient's knowledge, compliance and the occurrence of side effects of HA were not significantly different between IDD and NIDD. In NIDD, patients with complete compliance had better HbA1c (10.0±2.1 vs. 11.3±3.1 %, P<0.05) as compared with those without. In conclusion, poor compliance of HA is common in our diabetic patients and it is related to patient's glycemic control.

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EVALUATION OF A COMBINED EDUCATION/CLINICAL-EXAMINATION PROGRAM FOR NIDDM PATIENTS.

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An increasing amount of evidence suggests that patient education for people with diabetes should be an integral part of effective disease management. In this study, an education program for NIDDM patients, including basic self-care skills, basic physiology of diabetes and its complications was performed as part of an extensive clinical examination by a diabetes nurse, dietician, ophthalmologist and podiatrist. We evaluated the effect of this program on knowledge, self-care behaviour, disease perception, metabolic control and body mass index. Data was collected upon entry to the program, immediately following the education sessions, and after 6 months (follow-up). Metabolic control was assessed by glycosylated hemoglobin (HbA1c). Self administered questionnaires were used to assess patients knowledge, self-care behaviour and disease perception.

The study group consisted of 96 patients, of which 54% was female, treated by a general practitioner. At baseline the mean age was 64.5 years (SD 8), duration of diabetes 5.5 years, (SD 4.8) and mean Hba1c was 9.3% (SD 2.5). Self-care behaviour and disease perception improved after the program (for all dimensions $p<0.05$). The mean score for knowledge (range 1-23) changed from 8.5 at baseline to 12.4 and further to 13.6 at follow-up ($p<0.001$). Moreover, the patients showed an improvement of metabolic control after following the program. This effect was maintained, although it leveled off during the next six months (mean HbA1c decreased from 9.3% at baseline to 8.9% $p< 0.05$ to 9.1% in the follow-up). The percentage of patients with the poorest metabolic condition (Hba1c>10%) decreased (from 28% at baseline to 21% in follow-up, $p<0.001$). The body mass index decreased from baseline (28.7) to follow-up (28.2, $p<0.005$).

These results indicate that a combined education/clinical-examination program is able to improve both self-care behaviour and metabolic control in NIDDM patients. These improvements endured after the completion of the program, which suggests that it initiates lasting changes in the way patients handle their disease.

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EVALUATION OF A STRUCTURED 5-DAY EDUCATIONAL PROGRAMME FOR INTENSIVE INSULIN THERAPY.

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There is a clear consensus for improving glycemic control in most IDDM patients, but not on the methods to attain these goals, e.g. advanced (re)educational programmes or intensive DCCT-like outpatient follow-up. We retrospectively evaluated the impact at 1 year of a 5-day inpatient structured educational programme designed to help compliant IDDM patients with standard diabetes education to attain near-normoglycemia with the least consequences on hypoglycemias and quality of life. The programme included interactive sessions on treatment goals, injection techniques, diet and insulin adjustment, and early recognition and management of hypo and hyperglycemias. Quality and frequency of subsequent out patient follow-up were kept unchanged i.e. conventional visits every 3-4 mo. Seventy-three IDDM patients aged 42 ± SD 10 years, with diabetes duration 18 ± 10 years, were consecutively recruited. Most self-management indicators improved, the most significant differences being in the number of daily glucose testings (3.8 ± sem 0.4 vs 4.6 ± 0.2 at 0 and 12 mo), use of a log book (58 vs 88 % of the patients) and of an appropriate technique of injection (46 vs 80 % of the patients). Diabetes control indicators improved slightly though significantly : HbA1c 9.2 ± 0.3 % vs 9.0 ± 0.3, frequency of severe hypoglycemias 74 ± 28 per 100 pt-year vs 26 ± 12 i.e. the number of patients with satisfactory control (HbA1c < 8 % and no severe hypoglycemia in the last year) increased from 6 to 18 % ($p < 0.03$). Six of the 14 items of a DCCT-adapted quality of life questionnaire were scored significantly better at month 12 than at month 0. In conclusion, our results suggest that intensive diabetes management limited to a 5-day educational programme is able to improve patients attitudes and quality of life but has only modest long-term effects on diabetes control limited to a reduction of severe hypoglycemias.

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A study of syringe and lancet disposal practices among diabetic patients in a Singapore diabetes center

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The correct disposal of insulin syringes and lancets is an important issue because of the potential for accidental needle-stick injury. However, syringe and lancet disposal is often an area poorly understood by patients, and practices vary. We therefore conducted a study to better understand the practices adopted by insulin and blood glucometer users in Toa Payoh Hospital Diabetes Center. A questionnaire was applied to a group of 75 diabetic patients (39 males, 36 females). All 75 were using insulin, and 72 monitored their blood glucose regularly.The mean age of the group was 49 years, and 39% had glycated hemoglobin below 9% (NR 4.6 - 6.4%). 21% did not have any formal education, 40% had primary school education, 33% had secondary school education, the rest had vocational, pre-university or university education. 84% had monthly household incomes more than US$ 600. 55 patients (73%) were using insulin syringes, while 20 patients (27%) were using insulin pen-injectors. 79% of the patients were personally responsible for disposing of their syringes, needles and lancets, the remaining 21% depended on family members to take care of this task. 99% did not break the needle off the syringe prior to disposal. 95% recapped their needles. Only 5% put their used needles and syringes into a puncture-resistant container, 53% put these items in a plastic bag prior to disposal into the dustbin or rubbish chute, and 41% threw the used items directly into the dustbin or chute. For lancets, 26 patients (36%) did not bother with capping, throwing used lancets directly into the bin, whereas 21 (29%) collected the lancets first into a plastic bag before disposing of the bag into the bin. Only 4% got rid of used lancets using a puncture-resistant container. 6 individuals (8%) reported experiencing needle-stick injury. No needle-stick injuries occurred to household members. After the questionnaire, the patients were re-educated on syringe and lancet disposal safety issues. 89% of them resolved to use a puncture-resistant container in future, and 4% decided to use the safe-clip disposal device. We conclude that health professionals responsible for diabetes education programs should teach patients the correct ways to dispose of insulin syringes and lancets, and alert them to the potential dangers of needle-stick injury.

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BIBLIOTHERAPEUTIC PROGRAM FOR PEOPLE WITH DIABETES.
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In this study, 50 patients from Rural India were selected.They were not having adequate psychological adjustment with this disorder and were undergoing stress. The main aim of this study is to conduct a need oriented training program and evaluate through the gain in understanding and change in attitude before and after the training. Another aim was to investigate the misconception and superstition held by Indian families. Case history was collected for each patient. A questionnaiare was prepared to assess their knowledge about this matabolic disorder, the effect of this problem in their families and social circle, treatment with special diet, regular exercise, etc. A systematic training program was conducted by supplying bibliotherapeutic materials, counselling different members of family, providing information services, group discussion, etc. The pre and post test results, feedback questionnaire and also verbal feedback in an open session revealed significant gains made by the patients. Bibliotherapy proved to be an excellent mode of communication. The idea behind this procedure is to provide patients with information about diabetes in the hope of changing their attitudes, dietary habits, etc. The effectiveness of the program could be judged from the fact that the revisits of the patients are gradually increasing and message is reaching the unreached and the patients are improving by this innovative method. The author is hopeful that such training programs would go a long way, especially in India where there is an acute shortage of human and financial resources.

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FAMILY EDUCATION PROGRAM FOR THE HOSPITALIZED DIABETIC PATIENTS
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Patient education is the fundamental factor in the treatment of diabetes mellitus. Also education for patient's family seems to be indispensable to keep patient in well controlled codition.We assessed the role of family of diabetic patients on diabetic control. In 1995 we started the family education program for hospitalized patient's family. The program consists of five lessons: (1) What is Diabetes, it's etiology, symptoms, complications and treatment. (2)Nutrition and health. (3) Dietary cure of diabetes. (4) Planning of dietary menu. (5)Prevention and care for hypoglycemia. Care of insulin injection, if necessary. Sixty-three were invited to this program. 48 were partner of the patients, 14 were child, and one mother. The comments of the attendants to this program were: (1) it was very good time to consider how to do something better next day, (2) to recognize the necessity to cooperate with patient, (3) to obtain the knowledge of nutrition, (4) to correct the misunderstanding on diabetes, and so on ; while patients says that they were encouraged by family, that can easily get the help or support than before, that the condition of surroundings for cary out diet are improved. Mean HBA_{1c} of patients was improved from 8.5% on admission to 6.8% at discharge, and kept this value or below for 9 months, whereas in the case of 59 patients(age and metabolic state are matched) admitted before this program, mean HBA_{1c} was improved from 8.7% on admission to 6.7% at dischage, and exceeded this value after 5 months. Namely the duration of good control after discharge was doubled. These facts suggest that cooperation of family is indispensable for diabetic control and also family education is very important facter in the treatment of diabetes mellitus.

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UTILIZING FOURTH GRADE STUDENTS TO INCREASE DIABETES AWARENESS

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This project was originated by the Texas-Mexico Border Diabetes Registry Project (TMBDRP) to determine if fourth grade students were a suitable group to assist the TMBDRP register persons with NIDDM, raise awareness about the diabetes problem and delivering a diabetes message. Since NIDDM is found in epidemic proportions along the Texas-Mexico border of south Texas, it was determined that delivery of a NIDDM prevention message to as young a population as possible was important. The school pilot program was initiated with 1701 fourth graders from four school districts. School administrators and teachers cooperated with the project and were eager to assist since they themselves knew relatives and friends with NIDDM. Data from the project revealed that 916 out of 1701 fourth grade students who took part returned the diabetes enroll form as instructed and 808 forms out of the 916 were completed by persons with NIDDM. The 54% return rate indicated that closer attention to instructions needed to be followed by all involved. Results from the pilot program revealed that fourth graders are a suitable group to receive a diabetes message and they can responsibly assist in the enrollment of their relatives with NIDDM into the Diabetes Registry. Individual school campus responsibility for the return of all enrollment forms will be included in a planned future project which will involve 20,000 fourth grade students. It is estimated that at least 10,000 persons with diabetes will be added to the TMBDRP database as a result of the larger effort.

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SPECIFIC INSTRUCTIONS GAVE REDUCTION OF LIPOMAS AND IMPROVED METABOLIC CONTROL IN DIABETIC CHILDREN.

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Many diabetic children develop lipomas at the injection sites despite active education. The aim of our study was to see whether a specific instruction scheme lead to reduced lipomas, and if HbA_1c then decreased. First we examined 96 diabetic children and adolescents, aged 4-19 years (mean 12.6), with a diabetes duration of 1 month - 17 years. They all had multiple insulin therapy. 35 patients (27 boys and 8 girls) had lipomas, and they got traditional education in injection technique. After a three month run-in period 20 of these children with lipomas, 17 boys and 3 girls, were randomized into either a control group (C), who got further conventional advice, or into an experimental group (E). In addition to advice the E patients received a formal injection scheme which could be put on the injection area of the stomach, plus a diary for follow-up of their injection practice. Another 3 months later the area of lipomas was registered. HbA_1c was determined before and after the intervention (normal range <5.4%).
The E group got a more pronounced and frequent reduction of lipomas then the C group. The total lipoma area in the E group decreased from 107.6 cm^2 to 40.8 cm^2 ($p=0.07$), but was constant in the C group (89.9 resp. 82.2 cm^2). HbA_1c decreased in 9/10 E patients (mean went from 7.9% to 7.0%;), but only in 3/10 C patients (8.0% resp. 8.1%) ($p<0.001$). In both groups a decrease in HbA_1c was correlated to reduced lipomas ($p=0.07$).
We conclude that quite simple but distinct instructions may reduce lipomas and improve metabolic control in diabetic children and adolescents.

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BENEFIT OF IN-PATIENT DIABETES EDUCATION TO GLYCEMIC CONTROL

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It is a consensus that education is important in the management of diabetic patients. In our division, out-patients have educator and dietitian consultations using food models at their initial visit and subsequent visits if random blood glucose is higher than 11 mmol/L. In-patients have educator and dietitian educations, chi-kong exercise instruction, and daily diet with calculated calories. In this study, we investigate whether short-term admission for diabetes education improves glycemic control in those out-patients with poor sugar control. During July 1995 to June 1996, 53 non-insulin dependent diabetic out-patients (19 male and 34 female) were admitted for sugar control. The mean age, disease duration and body mass index was 58 ± 11 yrs, 8 ± 4 yrs and 23 ± 3 % in male patients; 60 ± 8 yrs, 11 ± 5 yrs and 26 ± 6 % in female patients. At admission, 51 % of them admitted irregular medication, all of them had higher daily calorie intake (38 ± 10 Kcal/kg body weight in male patients; 30 ± 11 Kcal/kg in female patients) and imbalance nutrition (carbohydrate 47 ± 10 %, protein 16 ± 3 %, fat 37 ± 14 % in male patients; 49 ± 9 %, 16 ± 3 %, 33 ± 7 %, respectively in female patients). Their mean of HbA_1c was 10.4 ± 1.7 %. After short-term admission (8 ± 4 days), HbA_1c level decreased to 8.9 ± 1.7 % ($p<0.05$ by paired Student's t-test) in 2-3 months while their dosage of oral hypoglycemic drugs were not increased In conclusion, diabetes care must repeat and repeat till patients can fuse these concepts and skills into their daily life. The diabetes educators and dietitians play important roles in the follow-up work.

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WHEN IS IT TIME TO RE-EDUCATE OUR PATIENTS? Sulway, M.J., Diment, A.D. and Harris, G., Royal North Shore Hospital, Sydney Australia.

Since the development of the Bodylink™ Teaching System we have been evaluating its long-term use in our adult I.D.D.M. programmes which are designed to improve understanding of and adapting to diabetes. The D.C.C.T which was conducted over an 8 year period has raised the question of long-term educational 'support' for patients. To investigate this reinforcement issue we analysed evaluation data on programme cohorts immediately before, after and at 6,12,18 and 36 month intervals on a 34 item questionnaire developed to assess both theoretical and behavioural, practical "skill" issues of diabetes management. A subset of course participants had three pre-course and three post-course HbA_{1c} measurements taken.

RESULTS. "Knowledge" scores - means ± S.D. (max. score = 34)

Whole group (n=259) Pre-course = 13.6 ± 5.2
Immed.Post-course = 28.3 ± 2.8

(a)	6 months	(n=71)	=	23.8 ± 4.7
(b)	12 months	(n=53	=	21.5 ± 3.2
(c)	18 months	(n=50)	=	19.8 ± 3.8
(d)	36 months	(n=38)	=	17.2 ± 4.4

Pre x (a) (b) (c). All significant at 0.001 level
(d) Not quite significant at 0.05.

Post x (a) (b) Not significant
(c) Significant at 0.05 level
(d) Significant at 0.001 level.

HbA_{1c} (means, n=56) Pre-course/post - 8.6% to 7.3% ($t = 4.32$ $P<0.001$).

These data indicate that knowledge scores having initially doubled, declined at a steady rate over the study period (3 years). It appear that between 2 - $2^1/_2$ years this "decay" shows there is now no difference between pre-course levels and follow up. On this basis we recommend some "formal" re-education between $2^1/_2$-3 years as an adjunct to the clinical and self-managed maintenance of the observed HbA_{1c} trends.

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DIABETIC EDUCATION : ASSESSMENT OF COMPLIANCE

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Compliance and metabolic control were ascertained in 13 diabetics (group A) attending education program of a University Hospital for first time, and in 20 (group B) and 28 (group C) diabetics who attended 10.9 ± 4.4 and 28.5 ± 8.7 similar programs respectively. Education Score (3.9 ± 2.2 in A, 6.7 ± 1.8 in B, 7.5 ± 1.5 in C /max 8) and Self-Care Score (4.1 ± 2.6 in A, 6.5 ± 0.9 in B, 6.5 ± 1.3 in C / max 7) were better in groups B and C. But, BMI (kg/m^2 22.4 ± 3.2 in A, 23.9 ± 2.6 in B, 24.2 ± 3.3 in C) and blood glucose in fasting (mg/dl 132.1 ± 48.7 in A, 119.6 ± 37.6 in B, 126.2 ± 30.8 in C) or at random (mg/dl 177.6 ± 43.6 in A, 194.9 ± 59.8 in B, 195.7 ± 72.9 in C) were similar in all. Self reported glycemic control (chi^2 4.6 n.s.) and diet adherence (chi^2 3.8 n.s.) were also not different among 3 groups. Monitoring of body weight, blood or urine glucose, GHb, lipids, ECG or fundus were not more frequent in group B or C. Elicited responses for poor compliance and control were inapt application of information (59.0%), inadequate education (27.9%), unrealistic treatment goals (23.0%), unawares of accompanying diseases (23.0%), uncommunicative health care team (19.7%) and limited resources (11.5%).

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Education for Professionals

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WESSEX MULTIPROFESSIONAL COURSE IN DIABETES CARE. EDUCATION OF DIABETES CARERS BY FELLOW PROFESSIONALS

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Good quality Diabetes Care is delivered by professionals from several disciplines working together as a multiprofessional, patient centred team. The Wessex Multiprofessional Course in Diabetes Care (W.M.P.C.D.C.) provides education of these professionals by their own Health Care colleagues. We aim to teach knowledge, practical skills, motivational techniques, coping mechanisms, and teamwork theory. The course is in 4 parts: a pre-course learning package, including learning activities in a course handbook; a 5-day intensive teaching block with 14 seminars, 4 practical workshops, and feedback sessions; a work based project done over 3 months; and a residential weekend to report on projects and discuss aspects of adult learning and team working. In 1995 and 1996 20 professionals, 10 podiatrists, 7 nurses, 2 dietitians, and 1 Prison Medical Services Nurse, have attended. In an evaluation questionnaire 75% found the precourse learning package and 66% the multiprofessional interaction in the 5 day block very useful, but 66% criticised one topic heavily, which has been withdrawn. Each presentation was scored (1-5, high) with mean values as follows: content 4.3, presentation 4.3, interaction 4.2, teaching aids 4.1, and relevance 4.3. The residential weekend was very valuable in understanding the roles patients and professionals play. The W.M.P.C.D.C. is a good model for multiprofessional education, which is important in trying to deliver good Diabetes Care.

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SUPPORTING PROGRAMME FOR EDUCATORS IN POLAND.

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A diabetes educator to be successful must be skilled in the teaching-learning methods and in process of planning, implementing and evaluating of local education programmes.The aim of this report was to identify the problems of educators and to develop a supporting programme for educational teams. Forty two educators (14 doctors and 28 nurses), aged 22-56 yrs, who has been working with diabetics 0.5-20 yrs (9.15±6.47, mean±SD) from different diabetes centres, participated in first step of the programme (3-day training course). All participants answered a 34-item questionnaire to evaluate their activity in the field of the patient education. The nurse's knowledge about diabetes was assessed by means of a multiple choice test with 20 questions at the beginning (A) and the end (B) of the course. The main barriers to the patient education were: the lack of the appreciation of the education by another medical staff, lack of formal programmes and financial problems. A significant improvement of nurse's knowledge was observed, global score changing from (A) 45.5±13.1 to (B) 70.7±9.6 ($p<0.001$). Next, all teams had to develop and implement own teaching programmes. As a result of 3-day training after 3 months, 18 of 19 local educational programmes for diabetics were implemented and evaluated with 237 of patients with diabetes involved. Conclusion: A first step of a long-term programme for the educators was very effective in the increase of motivation of educators and in the process of developing of local teaching programmes.This course was sponsored by the Eli Lilly Fund of the International Diabetes Federation.

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INTERNATIONAL EXCHANGES IN DIABETES EDUCATION :E Baumer Diabetes clinic Vienna Austria, M Schlaeppi Unité de diabétologie, Lausanne Switzerland and the Red Bus 1996 Global Medical Conference Project Group, USA. Representants from 23 varying countries attended the 2nd annuel GMC in Indiana USA.which incorporated models of patient care and the setout of varying training courses available throughout the world. During this Conference along with the sharing of ideas and experiences many friendships were made. The participants were then the guests of Eli Lilly at the 23rd American Association of Diabetes Educators Annual meeting in New Orleans. The Diabetes Educators from the following countries: Austria, Switzerland, Slovania, Croatia, Philippines, Aruba, Jamaica and Puerto Rico decided to create an International working group The first meeting of the "RED BUS PROJECT " working group was held with the objective of deciding exactly what the future plans were and who would be responsible for specific tasks. It was decided to open this group to all Diabetes Educators interested in the aims of international exchange. The main aims of this group are, to continue the International friendships and contacts made during the GMC, share and work together on research programs, encourage participation at future conferences, and the composition of joint written articles, abstracts and posters. The first project was the creation of this poster which would be an opportunity for as many representatives from the 1996 GMC to display their own personal gains, describe the work being done in their own countries and to explain their intentions with regards to future research studies between thier own and other countries. This poster shows a map of the world encircled by a Red Bus with pages of text attached to thier varying countries by ribbons indicating the knowledge and interest shared by each of the diabetes educaters. As Diabetes educators change thier roles from a source of all knowledge to that of disease managers they are also having to change the way they practice diabetes education, One very important way to obtain this new role is by sharing the experiences of other trained diabetes educators. Therefore the continuation and availability of these international educator conferences is very important in the ever changing domain of diabetes education.

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POST GRADUATE TRAINING IN DIABETOLOGY : RSSDI ACCREDITED PROGRAMS.

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To impart competence among primary care physicians in managing diabetes up to secondary level, Research Society for the Study of Diabetes in India (RSSDI) empowered National Accreditation Committee for organizing post graduate training programs. The program contents were uniform and comprehensive in the format of Continuing Medical Education, Workshop, Update, Panel Discussion, Open Forum, Post Graduate Course, Annual Scientific Meeting, Conference, Advanced Course or National Symposium. Since May 1995 in 18 months period, 31 programs were conducted in 13 Indian states including 5 Post Graduate Courses at teaching hospitals from South, North, East and Western regions of India. RSSDI designated post graduate training activity of 287 credit hours for these diabetes education programs - 14 for 5 credit hours each, 4 for 8 hours each, 7 for 10 hours each, one for 15 hours and 5 Post Graduate courses for 20 hours each. On average, each program benefited about 102 (45-325) clinicians and 3158 participants were trained for 6409 clinician-training-days over 52 program days for 6 hours a day. RSSDI accredited post graduate training in diabetology was effective in ensuring active participation of primary care physicians to facilitate coordinated diabetic health care system in the country,

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PRACTICAL DIABETES MANAGEMENT FOR FAMILY PRACTITIONERS

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The purpose of this program is twofold; to increase the diabetes knowledge and management skills of family physicians and to foster a collaborative working style between the local Diabetes Education Centre and key family physicians in the community. The program was developed under the auspices of the Canadian Diabetes Association and the Association Diabète du Québec with support from industry. A needs assessment supported addressing practical management issues in the care of the patient with diabetes. A 20 module manual was developed highlighting different aspects of diabetes management, with 10 modules considered "core". A handbook for the team teaching the program was developed, but the method of teaching was left to the discretion of the local group. It was expected that the local endocrinologist or other interested physician, the nurse educator and the dietitian would teach the program together. Three Diabetes Education Centres in various settings, ie teaching hospital, small urban setting were asked to pilot the program. Some centres indicated some difficulty in deciding who was to teach what and how to team teach when the participants were physicians and some of the teachers were not. The participants and the team teaching the program were asked for evaluations. Results of the pilot programs showed that more information was needed on nutrition and some refinement of the insulin initiation module was needed. The participants indicated an increased respect or trust in the Diabetes Education Centre and stated that they would refer more patients. Criteria for the selection of centres to host the program were developed. The first roll out of the program in the fall of 1996 resulted in 14 programs across Canada and 115 participants. Interest in the program has been very high, already a waiting list has formed both for new centres as well as for the original centres to repeat. Evaluations are being conducted at the finish of each program and will also be conducted after a 6 month interval. Results of these evaluations will be discussed in the presentation as well as the use of this program material in other related areas. Early results indicate that this program will be successful in addressing the education needs of family practitioners as well as encouraging better collaboration with the Diabetes Education Centres.

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DIABETES EDUCATORS TRAINING : INDONESIAN EXPERIENCE,

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Estimated Indonesian diabetic population in the year of 2020 will be up to 4 million, while there will be only 40 endocrinologists. As an anticipation of the forthcoming problems, Diabetes and Lipid Center through its Diabetes Information Center in Jakarta has conducted the diabetes educator trainings in 1992 and every year there after, and holding to the theme : *trying to live as a diabetic*. The first training was attended by 18 doctors, 19 nurses, 13 dietitians and 3 lay people from 20 hospitals. The hospitals are only allowed to send their participants to this training as one team. This course consisted of lectures, workshops, role play, learning by doing , case studies, problem solving and interactive learning. The system used in this course is a modification of the Steno Diabetes Center Course in Diabetology, education material given are modification of the DTTP program of Prof. Berger , Dusseldorf and Body Link from Australia. The second basic and the first advance courses in 1995 were co-sponsored by The Education Foundation of the IDF with participation of 14 specialists,17 GP's, 19 nurses, 18 dieticians, from 28 hospitals and 19 cities. The 3rd basic course in 1996 was held together with the 2nd advance course with 28 doctors , 30 nurses, 23 dietitians, 2 from pharmaceutical companies, from 31 hospitals (12 cities). All together there are now : 87 doctors, 100 nurses, 61 dietitians and 5 lay people , from 61 hospitals scatered in 23 cities in 15 provinces (from 27 provinces in Indonesia). At this moment some of these educators are doing their work in special diabetes education clinics. In Jakarta itself there are now 12 diabetes education clinics . The activities of these clinics will be reported in more detail.

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DIABETES MINI-RESIDENCY FOR HEALTH PROFESSIONALS: EFFECT ON KNOWLEDGE, ATTITUDES AND BEHAVIOR CHANGE. K. Berkowitz, L. Anderson, R. Panayioto, D. Ziemer and D. Gallina. Grady Health System, Emory University, Centers for Disease Control, Atlanta, GA, USA.

The aim of this study is to assess the impact of a diabetes mini-residency for health professionals on knowledge, attitudes and behavior change toward diabetes and its treatment. The one-week mini-residency is held in the Grady Health System Diabetes Unit in Atlanta, Georgia. The format includes lectures, interactive sessions, and case study presentations. There are 3 hours of clinical observation with diabetes team members, including nurses, dietitians, podiatrists and inpatient educators. Over the 13 month study period, 125 health professionals completed pre- and post-test questionnaires (79%) assessing knowledge and attitudes. Participants were asked to write 3 self-behavior change goals to be implemented into their practice within a 3 month period. Knowledge was assessed by a 40-item multiple choice questionnaire designed to reflect the content of the course. Attitudes toward diabetes and its treatment were measured by five subscales of the Diabetes Attitude Scale which assess attitudes toward: need for special training (α =.80), control and complications (α =.55), patient autonomy (α =.58), team care (α =.77), and impact of diabetes on patients lives (α =.59). Differences were found in pre-post knowledge scores (26.2 vs. 34.2, $P < .0001$). Attitudes were more positive after the course for each of the following subscales: need for special training, patient autonomy and team care ($P < .0001$), with a positive trend for the control and complications scale ($P < .02$). Most behavior change goals were in the areas of education (53%) or diabetes management (36%). Forty six percent of participants reported their 3 month goal completion; 67% of goals were > 50% achieved. Many goals were unrealistic, represented multiple goals or were unmeasurable. This study demonstrates that attitudes can become more positive after a mini-residency. Whether such changes will translate into behavior changes is not known and will need further study. Goal setting is not intuitive and may require structured training.

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DIABETES SELF-CARE REGIMEN SIMULATION AS A COMPONENT OF HEALTH PROFESSIONAL TRAINING. *BP Brackenridge, Learning Prescriptions, Phoenix, Arizona, USA.*

In caring for patients with diabetes, professionals make many recommendations for daily living. Although the recommended actions are known to be helpful in controlling diabetes, patients often do not implement the advice they receive. Although extensive research in the behavioral sciences describes a variety of reasons for such behavior, many health care providers persist in characterizing such "noncompliance" as a failure on the patient's part. Such "blaming of the patient" is not productive and may impair the health professional's ability to enter into a respectful therapeutic alliance with the patient. To help professionals gain insight into some of the practical and human reasons behind regimen nonadherence, the author includes a simulated diabetes self-care regimen in the educational design of an international training program which she conducts. Based on principles of experiential and discovery learning, the regimen simulation requires professionals to follow a diabetes meal plan, take three saline injections and four blood glucose tests daily, exercise, and keep a self-care record for a period of three days. Adherence data for 1015 professionals who have taken part in the simulation are comparable to adherence data reported for diabetes patients. The simulation impacts diabetes-related attitudes of participants, as measured by a sub-set of questions from the Michigan Diabetes Research and Training Center's Diabetes Attitude Survey for Professionals.

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DIABETOLOGIC IN-SERVICE EDUCATION FOR HEALTH PROFESSIONALS OF NON-DIABETOLOGICAL WARDS. E. Schipani, A. Piaggesi, F. Campi, F. Baccetti and R. Navalesi. Cattedra di Malattie del Metabolismo, Università di Pisa, Pisa, Italy.

To test the efficacy of in-service requalification interventions, we carried out a course on general knowledge on diabetes (GKD), bedside monitoring of blood glucose (BMG), insulin preparation and administration (IPA), diagnosis and treatment of hypoglycemic crises (DTH), hospitalization-related problems (HRP), to 171 health professionals (HP) of non-diabetological departments of our hospital. Knowledge before and after the course was evaluated with a multiple-choice questionnaire, while skills were evaluated with specific operational checklists. HP were compared with a group of non-trained colleagues (CG). Global knowledge on diabetes after the course, gathered from the percentages of correct answers in each questionnaire, significantly improved (61.82±23.64% vs 31.18±20.00%; $p<0.001$); separated analysis of different areas evidentiated improvements in GKD ($p<0.01$), BMG ($p<0.01$), IPA ($p<0.05$), and DTH ($p<0.05$), but not in HRP. Professional skills profiles of HP were significantly ($p<0.001$) better than those of CG, for BMG, IPA and DHT. Linear regression evidentiated a significant ($p<0.001$) correlation of skills and knowledge after the course either for BMG ($r2 = .49$), IPA ($r2 = .53$) and DTH ($r2 = .61$). The course produced a significant improvement of knowledge and skills on specific diabetologic items among participants.

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DOCTORS & STUDENTS KNOWLEDGE ABOUT DIABETES MELLITUS. D.J.S. Fernando, Colombo Medical Faculty, Sri Lanka.

We determined the level of knowledge on diabetes mellitus among final year medical students non specialist hospital doctors and general preactitioners from the Faculty of medicine, Colombo, National Hospital Sri Lanka, College of General Pratitioners of Sri Lanka. 120 randomly selected final year medical students (group A), 90 non specialist hospital doctors from the outpatient department and diabetic clinics (group B) and 80 general practitioners who attended a CME meeting (group C). A previously validated MCQ paper of 10 questions which assessed knowledge of diabetes on diagnosis, management, insulin therapy, dietery advice screening for complications management during acute illness and management of emergencies was administered to all subjects. The mean score were 53% SD 12.3 for A, 51% SD 14.3 for B and 52.3% SD 10.7 for C. Deficiencies were shown in diagnosis (34%A, 30%B, 40%C), management of non insulin dependent diabetes (35%A, 40%B and 45%C), Management during an acute illness (50%A, 58%B, and 54%C), Insulin therapy (59%A, 65%B, 60%C), Use of oral hypoglycaemics (45%A, 60%B and 55%C). Medical students had one lecture on diabetes in their 3rd year. Medical officers had no in service CME while general practitioners had attended lectures on diabetes sponsored by pharmaceutical industry. Knowledge required to manage diabetes mellitus is inadequate among medical students, hospital doctors and general practitioners.

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INJECTION-MEAL-INTERVAL: WHAT DO DIABETOLOGISTS RECOMMEND TO THEIR PATIENTS?

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In order to evaluate which suggestions diabetologists gave their patients regarding handling of the injection-meal-interval (IMI), we submitted a single-page questionnaire to the 72 hospitals organised in the "Working Group Structured Diabetes Therapy" in Germany. 55 (77 %) of the hospitals responded. In 16 hospitals (29 %) patients were recommended to keep a fixed IMI of 15 (0, 30) min (median (range)). The recommended minimal IMI was 0 (-10, 15) min and the maximal IMI 45 (20, 60) min. In the other 39 hospitals (71 %) patients were trained to use a flexible IMI. The suggested limits for the IMI was a minimal interval of 0 (-30, 15) min and a maximal of 45 (15, 120) min. The conditions influencing the length of the chosen time interval were evaluated by crossing: pre-prandial glycaemia 95 % YES/3 % NO, time of day 41 % YES/59 % NO, physical exercise 51 % YES/49 % NO, amount and type of meal 75 % YES/25 % NO, situation 85 % YES/15 % NO. In 18 (32 %) of the 55 hospitals a differentiation between patients with diabetes mellitus type I and type II with respect to the IMI was done. Answers to the question: "From which source do you have your information about the IMI?" were: literature 64 %, colleagues 56 %, textbooks 53 % and original articles 24 %. In 45 questionnaires other sources or comments were mentioned, "own experience" was named 24 times (53 %) and experience of the patients 9 times (20 %). This study shows, that the recommendations of experienced diabetologists regarding the injection-meal interval vary considerably.

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ASSESSMENT OF BASIC KNOWLEDGE RELATED TO THE MANAGEMENT OF DIABETES AMONG GENERAL NURSING STAFF (GNS).

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We observed that inadequate knowledge among general nursing staff (GNS) could explain a considerable number of problems related to the management of diabetes. To assess the knowledge of one of the premier hospital's GNS, we administered a questionnaire to 94 nurses. Only 39% of the nurses felt that they were confident in judging the type of diabetes, 39% whether patient needs insulin life long and, 47% regarding insulin formulations with different concentrations. 67%, 57% and 53% felt they were confident about their knowledge regarding type of insulin (according to the duration of actions), different syringe scales and diagnosis of hypoglycaemia (HG) respectively. However, only 31/94 stated that they could treat a patient having HG very confidently by themselves and only 2/31 knew the proper management of a patient found in the state of severe HG (unconscious). 56/94 thought they knew well how to prevent HG but 25 of these 56 subjects indicated inappropriate management of a diabetic who vomited immediately after insulin and food. 31%, 41%, 45% and 91% of the nurses felt: insulin should not be injected by diabetics themselves, should not be injected in adipose tissue, plain and lente insulin should not be mixed and patient should take meal immediately (no gap) after insulin injection.

Conclusions: A large section of general nursing staff have inadequate information and ill founded confidence regarding diabetes management issues. Continued medical education of nursing staff regarding diabetes management is urgently required.

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PERCEPTION AND KNOWLEDGE OF DIABETES MELLITUS AMONG NURSES WORKING IN A GENERAL HOSPITAL

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Nurses play a key role in providing education for individuals with diabetes (DM). This study was conducted in order to assess perception and knowledge of DM among nurses of our Centre. With permission from the authors (Drass et al,Diabetes Care, 1989), three adapted (translation and back translation) questionnaires: Demographic data (DQ), Perception (PQ) and Basic DM Knowledge (DKQ) were distributed to nurses who voluntarily participated in the study. The Centre is a 575-bed teaching hospital. An average 20% of inpatients admitted have the diagnosis of DM. The questionnaires were answered by 131 out of 320 (43%) staff nurses, 45% of them reported having attended a DM trainning course at least once, 37.8% had a family member or friend with DM and 46.5% felt themselves competent in caring for diabetic patients. DKQ was score 24.4±8 (54% correct). DKQ score was better in nurses working in the Endocrinology ward and those having a family member or friend with DM. DKQ score was not realted to previous attendance to a DM trainning course ($p<0.05$). Perceived and actual knowledge of DM were positively related ($r=0.37$; $p<0.01$). The study revealed that knowledge of DM among nurses of this Centre: needs to be improved although, its perception is satisfactory. Furthermore, these results, could be helpful in designing appropriate trainning programmes.

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INSTRUCTING NURSES ABOUT DIABETOLOGY

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178 Nurses were evaluated pre and post two days course about physiology, physiopathology, clinics and therapy of Diabetes Mellitus performed at Buenos Aires City and Corrientes City (Argentina). A multiple choice questionnary was given to them. The questionnary was the same pre and post course and contained 15 questions: 5 about Physiology and Physiopathology, 5 about Clinics and 5 about Treatment. Each good answer gave 1 point for the evaluation score. Statistical analysis was performed through T student test. The punctuation media at the pre-course evaluation was 11.77 and 41% of the students answered at least 13 questions. The punctuation media post-course was 12.77 but 65.1% of the students answered at least 13 questions. The difference between these two figures was statistically significant ($P < 0.001$). A high proportion of bad answers in the pre-course evaluation was in those questions about therapy. In two Argentine cities, the two days course for instructing nurses about Diabetology seems to be good in the sense of improving knowledge on Diabetes Mellitus. Therapeutical aspects must be focused specially.

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IMPACT IN THE MANAGEMENT OF D.M. TYPE II AMBULATORY PATIENTES BY A MULTIDISCLIPLINARY HEALTH TEAM.

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In the northeastern region of Mexico City the Mexican Institute of Social Security (IMSS) during six months in 1996 registered 23,216 patients with Diabetes Mellitus type II. With the object to implement the ambulatory management of Diabetes a team of Health Care Professionals (25 Family-Physicians, 22 Nurses, 21 Social-workers, and 9 Dietitians) was integrated, from 11 Medical Family Clinics of this area belongs to IMSS who were motived for the treatment of diabetic patients. After obtaining a consensus of the team about D.M. concepts and treatment. a group of 2036 ambulatory diabetic patients were selected; All of them with no more than 5 years of diagnosis, with persistent hyperglucemia in spite to eleveted glibenclamide and/or insulin administration. All patients recieved individual education in DM (diet, exercise, drugs, complicactions, etc) and were follow-up every week during two months in each clinic.A total of 1976 patients were able to evaluated at 2 mo showed excelent metabolic control and minimal doses of hypoglycemiant or insulin. Results:

	0	2 months	
FBS	237	122	
(mg/dl)	n=1976	n=1976	
Glibenclamide	25	7.5	0
(mg/day)	(n=1973)	(n=667)	(n=1126)
Insulin	35	20	0
(U/day)	(n=183)	(n=102)	(n=81)
Hb1Ac (%)	9.41	7.72	

These patients have been follow-up by 8 months and maintened this good control. These results emphasize the importance and the benefit of the multidiciplinary team for managment of DMII.

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DIABETES WORKSHOP FOR FAMILY PHYSICIANS

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AIM : TO CONDUCT DIABETES WORKSHOP TO IMPART MANAGEMENT SKILLS AND FORMULATE AN IDEAL PROGRAMME FOR DIABETIC WORKSHOP IN FUTURE.

MATERIALS AND METHODS : 5 diabetes workshops were held in different parts of Karnataka a South Indian State and interested family physicians registered in the year 1995 - 96. The workshop was for 2 days over the week end covering all topics of practical importance like OHA, Insulins, Monitoring, Diet & Exercise as group discussions and Etiology and classification, complications, pregnancy and diabetes as lectures. The knowledge was imparted by diabetologists and related specialists. The workshop initially consisted of 3 lectures and 7 group discussions. A pre post assesment were done and impression regarding workshop documented. The mean pre assesment mark 51.7% $\pm$ 14.4 and post assesment was 61.4% $\pm$ 17.4, T=2,995, DF=48,(P<0.001) Impression regarding the workshop 74% prefered group discussions 87% opined duration was ideal while 11% opined duration is short. All participants opined that content and arrangement was good.
The ideal number of deligates for a workshop should be 30-40 group discussion should be more ideal duration should be 1 1/2 days over the week end. This type of workshop increase level of knowledge in diabetes among family physicians.

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Nursing staff's understanding of diabetes in Northeast Zaire.

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Diabetes is an important medical problem seen at the Centre Médical Evangelique (CME) accounting for up to 5% of all adult medical admissions to the hospital. It is the nursing staff who are responsible for the initial diagnosis, treatment and daily supervision of these patients. In order to improve the quality of care CME offers patients with diabetes a questionnaire was sent to all 70 nurses employed at the hospital to assess their level of understanding about diabetes. 41 (59%) nurses returned a completed questionnaire. 36 (88%) understood diabetes to be a disorder of glucose metabolism but only 23 (56%) knew the normal range for blood glucose. Knowledge of the clinical features of hyperglycaemia, hypoglycaemia and severe ketoacidosis was generally poor. The majority of respondants understood that long-standing diabetes can compromise the eyes and cardiovascular system but knowledge of other complications was poor. There was a significent minority (34%) of respondants who considered that traditional/local medicine may be able to provide a permanent cure for diabetes. Knowledge of the various treatment options was good e.g 68% of respondants could give appropriate advice on a diabetic diet; but no respondant could provide a satisfactory treatment plan for the management of severe ketoacidosis. The majority (61%) of respondants considered diabetes a difficult condition to treat. Many of the nurses working in Northeast Zaire have been trained at the nursing school linked to CME and the responses generated by this survey probably reflect the level of healthcare received by many diabetic patients in the region. This survey shows the need for continuing medical education for nursing staff at CME and in the region if the level of care for diabetic patients is to be improved in Northeast Zaire. Of particular concern is the poor understanding of the treatment of severe ketoacidosis. This complication carries a high mortality in Africa and although lack of resources may be partly to blame this study suggests that a lack of education of the health-care team may also play a role. The introduction of standard treatment plans for ketoacidosis may help to improve the management of this complication.

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EDUCATED P.H.C. TEAMS - THE BASIS FOR SUCCESSFUL DIABETES TREATMENT

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For the past 3 years we have been organizing continuous education courses for primary health care physicians and registered nurses. The courses have been held twice a year, whereas in 1996 they are held 4 times a year, due to increased interest. An examination board of the Medical Faculty administers exams for the course participants, who then obtain a no.1 category licence for autonomous follow-up of NIDDM patients. The courses include lectures and round-table discussions, and practical work with a diabetes care team. Training of educators is carried out through work with small groups by means of meta-plan system and patient interviews. The training programme also includes the acquisition of skills required for self-monitoring and administration of pen injections. Since 1993, 172 primary care physicians and nurses from Croatia have completed the course. The final exam consists of both oral and written (test of 20 questions) form, together with comments on three examples of self-monitoring diaries. The results of the courses of continuous medical education are the active involvement of primary care physicians in the follow-up of NIDDM patients, founding of 22 new clubs of diabetic patients and the organization of educational courses for NIDDM patients in each GP team.

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Methods of Education

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CHILDRENS KNOWLEDGE OF DIABETES. AGE-RELATED GOALS, GUIDELINES AND EVALUATION METHODS.

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It is essential, that children with diabetes are ensured a continual learning process, adjusted and adapted to educational level, abilities and psychological development. This gives probability for improvement in the childs accept and regulation, thereby creating an increased quality of life, despite diabetes. The purpose of the present study is to define age-related goals for both practical abilities and knowledge of diabetes for children and young people, aged between 6 and 17 years. The investigation is undertaken by an interdisciplinary team consisting of teachers, psychologist and diabetes nurse specialists, attached to the childrens department. Detailed guidance material has been made for both children and parents to show, how the practical responsibility and theoretical insight in diabetes can gradually be transferred to the child. Furthermore the objectives have been used to develop an interview guide to ensure a broad evaluation of the knowledge and practice of the children. The evaluation is carried out from a structured interview by the teachers, while the family is informed of the results by the diabetic nurse specialists, who also assess the need for further teaching activities. A pre-pilot investigation was carried out with 10 children. The preliminary results generally show, that most children have adequate knowledge of diabetes, but that the material provides a good instrument to reveal areas, where there is a further need for teaching.

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BEHAVIORAL MEDICAL TREATMENT IN NIDDM IS MORE EFECTIVE THAN AN EDUCATIONAL PROGRAM: RESULTS OF A RANDOMIZED TRIAL

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Objective: In a randomized, prospective trial (MEDIAS II), sponsered by the German department for research and technology (BMFT, now BMFB), we testet, whether two newly developed behavioral medical programs are more effective than an evaluated structured treatment and teaching program for NIDDM (Group A; 4 lessons) on hyperglycemia, dyslipoprotinea and obesity. The behavioral medical programs were designed to enhance selfmanagement abilities to cope with the demands of the diabetes treatment (eating behavior, physical activity, home monitoring of urine glucose, diabetic foot care). One of the two treatments was performed as a group-treatment (Group B, 12 lessons), whereas the other program (Group C; 12 lessons) was designed to individualise the therapeutical approach by six single lessons. **Methods:** 193 type II diabetes patients were recruited, fulfilling following criteria (age 40 - 65 years, no insulin treatment, stimulated C-peptide >0,8 nmol/l; BMI > 26,7 kg/m²; no known psychiatric diagnosis, no cognitive impairment). These patients (age: 55,6 ± 6,3 years; diabetes duration 6,6 ± 6,2 years; BMI 32,1 ± 3,9 kg/m²) were randomised. At baseline (t1) i.e. glycemic control (GHb), triglycerides, total cholesterol, HDL-cholesterol and body weight were determined. The crucial evaluation (t5) was the course of these parameters 15 months after baseline.176 patients could be examined at t5 (drop-out rate 8,8%). **Results**:

Parameter	Group A		Group B		Group C	
	t1	t5	t1	t5	t1	t5
GHb %*	9,5 ± 2,5	9,7 ± 2,4	10,3 ± 2,7	9,3 ± 2,9	9,9 ± 2,4	9,5 ± 2,3
Triglyc. mg/dl*	207 ± 131	225 ± 150	206 ± 122	181 ± 102	207 ± 101	203 ± 190
Chol. mg/dl	231 ± 43	230 ± 39	233 ± 45	225 ± 42	237 ± 50	230 ± 37
HDL mg/dl	47,7 ± 14	45,4 ± 14	50,5 ± 22	47,4 ± 11	47 ± 12	44 ± 11
BMI kg/m²*	32 ± 3,5	31,5 ± 3,9	31,8 ± 3,9	30,9 ± 3,6	32,7 ± 4,2	31,9 ± 4,2

*Significant differences (<.05) between group A and B; no significant differences between group B and C (T-Tests or Man-Whitney-Test). **Conclusion**: The study demonstrated that a selfmanagement orientated behavioral medical approach (group B) is significantly more successful regarding the improvement of glycemic control, triglycerides and obesity than the educational program (Group A). Surprisingly a more indivualised approach (group C) had no further advantage.

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EDUCATIONAL METHODOLOGIES: AN ANALYSIS OF CHAOS
A 10 YEARS REVIEW OF PATIENT EDUCATION LITTERATURE

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Rationale: The therapeutic role of patient education has been widely documented in chronic diseases in general and diabetes in particular. Despite the awarness, limited progress is made in the field of educational methodologies and implementation of patient education programmes in diabetes care. We were interested to review papers dealing with patient education and to analyse which educational methods were described. **Methods:** We investigated a period of 10 years (1986-1996) using MedLine. *Key-words* were 1-«Patient Education (PE)», 2-«Diabetes Mellitus (DM)» and 3-«Randomised Trial (RT)». **Results:** A total of 9'111 articles were found on PE out of which 964 were on DM and PE (11%). A total of 57'400 were dealing with DM out of which 964 were on DM and PE (2%). Samples from the 964 DM and PE were taken: they spoke mainly about general concepts and role of education and its effect. We therefore tryed to analyse the studies which were structured in a scientific way and used the key-words [«Randomised Trial (RT)» and DM and PE]. Out of the total 964 papers on DM-PE, only 49 were RT (5%). Out of those randomised trials, 12 (25%) did not describe the randomisation. We therefore selected the 37 remaining papers where RT was clearly presented and analysed which educational methodologies was described: *no paper* described a meaningful and pertinent educational methodology used by the autors. Papers came from a total of 25 different medical journals out of which 14 (56%) used reviewers. Finally 2/3 of papers showed a positive effect of patient education, 1/3 of autors could not show any effect. **Conclusions:** 1-Diabetes is not any more the leading disease for patient education (11% of papers on PE). 2-In the field of diabetes research only 2% of papers deal with PE. 3-There is a dramatic absence of description of precise educational methodologies even in papers with scientific reviewers... This situation has urgently to be improved in order to analyse which educational methodologies are responsible for the outcome either positive or negative of those clinical studies. Therapeutic education deserves the same scientific methodological approach as that used for the study of any pharmacological agent.

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ALTERNATIVES TO ENHANCE SURVIVAL SKILL'S KNOWLEDGE

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In 1993 the need to explore alternatives to enhance diabetes education became one of our priorities. We developed a revised diabetes survival skill's program which consisted of 1-2 hours/day/wk sessions with audiovisuals. Among the skills covered by this program were: diabetes pathophysiology (DP), injection technique (IT), insulin kinetics (IK), glucose (GM) and ketone monitoring (KM), sick-days management (SDM), glycated hemoglobin (A_{1c}), and hypoglycemia (HYPO). A questionnaire was developed to ascertain for changes these interventions may have made. The latter was mailed to 143 patients with IDDM followed at the University Pediatric Hospital Diabetes Clinic between 1976-96. The data were grouped in patients diagnosed before 1993 with their parents (G-I) and those diagnosed afterwards (G-II). At the time of this submission 20 families have returned (12 in G-I and 8 in G-II) the questionnaire. Of these, we found: 74% of G-I answered correctly the GM questions *vs* 87% from G-II, both groups answered (100%) correctly questions about KM and IT, ≈ 1/2 answered correctly the SDM questions (58% and 62%, G-I and -II), questions regarding IK were answered correctly in 72% of G-I *vs* 68% of G-II, 92% of G-I and 88% of G-II answered correctly questions about DP, questions about A_{1c} were answered correctly by 88% from G-I but 69% of G-II, the lowest percentage was obtained for HYPO management where 17% of G-I and 25% of G-II answered correctly. In summary, patients diagnosed after 1993 and their parents, followed in our clinics, have continued accruing survival skill's knowledge as before. There was persistent lack of knowledge in hypoglycemia and sick-day management. We conclude that a more extensive survey is warranted to ascertain if above results can be extended throughout the population followed.

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EDUCATING DIABETIC PATIENTS: THE HAITIAN EXPERIENCE

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Diabetes can not be well controlled without education. Everywhere, educational programs are established to inform diabetic patients and their relatives. However, if the program is not adapted to the local customs and tabous, the goal of prevention and good control will never be reached. At Fhadimac, we have started an educational program since 1989 where the information is delivered daily in creole (language used by all Haitians) by a trained nurse, using posters adapted to the haitian reality, and person-to-person mean of communication for illiterate patients. The diabetics admitted to our popular clinic must attend all the classes and are periodically reevaluated on their knowledge. Well-trained patients inform others during group sessions. The final objective is to take away and diminish the impact of some of the ancestral concepts of our culture like the one of chronic diseases that doesn't exist. The patient doesn't know that he has the most important role in the good control of his diabetes. Furthermore, Diabetes at an early stage is silent, painless and doesnt render the patient inactive. Therefore the patient doesn't see any reason to refrain his urges. In conclusion, these methods of education are very demanding and ask for a constant and adapted follow-up; however the results are longlasting and efficient because we have seen a decrease in acute complications in patients coming to Fhadimac.

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EDUCATION CAPS CONTRIBUTION FOR YOUNG DIABETICS LIFE

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The aim of this study is show the education effect on young diabetic behaviour and life. It concerned two young diabetic groups controlled in the same clinical center (INN) group I (n=41) educated in ATD young diabetic caps and group II (n=59) did not recieve this structured education. The mean age and diabetes duration are respectively 14.1 ± 3.4 and 5.3 ± 3.4 in group I and 13.4 ± 5.4 and 2.9 ± 3.0 in group II. Our results show that patient knowledges according diet and self monitoring are significatively better in groupI than in group II. Patients in group I perform more frequently self urinary (85.5 %) and blood glucose monitoring (19.5 %) than patients in group II (55.9 % , 6.7 %) $p < 0.0001$. They adapt better there insulin doses (75.6 %) and take more regularly their monitoring note book (68.0 %) than patients in group II (38.0 % , 44.0 %) $p < 0.0001$. The mean hospitalization number and duration were significatively reduced in group I (3.0 ± 2.1 , 23.4 ± 13.9 days) compared to group II (8.6 ± 2.6 , 107.5 ± 53.3 days) $p < 10^{-5}$, $p < 10^{-7}$,delayed statural growth decreased from 40.6 % to 26.0 % in group I and increased from 33.3 % to 53.3 % in group II (p=0.04). School success is better in group I than in grou II. These results indicate that education improves diabetes control, self monitoring quality, statural growth and reduces the duration of hospitalisation and fees and regularly fill in their note book (68.0 %).

2451

Evaluation of Out-patient Group Consultation for Children and Teenagers with Diabetes and Their Parents.

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Backgroud: Since 1992 we carry on with outpatient group consultation in our clinic. The aim is that the families meet eachother and learn from eachother. Each time we talk about different subjects in order to gradually build up the knowledge of diabetes. We have done this evaluation to find out if this consulting is a good pedagogic method to pick up knowledge about diabetes.

Method: 52 children/teenagers participated in this evaluation. Together with Bengt Pettersson (educationist from the University of Gothemburg) we constructed a questionnaire with 17 questions. In the questionnaire we had medical, practical and dietquestions. If the children´s age was less than 7 years the parents filled in the form. If the children were between 7 and 12 years the children and parents together filled in the form. The teenagers filled in the form by themselves. The children and parents have without preparation filled in the form at two occasions. The first occasion was directly before group consultation and the second occasion was 3-5 months later at an ordinary visit. We gave the answers of the questions on the group consultation by playing a game.

Results: We found that the knowledge of diabetes was high from the beginning. 62% of the participants got a better result at the second test compared to the first. The families had the highest score on the practical questions, secondly the medical questions and thirdly the dietquestions. The result of the study indicates that outpatient group consultation is an useful method to pick up knowledge about diabetes.

2452

Club activity experience in motivating teenagers, suffering from diabetes,for study.

N.Goustova, Moscow Diabete Assotiation, Fund "Yours", Moscow, Russia.

The aim of program: to imprjve live of teenagers suffering from diabetes.
Participants: 30 teenagers and young people aged 14-20.
Duration of the program: 1 year, steps of the program - monthly.
The way of realization of the program:
- collective and individual meeting on diabetes control with an instructor of the program (doctor - diabetologist),
- execution of constant self-control along with keeping a self-control register,
- games, compeninions and tests in diabetes issues.
Results of the program:
-Kept self-control register befor participation in the competition-77%
constantly - 43% from time to time - 57%
-Keeping self-control register after participanion in the program-91%
constantly - 48% from time to time - 52%
-Participanion in the competition improved my life - 75%
-Improved their competence in diabetes issues - 51%
-Received additional information - 67%
-Would like to participate in continuation of the program - 75%

2453

Diabetology Course for Students in their last year of Medical School

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To improve the diagnostic and therapeutical approach on diabetic patients of the next medical school graduates, we set the following special goals: 1) To extend the knowledge of diabetes to students in their last year of medical school, offering a nonobligatory course. 2) To establish, by means of a pre- and post-course evaluation, the impact of this diabetology course on the acquisition of new information. The course was attended by 108 students, evaluated before and after each diabetology course, developed in 1995 and 1996. The program included: diagnosis of Diabetes Mellitus, methods of metabolic control (self-monitoring, laboratory), management of the Oral Antidiabetic Agents, management of insulin in IDDM and NIDDM, management of acute complications, detection of chronic complications. It was a two-day course. Pedagogical resources applied were 20-minute information presentations for descriptive programs, and dynamic approach Workshops, encouraging participation by the presentation and the resolution of prevalent clinical cases in diabetological practice. The same evaluation method was applied before and after each course. It consisted of a multiple choice and semistructured questionnaire with 11 questions: 4 on diagnosis, 2 on NIDDM therapy, 2 on IDDM therapy, 1 on acute complications, 2 on chronic complications. The general average of correct answers was 56 % before the course and 74 % afterwards, with a statistically significant difference of $p < 0.003$ (paired samples t-test). These results suggest the need to emphasize the development of diabetology programs intended for medical students, as an essential complement of the study program, apparently insufficient for the currently required approach to the care of diabetic patients.

2454

Educational and Support Groups for Type II Diabetes

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The goal of such groups is to improve self-management skills in Type II diabetes Type II diabetes is a chronic disease requiring life-style changes as the foundation of all treatment. Most patients are treated by a primary physician, who usually doesn't have the time or specialised knowledge to deal with life-style changes such as dietary discipline, the importance of physical activity, foot care, etc. Coming to terms with a way of life that has daily limitations is a constant battle and the emotional needs must be given on-going support. Maccabi's National Diabetic Centre has written a program for group support and education. Each course has six two-hour sessions and is counselled by a diabetic educator or nurse and a dietician, allowing adequate time for discussion. During the past two years, seven groups have taken place, including 80 participants, in the Herzlia Diebetic Centre. The following results are from 30 participants taken from the first four groups: Average HbA1 was 10.76% (4-7%) before the course and dropped to 9.42% after a period of between 6-12 months. 42% of all participants were exercising before the course and it rosé to 75% on follow-up, 6-12 months later. 25% of participants smoked before the course and after 6-12 months, only 17% continued to smoke. 47% lost weight on follow-up. A questionnaire given at the end of the course showed participant satisfaction and 80% expressed that the course was a help to them in managing their diabetes. A renewed sense of confidence motivated them to consider the importance of life-style changes. 98% agreed that they would recommend the course to a diabetic friend. Group meetings for diabetics are an important tool in meeting the emotional needs of living with a chronic disease. The use of guided discussions, allowing integration of theoretical material, must be applied by the educators. Flexibility and assessment must be used in order to determine to what extent the academic material is being understood, thus allowing necessary changes in the program.

2455

CHEF COMPETITIONS - AN INITIATIVE TO COMBINE HEALTHY EATING PRINCIPLES WITH FINE DINING.

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Recent consumer trends are to choosing foods lower in fat and salt and higher in fibre. People are eating more meals away from home and are dependent on public food establishments for an increasing proportion of their food and nutrition. Eating out often creates a barrier to compliance for those with specific dietary requirements, or those with a desire to follow healthy guidelines when dining away from home.

Diabetes is predicted to affect more than 173,000 New Zealanders by the year 2010 and cardiovascular disease is currently responsible for 44 % of deaths in New Zealand. Almost half of the population is overweight. The authors believed it was time to highlight the need for healthy eating to the industry and to influence hospitality and catering Chefs' attitudes about healthy eating and the methods they prepare food. Creating a supportive environment by making healthy choices easier and more accessible will encourage people to make better choices more consistently and thereby influence their overall health and future disease risk.

A new initiative of Diabetes Life Education in Christchurch, New Zealand, was sponsorship of a Healthy Fare category in the 1996 Canterbury Salon Culinaire. These are annual competitions for Chefs run by the Christchurch Polytechnic and supported by the industry.

On a national level, Diabetes Life Education and the National Heart Foundation of New Zealand co-sponsored a similar event in the 1996 New Zealand Culinary Fare. Participants were required to prepare a main course and sweet that met the criteria of being low in fat, sugar and salt.

This is the first time in New Zealand that health agencies have been involved in such competitions.

Work is proceeding in extending this initiative. Results and outcomes will be presented.

2456

DIABETES "TEACHING GUIDES" VERSUS "MAGAZINES": NEED FOR A DEVELOPING COUNTRY ?

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As part of a non-profit volunteer group involved in diabetes education and care, since 1987 we have continued to improvise and disseminate a variety of low cost self-care health educational materials [cyclostyled leaflets; printed booklets and books; structured diabetes "teaching guides" (a. "Diabetes: the answer is in your hands", 100 pages, English; b. "Diabetes - an introduction" - 14 pages, local languages); quarterly news letter/ "magazine" ("Challenge", 8 pages, English)]. Based on the recipients' (patients and families) responses to our publicational activities, and in comparison with responses to diabetes magazines, books etc., published from time to time by other Indian and International individuals/ organizations, we currently place emphasis predominantly on "teaching guides". The third edition of our teaching guide: (a) has been enriched with full chapters devoted to other, but critically interactive health problems (BP, dyslipidemia, smoking, heart disease, obesity and alcohol), highlighting a "holistic", rather than a "blood sugar" approach to total diabetes care. Simplicity (non-scholarly), clarity of expression, lack of ambiguity/ vagueness in the specified instructions and consistent format are qualities of good educational material. Conclusion: From a socio-economic point of view, inexpensive "teaching guides" (one-time; amenable for mass distribution) are more relevant and utilitarian to the current Indian society, rather than unstructured and fragmented diabetes "magazines" (periodicals).

2457

EDUCATIVE ACTIVITY IN A COMPLEX DIABETOLOGICAL CARE ORGANIZATION: FEASIBILITY OF A DIVERSIFIED INTERVENTION.

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Educative intervention needs to be diversified due to the variability of diabetic patient type and the consequent complexity of diabetological care organization. In Brescia, our model provides three different levels of intergrated assistance. 1st level: territorial nurses/GP; 2nd level: diabetological centre; 3rd level: hospital. Aim of this study is to demonstrate the feasibility of a diversified educative intervention in this model of assistance. We report here the 1995 data. 1st level: *receivers:* NID patients in absence of complications, with steady metabolic control; *aim:* diet information (correction of main errors) and feet (prevention of lesions); *operators*: nurses, GP; *methodology*: traditional lessons;3620 interventions. 2nd level: *receivers*: NID, NID obese and ID patients; *general aim*: informative/therapeutic education for specific aims; *operators:* diabetological equipe; *methodology:* traditional lessons and/or interactive didactics; individual or groups; monothematic or courses. 1013 individual interventions; 803 individual diet-therapy interventions; educative group activity (10-15 patients each): monothematic meetings (18 dedicated to foot care; 7 to diet); courses: 6 for NID patients recently diagnosed; 4 for obese NID patients and 4 for NID patients treated with insulin were realized. 3rd level: hospital activity in specific outpatient clinics, in day hospitals and with inpatients. *Receivers*: NID/ID patients at the beginning of disease; NID and ID patients with specific needs; ID adolescent patients transferred from pediatric divisions; pregnant diabetic patients; patients with gestational diabetes; *general aim:* therapeutic education; *operators*: diabetological equipe; *methodology*: single or group interactive didactics. 121 individual interventions; 27 individual courses for ID-NID outpatients; 735 for inpatient and 407 for outpatient alimentary education and diet-therapy interventions; 89 courses for pregnant patients and 6 courses for ID patients were realized. For each intervention (at 2nd and 3rd level) there is a verification (theoretical or practical; with interview and/or questionnaire; evaluation of behaviour; clinical parameters). If, we believe, education is a necessary therapy, it cannot be carried out occasionally or simply because we have sparing time. It is necessary to project management planning of educative activity specific for each reality. Our experience demonstrates the feasibility of a diversified educative intervention in a complex model of diabetological care organization. This model guarantees the realization and permits evaluation of efficent education, satisfying modern criteria for resource rationalization.

2458

A PRESENTATION OF THE NEW SWEDISH BROCHURE
"GOOD FOOD FOR CHILDREN - FOOD FOR CHILDREN WITH DIABETES"

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In 1996 the Nutritional Board of the Swedish Diabetes Association published a new brochure, for children with diabetes, in the age group 1-14 years and their parents. As indicated in the title the message is suitible also for healthy children. The brochure visualises the message how to obtain a balanced diet for children with diabetes in a simple way and consist of 36 pages and about 70 pictures. Headlines included in the brochure are: The plate model, the plate model shows the proportions of foods to include in the main meals. Menus for the three different age groups. What to consider when children have diabetes. Physical activities. What to do when children refuses to eat. Sickness: colds, infection and illnesses.
Foods for pick-nick and parties. Sweets and ice cream.
What is carbohydrate. What to drink. Nutrition labeling.
The brochure ends with about 40 recipes of dishes that Swedish children often appreciate.
The Nutritional Board of the Swedish Diabetes Association hopes that this brochure will be a toool not only in families having children with diabetes but also to other people who will meet these children, i.e. teachers and nursery staff, to increase the knowledge of nutrition and diabetes.

2459

HOW MANY HOURS ARE NEEDED TO TEACH PATIENTS TO MASTER THEIR TREATMENT ? AN INTERNATIONAL SURVEY

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WHO Collaborating Center - Switzerland

Rationale: Thousands of diabetes centers provide education of patients. Strangely enough even if different curricula are available, no clear data exist regarding the number of teaching hours necessary for therapeutical education of patients. This question is adressed by health care providers (HCP) as well as by health policy makers, medical insurance and hospital administrators. **Methods:** At time of several scientific reunions which all delt with patient education, participants were asked to fill a questionnaire which was asking two questions for type I and type II diabetes: how many hours are needed for the initial education for a newly diagnosed patient and how many hours during the first year follow-up. Participants were asked to define the minimum indispensable number of hours as well as the recommended time for education. **Results:** 419 participants answered the questionnaire (304 MD, 74 RN, 23 RD, 18 Psycho.). Participants originated from 46 different countries but the majority of them came from Occid. Europe (70%). ***Minimum indispensable hours*** *(mean +/- SD)*: Type I Diabetes: *Initial education*: 7.3 +/- 6 hours; *First year follow-up*: 9.2 +/- 7. Type II Diabetes: *Initial education*: 5.2 +/- 5 hours; *First year follow-up*: 7.0 +/- 6. As far as the ***recommended number of hours*** are concerned participants usually agreed to increase the minimal number of hours by about 70%. The analysis of the recommended hours showed little non-significant differences between doctors and nurses. Psychologists and specialists in education recommended 2 more hours than the average for each clinical situation. The coefficent of variation was in the order of magnitude of 79%. This certainly highlights the heterogeneity of the group of HCP on one side and of the great variability of the educational process on the other. **Conclusions:** This investigation has focused on the average number of hours needed for educating patients without diabetic complications in order to help them to self-manage their treatment. Based on those figures HCP may recommend to the appropriate health decision makers the number of hours needed for therapeutic education. These data may also help to better tailor the educational curriculum of patients.

2460

EFFICACY OF INTENSIVE GROUP EDUCATION PROGRAM ON METABOLIC CONTROL OF DIABETES IN TURKEY

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In this study, we aimed to investigate the efficacy of intensive group education program on metabolic regulation. Totally 38 patients who were not taken education before, 12 type 1 diabetics (mean chr. age: 34±10 yrs, diabetes duration: 9.6±9.5 yrs) and 26 type 2 diabetics (mean chr. age: 59±7 yrs, diabetes duration: 8.7±5.3 yrs) were included. All patients took a two days diabetes education program including definition and types of diabetes, diet planning, exercise, therapy with OAD and insulin, home monitoring, symptoms and prevention of acute and chronic complications, foot-skin-tooth care and special conditions related to diabetes for 14 hours totally 10 hours practical and 4 hours theoretical. All topics were given as 30-60 minutes theoretic and/or practical lessons to groups composed of 8-10 patients. A questionnaire including 24 questions were given to check the diabetes knowledge of patients at the beginning and after course. Answers were scored as +1 for right, -1 for false and 0 for empty replies. The values of blood pressure, body mass index (BMI), glycemia, HbA_{1C}, blood lipids 3 months before, at the beginning and at 3nd, 6th, 12th months after education were compared. No difference was observed between metabolic parameters and BMI values before and after education. In contrast HbA_{1C} levels were found to be significantly lower at 3rd, 6th, 9th months than the levels at entry ($p< 0.001$, $p<0.001$, $p<0.05$ respectively). But, improvement in HbA_{1C} was not lasted at 12th month. Compared to entry, diabetes knowledge level at 12th month was increased significantly ($p<0,0001$). No association was noticed between BMI and clinical findings with education. We concluded that intensive group education is effective on metabolic control up to 9th months, but to achieve good continuous knowledge in long-term, the education program should be repeated in regular intervals.

2461

COMPARISON BETWEEN TWO EDUCATIONAL APPROACHES IN OBESE NIDDM PATIENTS

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Weight reduction and normalisation improve the cardiovascular risk profile in cases of obese Type II diabetic patients. However, the majority of these patients experience certain difficulties in changing their eating habits and fail achieving calorie reduction. That is why the educational approach is very important in these cases. The aim of the study was to assess in a comparative way the effects of two educational approaches: a) 3-day-food-records (two working days and one Sunday) and b) traditional informative education on: food intake, BMI, glucose control and lipids. Sixty obese Type II diabetic patients were randomized to receive training in a 3-day-dietary-records educational program (group I) or in a traditional informative method (group II). Their mean age was 52.1±6.4 years and the duration of diabetes was 9.2±7.3 years. The first method included three times (at the beginning, after the 3rd and after the 6th month) dietary records analysis and reinforcement of the prescriptions. The second method included only the recommendations reinforcement for the same intervals. Fasting triglyceride, total serum-cholesterol, HbA_{1c} and weight were also measured three times. Significant differences were found between group I and group II concerning the percentage of energy derived from total fat (28.7±4.8% vs 37.5±7.6%); BMI (26.8±4.1 kg/m^2 vs 28.8±1.8 kg/m^2); HbA_{1c} (8.8±2.7 % vs 6.6±0.4%). Conclusion: Education based on the 3-day-dietary-records approach is simple, time saving and effective in weight reducing programs in obese Type II diabetic patients.

2462

DO WE REALLY HELP DIABETIC PATIENTS TO LEARN HOW TO MANAGE THEIR TREATMENT ? AN NEW EVALUATION METHODOLOGY FOR INTERACTIVE LEARNING TECHNIQUES

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Rationale: When Health Care Providers (HCP) teach patients, experience shows that they tend spontaneously to inform them about the disease rather than to help them to acquire self-management skills for their daily treatment. The concept of therapeutic education implies that HCP master educational skills which should help patients to *learn* how to treat themselves. We therefore developed an new evaluation methodology in order to quantify how much information and how much learning skills are involved during courses to patients. **The evaluation scale:** This validated tool is divided in 6 intermediate steps distributed from level «1» = pure information, to level «6» representing the most active level of learning. These evaluation scales took into account : 3 dimensions: 1) the interpersonal dynamics between teacher and patients, 2) the mental level of activity of the learners (patients) and 3) how HCP used the therapeutic knowledge when it had to be transfered to patients. **Methods:** This evalution scale was used for the educational follow-up of 15 HCP (6 RN+9 MD). The same course was videotaped weekly during 3 consecutive weeks. After the educational analysis of the course with the evaluation scale, a 45 minutes training was provided to each HCP. **Results:** Initial evaluation showed that HCP favoured information (mean of the 3 dimensions), score 2.9+/-0.1 SD. After those 2 supervisions the score rose significantly to 3.6+/-0.1 SD ($p<0.01$). This change documented that HCP had acquired educational skills which favoured the learning process of patients. Compared with nurses, results of physicians were significantly lower ($p<0.05$) showing that doctors tend to provide more medical information than to really help patients to acquire new therapeutic skills. **Conclusions:** This evaluation tool provides **1**-an objective and reliable system to evaluate the educational methodologies of courses to patients; **2**-it offers objectives ways for continuing education of the health care team involved in patient education; **3**-the use of this scale offers ways to analyse and compare which educational strategies are used between centers. Since patient education is a therapeutic approach such a scale may be used locally and to evaluate these new competences in patients' care.

2463

WRITTEN HEALTH EDUCATION MATERIAL SUPPORTING SELF-CARE OF IDDM DIABETICS

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Written material is important in patient education in self-care with in insulin dependent diabetes mellitus (IDDM). The purpose of this study was to find out how the IDDM guidebook is used and how it should be developed to support its users as well as possible. This study analyzed how the characteristics of the booklet were related with in its use, what kind of opinions and ideas the patients had of it and what kind of role it had in the process of becoming ill and self-care. In the study 4 diabetes nurses and 12 insulin diabetics were interviewed. The interviews were taped and written according to identified themes. In the first stage of the analysis the material was examined deductively according to the themes and subthemes. The frequencies of the similarities and differences of the opinions were then searched. In the second stage the material was examined inductively. The main results are related to the meaning of the guidebook for the patients in their process of becoming ill. The information of the booklet is of great significance for the patients who are just taken ill. The layout and illustrations and use of colours motivate to study the booklet but do not forward learning. Information about the balance of the therapy was the most central aspect for the elaboration of the content. Those patients who have had diabetes for longer periods solve their problems relating to the illness on the basis of their own and other diabetics' earlier experiences. The goal of the nurses and the patients in the treatment is common: good self-care. The nurses used the booklet to support the consultation especially in the early stages of illness. The use of the booklet of diabetics depends on their need of information, learning strategy, self-care motivation, individual characteristics, stage of illness as well as on other information available and the characteristics of the booklet. The booklet supports self-care by providing information to the patients. The booklet is also used by the family members of the patients from whom the patients get the most social support. The nurses give information as well as psychological support to the patients. Getting diabetes creates a crisis. In this situation the meaning of a booklet with relevant information is to help the patients to accept their illness and to put good self-care into practice.

Keywords: diabetes booklet, self-care.

2464

INTENSIVE DIETICIAN EDUCATIONAL SUPPORT CAN POSITIVELY INFLUENCE METABOLIC CONTROL IN NIDDM.

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The aim of the study was to evaluate the short(2), middle(4) and long term (12months) metabolic effects of two different educational Dietician-Patient approaches. 42NIDDM (25M,17F, age37-66yrs) were randomly assigned to two groups, balanced for age,BMI and disease duration:A(12M,9F, age48.2±12.4, BMI 27.2±3.2,diabetes duration 10.6 ± 6.4 yrs, 14 treated by oral hypoglycemic agents(OHA), 7 only by diet) and B (13M,8F, age49.5±11.1, BMI 27.2±4.4, diabetes duration 9.4±7.3, 14 treated by OHA,7 only by diet). *Group A* patients were allocated to a simple dietary instructions *support* by a dietician at the onset of the study and after 2 months. *Group B* patients were asked to fill an elaborate form, with questions about caloric intake,life style, food habits,"wrong believes"and more common quality and quantity mistakes, aimed to prepare a suitable personalized diet.The dietician checked patient's knowledges and behaviour correcting mistakes till the end of the study, every 15 days for the first 4 months and 30 days for the following 8 months(*intensive protocol*). BMI, HbA1c, fasting blood glucose level, total and HDL Cholesterol (HDL-Chol), triglycerides (Tg), serum Uric acid (Ua) (0,2,4 and 12 months) were evaluated.Group A: No tested parameters change occurred after 2 months; a significant fasting blood glucose(170±49 vs 257±43mg/dl, p< 0.01) and HbA1c(8.3±0.8vs9.7±1.4%,p<0.01)reduction occurred after 4months.Fasting glycemia decrease was also maintained after 12 months (201.8±42.6,p<0.005).Group B:Tg (216 ±62 vs296.1±91mg/dl,p<0.05) and Ua(4.1±1.0 vs5.1±1.1mg/dl,p<0.05) were reduced after 2 months, 4 months (Tg 187±62, Ua 4.3 ± 1.0, p<0.05) and all study long (12 months) (Tg 212.2±81.7, Ua 4.4 ± 0.67,p<0.05). Fasting blood glucose levels (183±55 vs234±72, p<0.05) and HbA1c (8.4±0.7 vs 9.8 ± 2.1, p<0.05) were also significantly reduced after 4 months. HDL-Chol levels increased after 12 months (53.3±15.5 vs 43 ±11[4 months] and vs 41±13 mg/dl [baseline], p<0.05). Comparing the groups, Ua after 2 months (B 4.1±1.0, A 5±1.0 mg/dl, p <0.05) and Tg after 12 months (B 212.2± 81.7, A 280.8±79.6mg/dl, p <0.05) were significantly modified.The *intensive protocol* caused an important Tg and Ua reduction, with an HDL-Chol increase. The caloric intake did not significantly vary to cause a weight loss.Therefore, these changes are mainly due to a modified behaviour and are suitable to reduce the cardio-vascular risk in NIDDMs, even if a remarkable charge of human resources was employed.

2465

CAN MAGAZINES ABOUT DIABETES SELF CARE CHANGE BEHAVIOR PATTERNS AMONG READERS?

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To the best of my knowledge, scientific research has not been conducted to pinpoint the effect of magazine reading on behavior. There are simply too many variables to account for, including the personality profile of the reader, exposure to other media, exposure to other sources of medical information, contact with health care providers, and comprehension of material presented. Even when the material is fully comprehended by a reader, it does not necessarily follow that he or she will act on the recommendations offered, especially when the matter under discussion is self care, and the means to that end entail sacrifice and lifestyle changes. In this session, the methods publishers and editors use to assess reader involvement, including subscription renewals, letters to the editor, responses to advertising, and feedback from experts in the field will be discussed. The magazine staff at the American Diabetes Association's Diabetes Forecast has experimented with other methods as well, including reader opinion surveys, contests, and rewards of books or subscriptions, to increase its impact on the health of its readers. There have been successes (hundreds of readers did initiate an exercise program following a contest) and failures (less than half a dozen readers took up our challenge to quit smoking cigarettes).

2466

A TEACHING TOOL ON INSULIN ACTION

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A tool was developed to help insulin-using patients achieve a greater understanding of insulin action, dosage and timing of administration in accordance with blood glucose profiles. A secondary benefit has been in nurse education programmes. Blood glucose readings and times are graphed. Fine overhead transparencies, colour coded to correspond with short, intermediate and long acting insulin (Novo Nordisk and Eli Lilly codings) are placed over the chart on the exact times of insulin administration, extending over onset, peak action and duration in hours. Episodes of hyperglycaemia and hypoglycaemia are examined in relation to dosage. The tool may be used on overhead transparencies and projector when group teaching. A pre-requisite to the use of the tool is that the person is adequately educated to recognise the factors which may affect blood glucose (diet, exercise, illness, stress, insulin dosage, drugs, injection sites) and accurate blood testing techniques. The tool was offered to 404 insulin-using patients, 242 (59.9%) elected to use it; 162 (40.1%) declined due to age, underlying illness or preferred the doctor to adjust their doses. Of the 211 nurses (6.6% students,93.3% registered) using this tool, 73.6% classified it as very good, 21% as good, 5.2% fair. This device was used by 59.9% of all insulin using patients successfully. It can be used by any insulin-using patients on any regimen.

2467

A LOCAL SUMMER CAMP IN JAPAN: CHANGES OF GLYCEMIC CONTROL AND INSULIN THERAPY OF IDDM CHILDREN AND ADOLESCENTS DURING 11YEARS

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The Oita summer camp for diabetic children and adolescents has been maintained since 1986. Clinical records including glycemic control, duration of diabetes, body weight, number of daily insulin injections, dosage of insulin, severe hypoglycemic episodes and diabetic complications were evaluated in 67 camp attendants between 1986, 1991and 1996. Incidence of single daily insulin injection was 35.7% in 1986, but that of 2 times daily injection was 64.3% in 1986, 68.8% in 1991 and 12.0% in 1996 ($p<0.05$, for each vs 1996). Incidence of 4 times injection was 0% in 1986, 31.2% in 1991 and 88.0% in 1996 ($p<0.05$). Average HbA1c level decreased from $10.0\pm1.8\%$ in 1986 to $8.8\pm1.9\%$ in 1996 ($p<0.05$). Decrease in number of severe hypoglycemic episodes during the summer camp was dependent on number of daily insulin injections, *i.e.*,4 times injection was superior to 2 times injection ($p<0.05$). Changes in Kaup index, Rohrer index and body mass index were not significant between the years. Introduction of the pen system for insulin injection was found to be a main cause to enable those young campers to maintain good diabetic controls.

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EFFICACY OF CALORIE COUNTER FOR GUIDANCE TO EXERCISE THERAPY IN DIABETICS

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Aim: Calorie counter has an advantage to determine not only number of walking steps but also consumption calorie levels during exercise and total consumption calorie levels. In the present study, we investigated usefulness of calorie counter for guidance to exercise therapy in diabetics. Methods and Subjects: Enrolled were 67 patients who were admitted to our hospital to receive education program for diabetes mellitus. In the group with calorie counter (n=44), patients carried calorie counter, whereby the consumption calorie levels during exercise and the total consumption calorie levels were determined to calculate daily calorie valances every day. Six months after discharge, questionnaire survey was performed to review the degree of understanding about exercise therapy of continuity, compliance status, plasma glucose levels and alterations of body weights . Results: 1.Even after discharge, most of the patients in the group with calorie counter positively employed exercise therapy and continued it. (89% vs 65%, $p<0.01$ by chi-square test) 2.The group with calorie counter attained more favorable trend toward maintenance of reduced body weights and controlling plasma glucose levels than the group without calorie counter. Conclusion: Incorporation of calorie counter into exercise therapy of diabetics was evidenced to be effective in enhancement of recognition of significance, continuity and positive attitudes toward exercise therapy.

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FIRST BLACKSEADIAB SUMMER CAMP FOR CHILDREN WITH DIABETES. M. Mota, K. Koprivarova, Z, Anestiade, E. Panteleeva, E. Toma, C. Panus, C. Ionescu-Tirgoviste. Diabetes Centre, Craiova, Romania.

The **BlackSeaDiab Working Group** was created with a view to promoting an exchange of experience in the field of diabetes care between the 12 countries surrounding the Black Sea in which the ethnic, social and cultural heterogeneity explains the great difference in the systems of health care. The first BlackSeaDiab Summer camp which took place in Breasta, Craiova in Romania between 10 and 20 July 1996 included 80 children with diabetes from three countries: Romania (59), Moldova (11) and Bulgaria (10). Mean age was 13 yrs. (range 7-18 yrs.); sex distribution - 38M/42F; mean duration of diabetes - 5 yrs. (range 1-9 yrs.). Before and after the camp, a score assessing the quality of diabetes care comprising a knowledge questionnaire, self monitoring, no. of injections/day, glycosuria, blood glucose and the glycated Hb. values was calculated for each participant. The maximum score possible was 40. The scores obtained at the beginning (stated in no particular order with a view to maintain confidentiality) for the three centres were : 34 (very good), 28 (good), and 17 (poor). The content of the programmes was categorised into three groups: (1) basic education programme; (2) recreation and sports programme and (3) social programme. Safety was completely ensured by following camp rules, permanent supervision and provision of translation. The main conclusions of this regional European Diabetes Summer Camp were: a) a large variability was noted with regard to the level of education and the qualities of treatment and metabolic control among the groups; b) in the low level group, an impressive improvement was noted at the end of the programme with regard to knowledge and treatment. In the group with the poor score at entrance, an improvement was noted from 17 to 24. An unexpectedly high level of enthusiasm was noted in the participants with regard to both the giving and the receiving of information, self-monitoring materials and the acceptance of a higher number of injections. Of the 16 receiving 2 injections per day, only 6 remained at the end. c) camping appears to have the best cost-benefit ratio d) camping standards should include special considerations for the different cultural backgrounds of campers if the participants are drawn from different ethnic groups.

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DIABETES CAMP AS MEANS OF EDUCATION FOR CHILDREN WITH IDDM

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Since 1992, the Foundation Cristian Serban from Timisoara has been organising every year summer camps for diabetic youngsters. A number of 71 children and teenagers aged between 7 and 22 years from 20 districts of Romania attended it last year. The diabetic team aimed at: a) enhancing self-esteem, independence in injections and blood testing; b) providing informal diabetes education (practical lessons, three-times a day self monitoring and adjustment of insulin doses); c) screening for microvascular complications (fundoscopy, Micral-test); d) improving short and long-term glycaemic control; e) providing education for diabetes care providers. The mean blood glucose values decreased significantly ($p < 0.05$), while daily insulin requirements slightly increased during the camp. The levels of HbA1c (chromatographic method) were 9.5% for all the participants, higher for the age group 15 - 22 years (10.7%) then for prepubertal children (9.12%). Home blood glucose monitoring equipment is available in 49.3% of these children, only urine strips in 26.7% of them, while 24% have neither glucose nor urine testing possibilities at home. As much as 29.5% of the children had Micral values $\geq$ 20 mg/l in two urine specimens and 11.2% had at least one microaneurism at fundoscopy, all of them with poor glycaemic control (HbA1c beyond 10%). We believe that diabetes camps are useful as means of medical education .

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"REAL LIFE" EXPERIENCE WITH IDDM DURING SUMMER CAMP. LESSONS TO BE IMPLEMENTED IN IDDM EDUCATION PROGRAMS.

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Our aim was to demonstrate that summer camps (SC) for children and adolescents with diabetes provide significant psychosocial benefits in terms of self-empowerment, improved attitudes towards diabetes, increased sense of responsibility and feelings of adequacy. Summer camps are highly recommended for IDDM youth as they enable camp activity under professional medical supervision. As a result of six years of cumulative experience in organizing and operating sleep-away camps for diabetic youth aged 9-16 years, we present our concept of a summer camp whose primary goals are fun and interaction with other children and counselors with diabetes; where there is no formal diabetes education, but rather education in "real life" using problem-solving methods. In order to evaluate the impact of this type of camp we compared 37 diabetic children attending SC with 21 not attending. An ATT-39 (Diabetes Attitudes and Adjustment Scale), which measures adaptation to illness, degree of positive attitudes and emotional adjustment to diabetes, and degree of alienation from society was administered to each child individually, without parental assistance, during a regular out-patient visit. Our preliminary results, based on chi-square analyses, show that young IDDM subjects who have attended SC show significantly better adaptation to their illness, have more positive attitudes towards diabetes and an increased sense of responsibility toward their condition when compared to those who have never participated in such activity. In conclusion, participation of young people with IDDM in summer camps using problem-solving methods of education is highly recommended and should be officially included as part of any diabetes education course curriculum; moreover, we propose that the medical team's participation in SC should be considered as an integral part of diabetes education work by the health care system.

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DAY CAMPS FOR MASS DIABETES HEALTH EDUCATION AND CARE : SOCIAL EXPERIMENTS IN A DEVELOPING COUNTRY.

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We [Health Care Team: HCT] have designed, standardized and implemented [through local sponsors [LS] collaboration], a voluntary, structured, 8 hours weekend (third Sunday of the month), intensive health education and self care training program for people and families, titled "Diabetes Self Care Intensive" [DSCI]. DSCIs have been provided at different urban and rural parts of the state of Karnataka and neighboring regions, upto a distance of 500 kms from our center (outreach program). A. Design [HCT]: (i) Creating syllabus and course content; (ii) Development of teaching and audiovisual aids [Diabetes teaching guides: English - 100 pages, local languages - 14 pages; 35 mm slides (n=220); modular lectures and group discussions (n=12); video tapes (n=3); health survey and multiple choice questionnaires; display posters (n=20)]; (iii) Model diabetes lunch, recipes and nutrition exhibits; (iv) minimal health screening (spot blood glucose, BP, ideal body weight), and (v) individual counseling. B. Implementation [HCT + LS]: (i) Requests from LS (Lions, Rotary, Medical Associations); (ii) Camp operations manual/ check list (4 pages) mailed out to LS 2 months in advance, followed by meeting and correspondence with LS representatives; (iii) Publicity, fund raising, printing, catering and other facilities organized by LS; (iv) HCT volunteers (doctors, nurse educators, dietitians, social workers) travel to and conduct the day camp at the outreach site; (v) Follow-up referral to specialized centers, as per need and stimulation of local programs. C.Results: (i) Camps conducted (1994-96; n=13); (ii) IDDM + NIDDM, 50-850 patients per camp; (iii) Education and training standards tailored to recipients capabilities and needs (literacy economics, motivation). Conclusion: A volunteer day camp movement has been successfully implemented for outreach community diabetes care, in a developing country with high disease burden.

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INTERNATIONAL CAMPS FOR JUVENILES WITH DIABETES 1987-1995

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Our aim was to organize camps for hitherto unprovided-for juvenile diabetics (over 16), in order to provide these generally overprotected individuals with a valuable experience away from home, yet in a safe environment where their independence and self-confidence in the areas of therapy and social contacts would be able to grow. We started 10 years ago with a large educational, medical and dietetic team, thus guaranteeing optimal care. A total of 186 juveniles came from 22 countries and three continents and used English as the language of communication. Sport, arts and crafts, the culture, history and sights of the host country and an exchange of experiences relating to treatment methods from the countries represented comprised the programme. The medical support and mutual supply of information led to a successful learning process for all, notable improvements in therapy owing to the relaxed atmosphere, motivation for improved diabetes management, promotion of international dialogue, improved English and a lessening of cultural prejudice. The success of the camps should be a motivating factor for other European countries to do likewise. The setting-up of self-help groups in both, Third World and Eastern European countries, could be stimulated by inviting juveniles and occasionally physicians from them to participate free of charge.

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LIFESTYLE INTERVENTION IN PEOPLE WITH INSULIN-DEPENDENT DIABETES MELLITUS (IDDM)

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Sixty one subjects participated in a randomised trial to determine whether a lifestyle intervention programme could further improve glycaemic control and lipoprotein mediated risk of cardiovascular disease in patients with insulin-dependent diabetes, amongst whom home blood glucose testing and insulin regimes had already been optimised. Subjects were randomised to a programme which involved intensive dietary advice and a programme of physical activity (Group 1) or routine surveillance (Group 2). After the 6 month randomised trial, Group 1 were followed for a further 6 months, with no further advice and Group 2 were given intensive advice similar to that received by Group 1 during the formal trial. Nutrient intakes, maximal oxygen consumption, weight, blood pressure, glycated haemoglobin, lipids and lipoproteins were assessed throughout the trial and follow-up period. During the 6 months trial, in Group 1 saturated fatty acids decreased significantly from a mean of 16% to 12% total energy ($p<0.001$) and carbohydrate plus monounsaturated fat increased from 55% to 60%. Protein intake remained relatively high at 17% total energy. These changes were sustained during the 6 month follow-up period. Some changes occurred in Group 2 during the trial but saturated fat only decreased to 12% during the 6 month follow-up period when they received intensive dietary advice. LDL cholesterol showed a small (0.2 mmol/l) statistically significant ($p<0.001$) fall between recruitment and randomisation. The improvement was sustained in Group 1, but not in Group 2 until they were given intensive advice in the post trial phase. Changes in HbA1 mirrored those in LDL cholesterol, average improvements of 0.6% ($p<0.01$) being observed with intensive advice. The lifestyle intervention programme resulted in modest changes in diet and exercise habits, sufficient to produce some improvement in glycaemic control and lipoprotein mediated risk of coronary heart disease over and above the benefits induced by optimising insulin regimes and home blood glucose monitoring. However despite intensive education nutritional targets were not met. More innovative approaches will be necessary to achieve the full benefit of lifestyle treatments.

2475

REPORT ABOUT PSYCHOLOGICAL AND SOCIAL APPROACHES BY PATIENTS USING CONTINUOUS SUBCUTANEOUS INSULIN INFUSION (CSII)

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CSII is since 10 years a safe and well known insulin delivery therapy,but still very seldom.The question is :Do the diabetics or the diabetologist fight against this intensifed insulin therapy? We asked 279 diabetics (142 female,137 male) by self-assessment questions. **Basic data:** age 34,9 years, diabetes duration 15,3 years, CSII treatment time 3,4 years. **Psychological fields:** *1.part:* pump user expections before CSII versus theire experiences with insulin pump. *2.part:* Does the self-esteem change under CSII?*3. part:* Do the users find a pronounced difference in quality of metabolic control,different egos and possibilities in active handling of this chronical disease? **Results:** most of the pumpusers are employes (55% male, 71% female) between 20 and 40 years (85% male, 81 female) with a diabetes duration between10 - 20 years (41% male, 27% female).For the decision to switch from discontinuous insulin delivery to pump therapy 46% need in middle 5,1 weeks.1. part: Flexibility: 6,8%expectation – 53% achieved, indepance from watch: 74,2% expectation – 57,7% achieved 2. part: 77,4% feel more independent,76% more liberal,68,1% more sense of responsibility, 42,7% more independent,36,6% more selfconfidens.3. part: better blood glucose 84,2% expectation – 52,0% achieved.Assessment of blood glucose quality: much better (24,9%), better (64%) same (10,3%), worse (0,8%)very bad (0%); improvement of HbA1/1c:: 84,2% expectation – 53,8% achieved.Assessment of HbA1/1c: much better (22%), better (60,2%) same 15%), worse (2,8%) very bad (0%).Body feeling:much better (15,4%), better (47,6%) same 34,6%), worse (1,6%)very bad (0,8%);Psych. feeling: much better (12,3%), better (34,3%) same (47,9%), worse (4,7%)very bad (0,8%).Other details will be demonstrated. **Summary:** The pump therapy is well accepted because it is a great advantage for the users.Body-psychological- and metabolic effects are better under CSII,if patients get the most possible professional support by diabetologists.

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ARE DOCTORS AND DIABETES PATIENTS READY TO COOPERATE?

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Cooperation and partnership between diabetes patient and doctor is one of the preconditions for the successful treatment of diabetes. Successful cooperation between diabetes patient and doctor is hindered by insufficient knowledge of diabetes patients about their ilness and limited possibilities of selfcontrol as well as their turn of a mind -- conviction that only doctor is responsible for their health. Doctors are not ready for the partnership with their patients either both for subjective (psychological) and objective reasons: there is insufficient number of endocrinologists in Latvia which results in their overload, they are insufficiently trained to educate diabetes patients; internists and family doctors are completely unprepared to solve problems connected with treatment of diabetes. Doctors view patients only as a material of their work and not as partners. Latvian Diabetes Association (LDA) turns a great deal of attention to improvement of partnership between diabetes patients and doctors by improving the patients' knovledge of their ilness, trying to raise their self confidence, informing doctors endocrinologists and other medicine professionals about different aspects of diabetes. To improve the cooperation between doctors and diabetes patients, LDA recommends: to create well structured, understandable for different target groups of patients educational program; to advance the knovledge of doctors about diabetes; to reach an understanding that selfcontrol equippment should be paid by state; to advance the psychological preparedness of doctors in creating a partnership relations with patients; to advance the creation and adoption of programs about the diabetes patients' education for doctors endocrinologists, internists and family doctors.

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PANIC DISORDER AND DEPRESSION IN INSULIN DEPENDENT DIABETES MELLITUS

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Panic disorder with concomitant depression becoms to be frequent in patients with insulin dependent diabetes mellitus. 25% with panic disorder and 41% depresed was found in the study of 44 patients with diabetes mellitus acording to th DSM IV clasification of mental disordes. Most frequent symptoms were shortness of breath (dyspnea), trembling or shaking, sweating, fear of doing something uncontrolled, early awaiking, loss of interest or pleasure in activities, excessive guilt, fatigue or loss of energy, decreased appetite. 81% had common panic disorder and depression, so it was necessery to make good psychological assesment and consequent psychotherapy and psychopharmacological treatment. The prognosis of insulin dependent diabetic patients with panic disorder and depression is unsatisfied becouse these patients could not rich good glycemic control and complications are very frequent (especially polyneuropathy and angiopathy). The complications were found in 96% in this group.

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PSYCHOSOCIAL IMPACT OF TYPE 1 DIABETES ONSET IN A COHORT OF DIABETIC PATIENTS.

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The psychopatological impact of diabetes onset was analyzed in a group of type 1 diabetic patients (IDDM). Forty five IDDM patients were studied in the first month of their diabetes onset The mean age was 26 ± 6 years, 66,6 % were men and 33.3 % women. Social and psychological impact of the diabetes were evaluated by three test: V.S (social events), Beck-Pichot and H.G.Q. Golberg.

Results: It was observed in V.S. study that 84 % of patients considered satisfactory their social situation without any adverse social events, 73.3 % had good adaptation to the new situation of diabetes onset, 93.2 % and 90 % manifested a great family and social support. In the Beck-Pichot test, it was demonstrated anxiety crisis in 9 % of patients and moderate anxiety in 11 %, (Normal range scale 0 to 9), serious and moderate depression symptoms was manifestated in 7 % and 13 % of patients (Normal range score: 0 - 11). In Golberg test, general psychopathological manifestations was observed in 47 % of all patient group (Normal modified scale 0 - 14). A very good correlation was observed between Golberg (general psychopathological manifestations) and Beck tests (anxiety and depression) ($r=0.78$, $p < 0.001$ and $r=0.82$, $p < 0.001$, respectively) and no correlation with V.S (social events of life).

In conclusion, the Beck-Pichot and Golberg test demonstrated an important psychopathological manifestations in patients at the onset of the type 1 diabetes although they did not detected any problems in their social events.

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DO DIABETICS NEED PSYCHIATRIC EVALUATION FOR THE GOOD QUALITY OF LIFE?

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The aim of our study is to evaluate the pysiciatric status in patients with well(HbA1C<%6,fasting blood glucose<%120) and poorly(HbA1C>%8,fasting blood glucose>%140) controlled diabetes.150 patients(70 female,80 male,mean age 45.3±13.6) are evaluated psychiatrically by using SCID-OP(Structered Clinical Interview for DSM III-R-outpatient).Depression degree was calculated by using Hamilton depression scala. The parameters that we observed also included age,sex,marital,social,economic status,number of stillbirths,degree,duration type and therapy of diabetes, associating psychiatric and organic disorders caused by diabetes and the presence of diabetes and psyciatric disorder in 1st degree relations.

The frequency of psychiatric disorder in the past was %21.3, depression at present was %30.7,depression and anxiety at present was %15.3, psychotic symptoms at present was %2 respectively.Among the patients with anxiety 5 had panic attacs with agorophobia, 28 without agorophia, 10 of them had obsessive compulsive disorder,15 had generalized anxiety disorder , 5 had social phobia and 4 had simple phobia.When correlated to each other, significant correlations have been found between the group with depression and degree of diabetes($p<0.05$) and proffesion distribution($p<0.05$); between the group having depression and anxiety with treatment of diabetes($p<0.05$) and between the group of anxiety disorders with sex($p<0.05$.

So, we emphasize that in order to manage diabetics appropriately pysciatric analysis should be kept in mind for the good control of diabetes.

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Gender Equity in Swedish Diabetes Care: the Patient Perspective.

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As perception is the step that psychologically precedes response and action, the patients´ subjective experience of diabetes mellitus, and the care they receive, is an issue of great importance.
A number of studies in Sweden indicate gender differences in sickness perception, sickness impact, experienced quality of life and satisfaction with care among diabetic patients. A population-based study in northern Sweden (Gåfvels et al) showed that diabetic women worried most about symptoms and future complications, while men were more troubled by the regulated life-style imposed by the disease. Depression and anxiety, in combination with a low general well-being and a high subjective sickness impact, are found to be more common in diabetic women than in men (Wredling et al). Gender differences in perceived health, such as that women are less satisfied with their health status and physical capacity, are also found (Jonsson et al). Regarding experience of diabetes health care, more women than men reported dissatisfaction with the way they were met and the accessibility and service they were offered (Jonsson et al). Women in hospital care were less satisfied with their doctor-patient relationship and reported a greater dissatisfaction with the treatment and the time for discharge than did men (Östlin et al).
We suggest that, because of a different sickness perception and subjective sickness impact, due to gender-based or biological differences, men and women have different needs and expectations of diabetes care. The model of practising diabetes health care today may suit men better than women.

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DIABETES EMPOWERMENT SCALE (DES): A MEASURE OF PSYCHOSOCIAL SELF-EFFICACY

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The Diabetes Empowerment Scale (DES) is a 34 item questionnaire developed to measure the psychosocial impact of a patient empowerment program. The empowerment program is a six-session (2 hours each) group education program focusing on psychosocial issues related to living with Diabetes Mellitus (DM). The DES has eight subscales: assessing satisfaction, setting goals, solving problems, emotional coping, managing stress, obtaining support, motivating oneself, and making decisions. The overall reliability of the DES is .94. Subscale reliabilities (Chronbach's coefficient α) ranged between .57 and .85. To provide evidence of validity the pre/post empowerment program change score for patients on the DES was compared with their change scores on independent measures of positive and negative attitude toward having diabetes (Correlations of .65 and -.51 respectively, p <.001), and glycosylated hemoglobin levels (Correlation =.31, p = .03). Also, baseline DES scores were correlated with the patients' self-reported comfort asking questions of their doctor (.29, p =.02), and self reported positive adjustment to DM (.50, p <.001). Test/retest reliability was determined by comparing the scores of the control group on the DES from baseline and at six weeks. The test/retest correlation was .79 (p <.001). These findings provide evidence that the DES is a valid and reliable measure of diabetes-related psychosocial self-efficacy. The DES is suitable for clinical assessment and program evaluation.

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PSYCHOEMOTIONAL STRESS AND TYPE 1 DIABETES : THE POINT OF VIEW OF PATIENTS

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Chronic patients relate frequently their health problems to stressful events. In this study - using a questionary - we compare the point of view of type 1 diabetic patients, (50 patients, 23 male 27 female, ranging in age from 15 to 25 years mean : 22,5 years; duration of diabetes between 1 and 10 years mean duration : 4,6 years) and the point of view of a control group (50 patients with acute chirurgical illness, 28 male, 22 female, same range of age) about : 1 - psychoemotional stress in every day life. 2- stressful events and onset of disease. We also ask diabetics about : 3 - relation between stress and metabolic control. The results show 1- number of psychoemotional stressful events in diabetic patients was higher than in control group (44 versus 15); different types of stressful events were reported: death in the family, problems with divorce or separation, legal problems, difficulties with family members, school and job problems. 2- 70% diabetics patients and only 26% control patients said they have lived stressful events before illness onset ($p<10^{-4}$). 3- 25 diabetic patients said that stress impairs metabolic control producing hyperglycemia alone (20 patients) or hyperglycemia and ketonuria (5 patients). These data suggest the importance of educating type 1 diabetic patients about relation between stress and their disease.

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MULTIDISCIPLINARY BOARDING IN EDUCATION OF THE DIABETIC CHILDREN AND ADOLESCENT , PSYCHOLOGICAL ASPECT

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It describes a modality of interdisciplinary attention that emphasized the psychoterapeutic boarding to identify the psychological aspects that interfere in the education process and in the treatment. It was worked with 150 diabetic children and their families Their ages are of 6 to 15 years from june 1994 of until july 1995 It was realized a psychological evaluation that was formed of individual and familiar interview,proyectives, graphic and verbal tests. Of the material to extract graphic statistical as holding to the theorist postulates. The results to which arrive:the psychological characteristics were in a 100 % depressive elements of the personality and dependence- passivity ; in a 80 % impulses auto-heteroagresives and negation of the sickness and in minor percentage 60 % difficulty in the acceptance of the limits and to be integrated to the group of even. As soon as the parents the 74 % label to the sons as the" poor thing" and a 20 % as alone" to be diabetic", the remainder see to the sons in normal manner. Fit into to spotlight that to measure that the treatment advances, these psychological characteristics have improved in a 80 %. This permits us to conclude that the interdisciplinary boarding permits advances in the boy and the family.

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THE THIRD VERSION OF THE DIABETES ATTITUDE SCALE (DAS -3)

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The purpose of this study was to improve the psychometric properties and clinical relevance of the Diabetes Attitude Scale (DAS). The DAS is a general attitude scale that can be used to compare diabetes-related attitudes of nurses (RN's), physicians (MD's), dietitians (RD's), and patients. Content validity was assured by having the new items for the DAS-3 generated by a panel of 22 diabetes experts (including physicians, nurses, dietitians, social workers, and patients) at the University of Michigan. A total of 210 new items were generated. A modified Delphi process was used to reduce the list to 17 new items. The 17 new items and 18 clinically relevant and psychometrically sound items from the previous DAS were combined to create the DAS-3 with 35 items. The DAS-3 was then completed by 531 RN's, 567 RD's, 320 MD's, and 378 patients with diabetes (Total n=1796). The revised subscales (along with their Chronbach α's) are: need for special training to treat Diabetes Mellitus (DM) .67; seriousness of Type II DM .80; value of tight glucose control .72; psychosocial impact of DM .65; and patient autonomy .76. These subscale reliabilities are significantly (p<.0001) better than the two earlier versions of the DAS. The validity of the DAS-3 is further supported by the finding that specialists in treating diabetes had significantly (p < .0001) more positive (as defined by the expert panel) attitudes than nonspecialists on all five DAS-3 subscales. The DAS-3 is a valid and reliable general measure of diabetes related attitudes that can be used to compare the attitudes of different groups (e.g. patients, health care professionals) and for program evaluation.

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DIABETES & STRESS: THE FORGOTTEN LINK

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As part of our program "Consciousness of Being", patients and relatives (150n) have praise since 1987 the benefit of regular practice of Mental Exercises (muscular relaxation, deep breathing and visualization). To evaluate the objetive /subjective impact of these experiences we did a 20min. - early morning practice, randomly choose 30 non-trained patients and made measurements in glycemia, pulse, systolic/diastolic pressure and changes in physical and emotional complaints. The group A, with hypertension(61yrs.age, 10yrs.DM evolution) and oral treatment had a glycemia derease 22mg/dl, pulse decrease 06rpm,a systolic increase15 mmHg, no change in diastolic pressure. The group B,with out hypertension(54yrs., 08yrsDM) and oral treatment had glycemia decrease24mg; pulse, systolic& diastolic with no change.The groupC,without hypertension(22yrs,05yrs DM) and insulin treatment had glycemia decrease 69mg, pulse decrease 02rpm, systolic decrease12mm, diastolic decrease 19mm.Of all groups, 20% had physical complaint (75%improved)and 60% had emotional moan(85% improved). This short-term result expose the usefulness of Mental Exercises to get a well being state, condition that seem related with - moderate glycemia decrease. We do recommend Mental Exercise, as daily therapeutic tool in the diabetic treatment.

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ASSESSMENT OF DIABETES-RELATED DISTRESS IN IDDM vs.NIDDM INSULIN TREATED PATIENTS

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The study intended to identify the levels of diabetes and insulin treatment-related distress. **Patients**: 86, IDDM-66,NIDDM insulin treated-20, mean age-37.06 ± 18.23years, duration of DM-7.74 ± 6.03 years, number of injections/day-2.5 ± 0.98, with the same education. **Method.** Psychometric instruments have been used: PAID-test (Problem Areas In Diabetes Survey-H.Polonsky) and a structured interview assessing insulin treatment-related emotional distress. **Results.** The general PAID-score, was 50.44 ± 21.12, higher in NIDDM than IDDM patients (57.1 ± 13.27 vs 48.42 ± 17.03).As serious problems were reported the diabetic complications (63.6% IDDM vs 70% NIDDM) and the guilty feeling when the diabetes management was not followed (54.51% IDDM vs. 40% NIDDM). The insulin requirement for the well being status, was recognized by 70% of IDDM patients vs. 30% of NIDDM patients; 42.42%IDDM and 40%NIDDM patients are dissatisfied with the meal and insulin regimen.The acceptance of the disease and the treatment, was present in 48.48% IDDM vs. 60% NIDDM patients. Among them, 71.42% IDDM and only 28.5% NIDDM patients were worried about the future. In spite of the worry about complications,41.86% are not satisfied with the diabetes regimen, a possible obstacle for a good care.There is a significant relationship between emotional distress and worry about complications. **Conclusions.** Insulin treated patients, especially NIDDM patients, have a high level of distress, the main problem being the future complications. A small number of NIDDM patients recognised the necessity of insulin; even if the majority of them are concerned about complications; they hardly agree with the meal and insulin regimen.The negative reaction towards insulin therapy is an obstacle for a good metabolic control; it is need to provide education according to psycho-emotional identified problems. The IDDM patients, have a higher acceptance of insulin regimen and meal planning, to avoid diabetic complications. PAID- test could be useful for an efficient education and treatment approach.

2487

GENDER DIFFERENCES IN QUALITY OF LIFE AMONG PERSONS WITH DIABETES MELLITUS IN S INDIA. Madhu K, Veena S and Sridhar GR*. Andhra University and *Endocrine & Diabetes Centre, Visakhapatnam, India

We have studied the quality of life (QOL) using diabetes quality of life measure employed in the DCCT. The scoring was done according to the modified method of Jacobson et al (1994), where raw scores were converted into 100 point scale, with 100 representing the highest possible score (ie best quality of life). The random selection of patients (n:227; 144 men, age 49.22 +/-10.78 years, duration of diabetes 5.4 +/-4.88 years; 83 women; age 49.22+/-10.78 years, duration of diabetes 6.28+/-5.67 years), was done from three Diabetes clinics of Visakhapatnam. The women had a consistently poorer score (ie worse QOL) than men on all aspects in quality of life -- satisfaction core (62.17+/-7.5 in men; 59.14+/-6.84 in women; p<0.01), social and diabetes worry (26.94+/-5.77 in men; 24.17+/-6.31 in women; p<0.01) and total quality of life score (174.15+/-20.19 in men, 166.47+/-15.72 in women; p<0.01). Women as a group felt their health was poorer compared to other women of their age group. In summary, we have shown that women with diabetes scored lower on quality of life score, compared to men with diabetes.

2488

VALIDATION OF THE DIABETES-39 DISEASE SPECIFIC QUALITY OF LIFE INSTRUMENT IN DANISH, FINNISH, NORWEGIAN & SWEDISH

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Measurement of health-related quality of life is increasing in importance for assessing patient perception of therapy and for evaluating interventions and care programmes. The Diabetes-39 has recently been developed in English for use in all people with Type 1 or Type 2 diabetes - whether managed with insulin, oral agents or diet alone. Due to differences in culture, attitude and perception, the properties of any quality of life instrument must be assessed separately for each translation of the instrument. This study evaluated the reliability and validity of new translations of the Diabetes-39 into Danish, Finnish, Norwegian and Swedish. Translation was by a process of two forward and one backwards translations: an accepted methodology. Patients enrolled in a clinical trial of a novel antidiabetic agent were asked to complete the Diabetes-39 at baseline and after three and six months of the study. The SF-36 generic quality of life instrument was also administered. Psychometric evaluation of baseline responses was performed. 467 patients completed evaluable questionnaires at baseline: 68 in Denmark, 180 in Finland, 118 in Norway and 101 in Sweden. The translations of the Diabetes-39 were found to be reliable and valid and their psychometric performance was consistent with the original, US English, version. Internal reliability of the instrument's five scales (Diabetes Control, Anxiety and Worry, Social Burden, Sexual Functioning, Energy and Mobility) was supported by Cronbach's alpha of >0.7 for all scales in all countries (range 0.78-0.93). The results of other psychometric tests confirmed the validity of the translations: definite scaling error occurred for less than 1% of assessments; construct validity, assessed by intercorrelating scales of the Diabetes-39 and correlation with the scales of the SF-36, was consistent with *a priori* expectations. The Diabetes-39 is now validated for use in patients in these countries.

2489

BULIMIA DIABETICA

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Main aim: To help diabetes care teams prevent, identify, and handle bulimia among patients with IDDM, and to develop psychotherapy methods. **Background:** In one psychologist's practice, 40% of clients suffered from eating disorders, responding well to psychosocial support and information; those with IDDM and bulimia did not: some requested psychotherapy. Bulimia prevalence in IDDM was 6% in one report, agreeing with the author's experience of people with IDDM, but higher than commonly expected. In individual psychotherapy sessions, clients with IDDM described their compulsive eating and purging as extremely shameful. They hid these habits from doctors and nurses, often even from family members. **Methods:** Twenty group meetings were held in Swedish cities, with a total of 300 participants (persons with IDDM and members of diabetes care teams). ***Bulimia diabetica*** was suggested as a descriptive term for the syndrome under discussion. **Results:** During the meetings, persons with IDDM described how they, from the time of onset of the disease, had been taught to weigh all their food (and themselves), initially achieving near-normoglycaemia; in later years, their FBG concentrations were low, levels of glycated haemoglobin high. Strenuous efforts were made to keep body weight low through physical exercise and hyperglycaemia with glucosuria. Since eating caused anxiety, they withheld insulin. Some kept to a strict regimen of training, vomiting, and expectorating. **Conclusion:** Diet teaching may be a risk factor for eating disorders. Bulimia is frequent in IDDM, though often hidden; thus prevalence can be underestimated.

2490

PSYCHOLOGICAL IMPACT OF ISLET CELL ANTIBODY SCREENING.

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The purpose of this priliminary study was to evaluate the psychological impact of Islet Cell Antibody (ICA) screening and its results on at risk individuals and their family members. ICA+ individuals were identified through a large scale screening program conducted at our institute. The sample consisted of 9 families in whom 10 youngsters with ICA+ were identified. The 7 boys and 3 girls ranged in age from 6 to 18 years (mean age 11.8 median age 10 years). Both parents participated in the study. Eight diabetic youngsters (mean age 15.2 median age 16) as well as 9 healthy sibling were also included in the study. (One family had 2 diabetic children and in two families one of the parents was diabetic). Reactions to positive results of ICA were assessed by a structured questionnaire and with the Impact of Events Scale (IES). The IES was answered twice : within a week from the disclosure of the ICA+status and 3 months later. Although mothers' reactions on the IES were higher than that of the fathers ,no significant difference was found between parents.

Impact of events scores at diagnosis and three months later

	Intrusion		p	Avoidance		p
parents	14.6 ± 6.2	7.1 ±3.5	.004	16.6 ± 8.2	11.3 ±5.5	.04
diabetic	4.3 ± 2.1	2.0 ±1.0		3.6 ± 2.2	3.6 ±1.9	
ICA+	7.5 ± 1.7	3.5 ±1.4		2.0 ± 1.3	1.0 ±0.7	

The results suggest that learning one's ICA+ status induces significant anxiety especially in parents of ICA+ youngsters. Although this initial anxiety is diminished over time, it still remains quite high after 3 months. Although preliminary, these results highlight the importance of maintaining sensitivity to the impact of ICA screening upon the entire family.

2491

COMMITMENT TO SELF-CARE AND METABOLIC CONTROL AMONG IDDM PATIENTS

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The aim of the study was to assess the relationship between commitment to self-care and metabolic control among adult patients with diabetes in Finland. The data were collected from 213 patients (age ≥ 17 year) using questionnaires. The response rate was 75 %. Most of the respondents (63 %) were on flexible insulin therapy (>3 injections per day or an insulin pump). According to the results, the patients had no problems with their insulin treatment, though they had some problems observing the diet. A fifth of them (21 %) ate daily whatever they wanted without thinking of the nutrient content of the food. Many (82 %) mentioned some difficulties with evaluation of the food consistency. 39 % did not monitor their blood glucose regularly, and a tenth of the patients did no monitoring at all. In spite of this, SMBG was not perceived as being problematic. Altogether, 48 % of the patients did not mention any difficulties in their daily care, but reported more problems related to special situations, such as attending parties. The mean of glycosylated hemoglobin as a measure of metabolic control was 8.1 % (sd 1.94). The patients who had had diabetes for a longer time had poorer GHbA1c ($r=.23$, $p<0.001$) than the others. Those with good metabolic control (under 8.0 %) reported better observance of diet regimens than those with poor metabolic control ($p<0.001$). There was no statistically significant relationship between metabolic control and SMBG, exercise, or insulin treatment. Nevertheless, although if the patients evaluated themselves to be active and responsible in the care no response was seen in metabolic control in this population. In conclusion, the results suggest that patients with diabetes can have better metabolic control with commitment to the observance of diet regimens than to the other aspects of diabetes care, although there are many other variables relating to metabolic control as well.

2492

PROSPECTIVE STUDY ON QUALITY OF LIFE IN IDDM PATIENTS AFTER PANCREATIC-RENAL GRAFTING

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To investigate the effects of successful combined pancreas and kidney transplantation on quality of life (QOL) a prospective study was performed. Therefore a multi-dimensional questionnaire was mailed twice in 1989 and 1995 to all patients in the transplantation program in a single transplantation center. In 41 IDDM patients data of both evaluations were available: **Group A** (n=12): patients on the waiting list for transplantation in 1989, meanwhile successfully pancreas and kidney grafted: age 40,7±9,6 yrs, duration of diabetes 28.3±4,7 yrs, duration of dialysis 46,7±20,2 m, time posttransplant 3,8±1,7 yrs. **Group B** (n=15): patients after successful simultaneous pancreas and kidney transplantation at both evaluations (age 44,1±9,6 yrs; duration of diabetes 29.3±5,1 yrs, duration of dialysis 31,2±22,1 m, time since transplantation 8,7±1,8 yrs. **Group C** (n=14): posttransplant patients with functioning kidney graft and under insulin therapy at both evaluations: age 45,2±7,2 yrs, duration of diabetes 34.1±10,1 yrs, duration of dialysis 32,5±37,2 m, duration of renal graft functioning 9,3±2,2 yrs. Questions for satisfaction with different aspects of QOL were answered using a rating system from 1 (very dissatisfied) to 5 (very satisfied). Means ± SD were calculated for each item separate for each group and both evaluations respectively. For testing significance of intraindividual changes the Wilcoxon signed ranks test for paired samples was performed. In **Group A** a marked improvement of QOL scores was observed in 7 of 9 different aspects of QOL (f.e. physical capacity: 1995 3.1±1,4 vs.1989: 2.1± 0,8 ;partner relationship:1995: 4.3±0,9 vs. 1989: 3.9± 1,5 ; sexual activities: 1995: 2.8±1,5 vs. 1989: 1.9±1,1) , in two items values remained stable. However with respect to overall QOL a significant improvement was observed (1995: 3.8±0,6 vs. 1989: 2.5±0,9 ; $p<0.05$). **Group B** also showed an improvement in 5 aspects of QOL plus in overall QOL (1995: 4.3±0,7 vs. 1989: 3.8±0,9), but this was insignificant. In 4 aspects (mental capacity, family life, number of friends, leisure time activities) the satisfaction deteriorated. Patients in **Group C** remained exactly or nearly stable in all aspects of QOL (f.e. overall QOL 1995: 3.7±0,6 vs. 1989: 3.9±0,8). These data indicate an additional positive influence of pancreatic grafting on QOL when compared to single kidney transplantation in diabetics.

2493

EQUIVALENCE OF THE PAPER-AND-PENCIL AND THE COMPUTERISED ADMINISTRATION OF THE WB-Q AND THE DTSQ.

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To monitor well-being and satisfaction with treatment in patients with diabetes, the St Vincent Declaration recommended the use of two instruments, the Well-being Questionnaire (WB-Q) and the Diabetes Treatment Satisfaction Questionnaire (DTSQ). It is argued that clinical practice benefits from computer-assisted administration of both questionnaires. Aim of the current study was to investigate whether the paper-and-pencil assessment and the computerised assessment of the WB-Q and the DTSQ can be considered equivalent. One hundred and five patients with diabetes, treated at the outpatient clinic of the Academic Hospital of the Vrije Universiteit (Amsterdam), were invited to take part in the study. A randomised crossover design was employed. Seventy six patients completed both a personal computer and a paper-and-pencil version of the WB-Q and the DTSQ in a randomised order, with a mean interval of 7 days. Mean age was 48.1 years (sd 17.9), 61% was female. Data suggest that the traditional paper-and-pencil questionnaire and the computerised version of the WB-Q and the DTSQ can be considered equivalent according to criteria formulated by the American Psychological Association. The scales showed high test-retest correlations and the means, dispersions, kurtosis and skewness were found to be approximately the same in both versions. In both ways of assessment, the WB-Q Depression scale and the WB-Q Energy scale proved to be sensitive for carry-over effects, resulting in better well-being scores at the second measurement (one week later). Although even 58% of the subjects reported that they had rarely or never used a personal computer, 99% of the subjects found the questionnaires (very) easy to complete on the personal computer. It is concluded that norms and cutting scores obtained from paper-and-pencil assessments can be used in computerised versions of the WB-Q and the DTSQ.

2494

IMPEDIMENTS TO DIABETES CARE IN PRIVATE MEDICAL PRACTICE IN A DEVELOPING COUNTRY.

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Background: Medical care is not state funded for most citizens in India. Most diabetics are followed with private medical practitioners (family physicians, specialists).
Aim: In order to plan a diabetes education module for patients and to explore for issues not addressed in KAP surveys. ***Study subjects:*** 20 private medical practitioners at their clinics in different areas of Delhi, and different educational levels and 20 diabetics followed up in private medical practice. ***Design:*** a cross-sectional, multiple, free flowing, friendly, guided interviews (mean: 3 hours per interview). ***Results:*** **Doctors believe** that the impediments to effective diabetes care are: 1. patients perception that diabetes is curable (20/20); diabetic tablets and insulin are addictive to the body (20/20); should be avoided as far as possible (19/20); faith in alternative system medicine (20/20); patients getting lost after first few consultations; patients reluctance to spend $6 for fundus examination. AND 2. problems at practice: inadequate continued medical education including lack of awareness of basic skills of insulin injection technique; unawareness regarding adverse effects of oral hypoglycaemic agents (4/20); unaware of retinal and feet check-ups (4/20); prescription of overdose and multiple sulphonureas; prescription of alternative system of medicine (4/20) [with a belief in its effectiveness (2/20), or sheer fear of loss of patients (2/20); inadequate time ("can't spend more time with a fees $0.2-$0.5 per patient"). **Patients believe** that there is no relation of DM to obesity (16/20); or CHD (19/20); diabetes can disappear on its own (19/20); diabetes can be cured if properly treated (20/20); people who do not eat sugar need not test for diabetes and its control (19/20); one cannot have high BP if one is feeling fine (6/20); alternative system medicine are effective (17/20) [should not be taken with oral hypoglycaemic agents (8/20)/ can be taken (9/20)]; unaware of incurable blindness (retinopathy) (18/20) associating poor DM control with spectacles ; take an extra oral drugs when one eats more than usual (6/20).
Conclusions: an understanding of the patients' and their health care providers' constraints and beliefs have been uncovered.

2495

DIABETES RELATED DEPRESSION IN YOUNG INSULIN DEPENDENT DIABETES MELLITUS (IDDM) PROBANDS

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The study was aimed at comparing factors of depression in children with IDDM, their normal siblings and normal controls. The IDDM samples consisted of 74 boys and 76 girls, their normal siblings 21 boys and 23 girls,the normals controls 51 boys and 58 girls. The normal controls were drawn from neighbouring schools. All subjects were in the age group 7 - 18 years. All groups were matched for relevant demographic variables. Kovac's children depression inventry was used to measure depression scores. The data were processed using 3*2 factorial analysis of variance followed by one-way analysis of variance and t tests. None had scores diagnostic of depression. But within normal range of scores on depression factors the IDDM probands were significantly less depressed, lower in negativemood, ineffectiveness, anhedonia, and negative self-esteem than the normal controls (P=0.001). Results indicated that the IDDM children had developed good coping devices and made psychologically healthy adjustments. It is to be noted that IDDM probands and their parents had the advantage of diabetes education programme and counselling.

2496

TREATMENT SATISFACTION IN 657 TYPE 1 DIABETIC PATIENTS.

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Assessment of Treatment Satisfaction is an integral part of the evaluation of new treatment strategies. However, there are no validated measures taking account of individual treatment goals in order to help tailor treatment strategies to individual patient needs.

In a population-based study, a treatment satisfaction scale (TSS) was validated in 657 adult IDDM patients (41% women, age 36 ± 11 years, diabetes duration 18 ± 11 years, HbA_{1c} 8.0 ± 1.5%; mean ± SD). The TTS comprises 20 items: patients rate the desirability of 10 different treatment goals (e.g. stability of blood glucose, diet flexibility, avoidance of mild or severe hypoglycaemia) on a 6-point Likert scale and, accordingly, the degree of satisfaction with the achievement in these treatment goals. The product of both ratings provides a preference-weighted treatment satisfaction score. TSS score was moderately associated with HbA_{1c} ($r = -.22$; $p < .001$) and quality of life ($r = .42$; $p < .001$). Patients with more than two episodes of mild hypoglycaemia / week ($p < .001$), those with HbA1c values above 9% ($p < .001$), patients with late complications ($p < .05$) and patients living alone ($p < .01$) perceived less treatment satisfaction. Kind of insulin therapy was not associated with treatment satisfaction. ANOVA revealed a two-way interaction between treatment goals and insulin therapy on treatment satisfaction ($p < .01$). Patients with unrealistic treatment goals (aiming at good and stable blood glucose levels but at the same time intending to avoid frequent self-monitoring, to avoid even mild hypoglycaemia and to conceal their disease from other people) achieved the lowest scores under intensified insulin therapy. Patients with realistic goals (categorisation by cluster analysis) achieved the highest scores when intensively treated. Unrealistic treatment goals were more frequent in patients with a low social status and patients with conventional insulin therapy ($p < .001$).

The TSS is a valid instrument to assess preference-weighted treatment satisfaction. High scores do not necessarily indicate a good treatment. Assessment of individual treatment goals may be helpful to identify motivational deficits and to tailor individual treatment strategies.

2497

SMBG measurements in unstable diabetics do not correlate well with their HbA1c

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Measuring SMBG and HbA1c are useful methods to estimate long-term glycemic control in diabetic patients. However, the correlation of the SMBG and HbA1c measurements in unstable diabetic patients is not clear. Therefore, we compared the theoretically predicted HbA1c values from SMBG measurements and the measured HbA1c values in unstable diabetics. Subjects were 5 IDDM patients. They made SMBG measurements 3 times or more every day, however their blood glucose measurements fluctuated very much. The calculated mean of the SMBG measurements of a day was assumed to be the mean blood glucose of the day. HbA1c had been indicated theoretically to be proportional to the weighed mean of the blood glucose for 120 days. The weighed mean of the blood glucose was calculated using the calculated daily mean blood glucose for 120 days. The relationship of the measured HbA1c values and the calculated weighed means were then analyzed. There were no correlation between the measured HbA1c and the calculated weighed mean of blood glucose in all subjects. Therefore, we concluded that the calculated mean of blood glucose of a day from SMBG measurements was not a correct measure of the real mean of blood glucose of the day in unstable diabetics.

2498

PATIENT AND DOCTOR'S PERCEPTION OF DIABETIC CONTROL

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We aimed to study patients' perception of their diabetic control. 133 patients agreed to participate in the study. Patients perceived a mean level of diabetic control on a scale of 0 (poor) to 7 (well controlled) was 4.7±1.6 (mean±SD) when HbA1c was 8.0±1.5. Patients who perceived their health as poor had higher HbA1c than those who perceived their health as excellent (9.2± 2.1 v 7.7± 1.5 %, $p<0.01$). Patients who perceived their control as good also perceived better general health ($p< 0.001$). Perceived diabetic control was negatively correlated with HbA1c at the beginning and end of the study ($r = -0.39$ and $r =-0.41$ $p<0.0001$). Patients describing themselves as well controlled were better controlled. They were also more satisfied with their consultation. Doctors perceived consultations with these patients as more satisfactory and thought the patients would be more likely to comply. Patients complying with advice on weight perceived their general health and diabetic control to be better after three months. However patients perception of compliance did not correlate with improvement in HbA1c. In conclusion patients are good at perceiving their own control and this correlates with wellbeing. Doctors perception of compliance and actual compliance may be at variance.

2499

QUALITY OF LIFE OF THE PATIENTS WITH DIABETES MELLITUS AND ITS INFLENCE FACTORS

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The aim of this paper was to investigate the influence factors of the quality of life.

Material and Methods: There were 143 patients with diabetes mellitus, based on WHO diaghosis standard. Investigation table included 156 questions (3 aspects) in total. The content of quality of life cocsisted of 14 items, covering physiological, psychological, social function and Living conditions.

Results and Discusstion: The results showed that mang abnormal changes of quality of life were found in the patients with diabetes mullitus . Among them the decrease of memory and lack of attention concentration were the most obvious . And less important changes were abnormal emotional reactions, reducing of entertainment activities, social adaptation and work ability . The age, degree of aducation, condition of the illness, complications, social and family background have effects on the quality of life.

2500

GLYCOSILATED HAEMOGLOBIN CORRELATES WITH COGNITIVE FUNCTION IN PATIENTS WITH INSULIN DEPENDENT DIABETES MELLITUS

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This study investigates the correlation between the glyco-metabolic control of Diabetes and Cognitive Function (CF) in patients with Insulin Dependent Diabetes Mellitus (IDDM) younger than 50 years and without any primary and secondary illness. IDDM patients were between 20 and 50: schooling ≥5 years; onset of disease before the age of 35: no concurrent diseases that could justify cognitive deficits as hypoglycemia before and after test, alcohol abuse, uremic and hepatic encephalophaty, thyroid disfunction. In all the patients a brain computerized tomography (CT) and E.E.G. were negative. CF were analyzed using a battery of neuropsychological tests for the following intellectual functions: memory, attention, visuospatial analysis, language, praxia, perceptual recognition, "frontal function", visuomotor coordination (Raven's CPM 47, WAIS digit span, Corsi cubes, prose memory, Rey's Figure A, WAIS digit symbol, Wisconsis card-sorting, Buschke-Fuld verbal learning, Benton Judgement of Line Orientation, Grooved Pegboard test A and B, Stroop test, phonological fluency, sematic fluency). Performance was compared with the normative data from control subjects equivalent in age and education. The patients with highest HBA1C had the highest compromission of CF. This result introduces a new and not very analyzed problem in the communication amongst the doctor, his staff and the patients: 1) The patient may have difficulty in learning what the doctor and his staff teach. 2) the doctor and the staff may be frustrated and distort the education of the patient Since education is generally considered the most important form of therapy, its alteration represents a serious damage for these young patients.

2501

TYPE 2 DIABETES AND ITS INFLUENCE ON THE PATIENTS' PSYCHOLOGICAL WELL-BEING.

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The aim of the study was to evaluate the influence of type 2 diabetes on the quality of patients' life from the psychological point of view. 100 type 2 diabetics (average age 59.6 yrs, average duration of diabetes 11.5 yrs, 50 on oral therapy and 50 on insulin), attending our out-patient diabetes clinic, participated in the study. The patients who were divided into groups regarding age, sex, level of education, diabetes duration, therapy, metabolic control, and presence or absence of chronic complications answered the ATT 39 questionnaire which serves for the appraisal of the patient's adaptation to diabetes. According to these results, our patients are well adapted as it can be deduced from the positive direction of the score of the total 39-item scale and of its parts. Interestingly, more than 75% of the patients gave identical answers to 1/3 of the questions. Positive influence of the regular education of our patients was shown, as the majority of them feel capable of coping with their illness, are cooperative, and in 99% establish good relationship with their therapist. In general, we have not found any statistically significant differences between the groups concerning the studied parameters, although replies by males, younger patients and patients with shorter diabetes duration tended to be more positively oriented. The illness was more stressful to the insulin-treated patients and to those with advanced chronic complications. The latter have, expectedly, reconciled themselves with every-day measures necessary for control of their diabetes.

We conclude that diabetes is not inevitably associated with poor psychological or physical feeling. Probably, education of patients as well as good relationship between the patient and the therapist play an important role.

2502

PERSONALITY PROFILE OF INSULIN NON DEPENDENT DIABETICS

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The psychosocial structure of NIDDM patients is of great importance for the education and the achieving of satisfying therapeutic effects. The psychosomatic model of approaching internal diseases is also applied to diabetes mellitus. 21 persons ,11 female [f] and 1o male [m] had participated in this research. Psychiatric interviews had been accomplished with each patient, as well as EPQ- Eysenck test which considers 4 personality features: P- psychoticism [rigidity], E- extravertness, N- neuroticism [emotional instability] and L- longing for social acceptance. Eysenck differentiates the characteristics of 4 classical temperaments: sanquinic, melancholic, choleric and phlegmatic. In total score , our patients had: P values 9,17% lower, E values 3,71% higher, N values 11,17% higher and L values 20,77% higher than normative values. The single analysis of each test result gives the following picture: 7 melancholics [5f, 2m], 7 sanquinics [4f, 3m], 5 cholerics [1f,4m] and 2 phlegmatics [1f, 1m]. There is no statisticaly important difference between groups [Fp=1,014, p=0,412 , p.>0 ,05] and sexes [Fp=0,364, p=0,782, p.>0,05].,tested by ANOVA. The longing for social acceptance is remarkably increased.

2503

THE GLUCOCARD MEMORY 2 BLOOD GLUCOSE ANALYSER. AN EVALUATION OF ITS ANALYTICAL PERFORMANCE.

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The Diabetes Complications and Control Trial demonstrated for the first time the beneficial effect to the diabetic patient of good glucose control. It has been shown that self glucose monitoring is of central importance in achieving good glycaemic control. The Glucocard Memory 2 glucose meter (A. Menarini Diagnostics, Florence, Italy) is a new meter designed for patient and clinic use; it uses a biosensor strip which utilises glucose oxidase to estimate blood glucose. Analysis is initiated by the application of blood, which is introduced into the strip by capillary action. Performance was investigated by using either fresh blood, blood collected into EDTA or Fluoride Oxalate. Imprecision was assessed by 20 repeat measurements of samples. Linearity was investigated by measuring glucose (in triplicate) in a mixture of whole blood samples with a low and a high glucose concentration. Samples analysed on the Glucocard Memory 2 were additionally analysed for glucose using a hexokinase method on a main biochemistry analyser (ILab 900). Results demonstrate good precision throughout the analytical range of the meter, typically 4.5% CV at a glucose concentration of 1.2 mmol/L (22 mg/dL) and 3.0% CV at a concentration of 10.0 mmol/L (180 mg/dL). The analyser was found to be linear (y = 1.00x - 0.32; r = 1.00) throughout the quoted analytical range (1.1 - 33.4 mmol/L; 20 - 600 mg/dL). Good correlation was demonstrated (y = 1.1x - 0.5; r = 0.99) between the Glucocard Memory 2 (y) and the hexokinase reference method (x). Haematocrit ranging from 35% to at least 68% had no significant effect on the result obtained, this meter can confidently be used with paediatric samples. The ease of use of this meter (especially the ease of sampling) and its excellent analytical performance will result in this meter becoming an important new tool in self monitoring, and because of the sampling process will play an important role in the diabetic clinic.

2504

HEALTH-RELATED QUALITY OF LIFE IN PATIENTS WITH INSULIN-TREATED DIABETES MELLITUS IN SUDAN

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To determine health-related quality of life (HRQL) in people with insulin-treated diabetes mellitus in Sudan, a total of 89 patients aged 25-55 years and with ≥ 5 years diabetes duration was studied. HRQL was measured with a 68-item questionnaire from the Medical Outcomes Study. Late diabetic complications were assessed, and haemoglobin $A1_c$($HbA1_c$) was measured to assess the metabolic control. Of the patients (m=36; f=53), only 13.5% had good metabolic control ($HbA1_c$ < 7.5%). These patients rated their HRQL as worse than patients with poor metabolic control ($HbA1_c$ > 10%). However, the latter were significantly younger, had shorter diabetes duration, and were free from late complications. Overall, 49.4% of the patients had one or more of the late diabetic complications. These patients rated their HRQL significantly lower when compared with patients without complications. Older age and the presence of late diabetic complications were the most important predictors for HRQL. The results indicated that self-rated HRQL in this group of patients is generally low. Improving diabetes knowledge and the metabolic control since early in the course of the disease, will not only retard the development of late complications, but will certainly improve the HRQL of these patients.

2505

PSYCHOSOCIAL WELLBEING OF THE DIABETIC; THE DIFFERENCE BETWEEN THE IDDM AND NIDDM DIABETICS

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The study examines the wellbeing of the diabetic, the differences in psychosocial factors between IDDM and NIDDM, the self-image and life control and compare the characteristics of IDDM diabetics and heart diseases type A and NIDDM and type B. Teory: a model considering the quality of life on human being as a whole, symbolic interaction, coherence feeling and self-image. The material of the empirical part was collected in 1992 by a questionnaire, aged 16 and 64,in Turku. Analysing methods were the factor and the wariance analysis. Conclusions: Psychosocial factors differences were found in social life situation, health factors, working-life factors, knowlegde level of the one's illness and life control. Social and psychosocial coping on IDDM is significally better than that of NIDDM. The IDDM diabetics have a stronger self-image and better life control. The prominent characteristics were autonomy, balance, sociability, courage and energy. Long-term illness develops character. It seems that diabetes is a high-living-standard and way-of-life illness. It is easier to prevent NIDDM than IDDM by changing way-of-live.

2506

ASSESSMENT OF GENERAL AND DIABETES SPECIFIC FEARS IN INSULIN REQUIRING ADULT DIABETIC PATIENTS.

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Literature suggests that anxiety disorders occur more often among people with diabetes than in the general population. Aim of this study was to assess both general and diabetes-specific fears in diabetic patients. General fear was measured by the STAI-DY-2, a questionnaire referring to trait anxiety. Other measures included were diabetes-specific: the Diabetes Fear of Injecting and Self-testing Questionnaire (D-FISQ), which was developed to measure the degree of fear of self-injecting and fear of self-testing of blood glucose, and the Dutch version of the Hypoglycemia Fear Survey (HFS). Also, patients were asked how concerned they were about having (further) diabetic complications. A sample of 266 insulin requiring adult diabetic patients (50% ♂, 72% type II) filled in these questionnaires. Intercorrelations between the questionnaires are as expected, ranging from .41 to .66 (P<.001). Results show that 49 (18%) patients (27♂, 39 type II) have one or more high scores on the measures; almost half (23) of this group obtained multiple high scores. Fourteen patients scored ≥ 2 s.d. above mean on the trait-anxiety scale. On the specific questionnaires, respectively 13 and 14 (9♂) patients scored in the top 5% on fear of injecting & fear of self-testing. 14 patients had scores ≥ 2 s.d. above mean on the HFS. Twenty patients were "always" worried about complications. Fear of injecting and fear of self-testing seem to be different phenomena as highscorers overlap in only 7 cases. Patients scoring high on trait anxiety often also show diabetes-specific fears (12 out of 14 cases). However, a large number of high scorers (35, 71%) had (multiple) high scores on specific diabetes fears, but showed no elevated trait anxiety. A high prevalence of anxiety was confirmed in this study, demonstrating the psychological burden of diabetes and underscoring the importance of psychological assessment in diabetes care.

2507

ASSOCIATIONS AMONG DIABETES SELF CARE PRACTICES AND ILLNESS-SPECIFIC HEALTH BELIEFS: DIFFERENCES BY DISEASE AND TREATMENT TYPE.

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This study aimed to examine whether associations among illness-specific health beliefs and self care practices varied among different diabetes patient groups. The sample comprised 107 patients recruited from the diabetes centre, and was divided into three groups on the basis of patient diagnosis and treatment type: Type I patients undergoing conventional insulin therapy (n=27), Type I patients undergoing intensive insulin therapy (n=38), and tablet treated Type II patients (n=42). Self care practices and illness-specific health beliefs, including diabetes self efficacy, diabetes locus of control, and hypoglycemic fear, were assessed using self report instruments completed by patients during a routine visit to the diabetes centre. Analysis of the reliability and validity of the health belief measures supported their use in Australian patient groups. Factor analyses of the diabetes self efficacy and diabetes locus of control scales revealed the multidimensionality of these constructs, and supported an examination of associations among the components of these health beliefs and diabetes self care practices. Analysis of variance procedures comparing group means on the self care scales suggested that patient groups differed only in their diet self care ($p<.05$) and glucose monitoring ($p<.05$), with conventionally treated Type I patients reporting the most positive self care in these areas. Between group differences were also observed on several of the health belief scales. Intensively treated Type I patients demonstrated greater confidence in their ability to manage their treatment demands ($p<.05$), less fear of hypoglycemia ($p<.05$), and were less likely than other groups to consider medical professionals ($p<.05$) and others' support ($p<.05$) as essential for maintaining their health. As hypothesised, correlational analyses revealed that associations among health belief scales and self care practices differed among patient groups. Findings support a more complex conceptualisation of the structure of illness specific health beliefs, and suggest potential targets for interventions aimed at improving self care practices in different patient groups.

2508

KNOWLEDGE, ATTITUDE AND PRACTICE OF KHARTOUM DIABETICS IN RELATION TO THEIR DIABETIC STATE.

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Diabetes mellitus with its concomitants has become a major health problem in the Sudan. The aim of this work is to assess patient disease related knowledge and practice and study patient attitude towards their disease condition. Two hundreds middle aged alert and ambulatory diabetes were randomly chosen. Most patients were illiterate. A structured questionnaire with closed end questions was used to obtain patient knowledge about his disease state, complications, diagnostics tests and management. Further information were obtained about daily activity, hygienic measures, reaction to disease and dietery intake. The level of knowledge and practice was poor. Most patients accepted their disease state. Patient compliance depends on his cooperation and understanding of his disease. Illiteracy may account to the lack of knowledge. This is not being helped by fragmented information given to them. The general acceptance of this disease suggest lack of appreciation of the problem. This coincided with faulty dietary practice. This study calls for urgent measures to establish patients' education programs.

2509

A DEVICE FOR RECORDING INSULIN USAGE IN DIABETIC PATIENTS WITH VISUAL IMPAIRMENT.

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Insulin delivery systems (pens) and 'talking' blood glucose meters allow greater independence for the visually impaired diabetic, however a means of recording insulin usage in order to calculate how much insulin is left in a 300 unit pen cartridge was needed. A small unit was devised consisting of 3 horizontal rods with 5 unit graduations secured inside the lid of a Novopen 3 case. Each rod has a small marker which is progressively moved to the right (in multiples of 5 units) to a total of 95 units each rod. When all markers on the 3 rods are moved to the extreme right, the cartridge is spent. Built in to the mechanism is a 15 unit excess allowing for patient error. Where 2 insulin pens are used they are identified with Braille or a tactile material such as Velcro to differentiate between insulins. Extensive education is needed in the precise and safe use of the talking meter and the insulin pen. A studyof 404 insulin-using patients was conducted; 62 (15%) were visually impaired, 35 (8%) were using insulin pens and needed assistance with dose recording. Trials involving nursing staff and patients were conducted over 2 years. All device users maintained their independence.

2510

RELIABILITY OF THE SPANISH VERSION OF THE DIABETES QUALITY OF LIFE.

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Results of the Spanish version of Diabetes quality of life (Sv-DQOL), evaluated in 105 diabetic patients from Terrassa (Spain) have been published recently. Our aim was to assess the reliability of the Sv-DQOL in the whole country and to find out about differences according to sex, age, diabetes duration and place of residence. From all the Spanish regions, 874 diabetic patients (mean age 43.5 y, 48% men, mean diabetes duration 10.2 y and 95% insulin-treated) answered the questionnaire and 496 of them, answered the retest. Sixty percent defined themselves as IDDM and 40% as NIDDM. Reliability was assessed for: Internal consistency (Cronbach's α) and Test-retest by Pearson's correlation. Globlal Cronbach's α was 0.88 and 0.84, 0.76, 0.8 and 0.68 for Satisfaction, Impact, and Worry: Social and Diabetes-related subscales. The test-retest correlations were in the 0.52-0.81 range ($p<0.001$). Except for Social worry, quality of life was better in the youngest group (16-25 y) than in the oldest (56-69 y) ($p<0.001$). Diabetes-realted worry was higher in women than men ($p<0.01$) and Social worry was higher in patients with longer diabetes duration ($p<0.05$). No inter-regional differences were found. The Sv-DQOL is highly reliable for both IDDM and NIDDM. The absence of inter-regional differences suggests it could be useful in other Spanish speaking countries.

2511

DIFFERENCES BETWEEN AFRICAN-AMERICAN AND CAUCASIAN PATIENTS IN SELF-REPORTED FUNCTIONAL HEALTH STATUS

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We obtained data on functional health status in a sample of 415 patients with either NIDDM or IDDM using the RAND SF-36 survey. Scores on the eight SF-36 dimensions were compared for African-American (N=129) vs. Caucasian patients(N=73) with NIDDM. (Too few African-American patients with IDDM were available for analysis.) Median scores were lower for African-American patients on all dimensions; these differences were statistically significant for Physical Functioning, Social Functioning, Role-Physical, Pain, and General Health. Logistic regression methods were used to adjust for potential demographic or clinical confounding variables. After adjustment for these variables (e.g., duration of disease, age, gender), significant differences only remained for Physical Function. We conclude that apparently large differences between racial groups may reflect the influence of a complex mix of clinical and demographic factors, but that a residual effect of race remains in at least one SF-36 dimension after adjustment for other factors.

2512

THE VALUE OF PREDICTIVE TESTS FOR FAMILIES WITH IDDM

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Before widespread clinical predictive testing for IDDM occurs, it is important to study the impact of testing on family members and to identify their concerns. The aim of this study was to gather qualitative data about the value of predictive testing for adults and children when a family member has IDDM. Two leaders ran five focus groups with a total of 31 participants (males=9, females=22) from three diabetes services in Melbourne. Answers to prepared questions were tape-recorded and responses categorised. Groups:- 1. Teenagers with IDDM (n=9); 2. Young adults with IDDM (n=6); 3. Young parents with IDDM (n=6); 4. Older parents with IDDM (n=3); 5. Parents of children with IDDM (n=7). In addition, data from telephone interviews of siblings of children with IDDM (n=5) were used in the study. A broad range of views were given, and some common themes were identified including 1. The value of predictive tests is limited as prevention is presently not possible; 2. However, many indicated they would modify lifestyle in an attempt to prevent or delay onset; 3. There is no value for young children in knowing test results, but anxiety could be reduced for some adults and parents, raising the issue of children's right to decide; 4. For most, a false negative result was not as devastating as a false positive; 5. Insurance companies and employers should be denied access to results. The study provided valuable insights into the way predictive tests are viewed by a range of family members. The impact of test results on individuals and their relationships, the complexity of handling risk statistics, and the range of associated ethical issues requires that pre and post counselling protocols are established. Quantitative studies are now needed to assess possible uptake of testing, and to plan specialised services.

2513

KNOWLEDGE, SEVERITY BELIEFS AND HELP SEEKING BEHAVIOURS: NIDDM COMPARED WITH NON DIABETICS.

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Appropriate self care, seeking treatment early and regular screening can limit diabetic complications. They depend on the person having the appropriate knowledge a belief that diabetes is serious and that they are vulnerable to its effects. A cross sectional survey using a self completed questionnaire was undertaken to determine knowledge about diabetic complications, if subjects considered diabetes to be serious and how long they would wait before seeking help for chest pain. Subjects were randomly selected from a) the hospital diabetic Outpatient Department (NIDDM n =50) and b) non diabetics from a suburban general practice (non DM n = 51). 46% NIDDM and none of the non DM had received formal diabetes education in the six months preceding the study 78% of NIDDM were able to list at least one complication compared with 35% of non DM. Both groups considered diabetes to be a serious disease and 48% of NIDDM believed they were likely to develop a complication particularly eye disease. Females were more likely than males to seek treatment within 30 minutes for severe chest pain, ($p<0.05$). The NIDDM group were more likely to seek treatment than non-DM, ($p<0.05$). There was no significant relationship in NIDDM between seeking treatment immediately for chest pain and diabetic treatment, duration of diabetes or age. Reasons for not going to the doctor included work commitments; hoping the problem would resolve; fear; not to worry the doctor and cost. NIDDM have a good knowledge of diabetic complications, believe diabetes to be serious and are likely to seek help quickly for chest pain. Non DM consider diabetes to be a serious disease but have limited knowledge about it complications.

2514

EVALUATION OF AN ENHANCED ELECTROCHEMICAL GLUCOSE METER SYSTEM BY DIABETICS AND NURSES AT THREE CLINICAL SITES

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The performance of enhancements in the Glucometer ELITE® blood glucose monitoring system; including a 30 second reaction time, 1.11 to 33.3 mmol/l assay range, 20 result memory, temperature compensation and improved ergonomics; were evaluated in the hands of 61 diabetics and 3 nurses. Each participant assayed controls and fresh capillary bloods on new and current ELITE® meters. Capillary plasma glucoses and hematocrits were done by study site laboratory staff using appropriate handling care. The 61 diabetics and 3 nurses, using 9 meters, obtained CV on three controls that ranged from 3.3 to 4.6% and 3.1 to 4.3%, respectively. Replicate analyses on 119 fresh capillary bloods at the three sites, by the 64 testers, had a CV of 4.7%. For three comparative methods (Abbott Vision and Hitachi 911 hexokinase, and Beckman CX3 glucose oxidase methods), combined regression statistics were:

Analyst	n	Regression	Syx (mmol/l)	r	Bias
Diabetics	116	y = 0.96x + 0.13	1.02	0.978	(-) 4.6 %
Nurses	116	y = 0.95x + 0.12	0.82	0.985	(-) 4.0 %

Nurses had 100% of blood glucoses within 20% of laboratory results and diabetics had 97.4% within 20% of laboratory results. In comparisons of new ELITE to current system by nurses and diabetics at the three sites, result ranges were; n = 38 to 42, slopes of 1.02 to 1.06, y-intercepts of -0.58 to 0.03 mmol/l, Syx of 0.38 to 0.78 mmol/l, r =0.988 to 0.997 and biases of -1.2 to 1.5%. Both diabetics and nurses had 100% of new ELITE glucoses within 12% of current ELITE results on bloods and controls. The new ELITE system compared well to laboratory and current ELITE glucose results. The 61 diabetics and 3 nurses at the three sites found the new Glucometer ELITE® to be attractive, ergonomically improved, precise, accurate, convenient and easy to use.

2515

Self-management of young diabetics in reality.

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To determine, whether young diabetics follow the most important rules of diabetes educational programs as forgetting "carrying sugars" and insulin shots in their daily life, the analysis using 263 simple and anonymous questionnaires was performed. The study involved 183 IDDM children from Kansas-USA (76 boys, 107 girls) and 80 IDDM children from Silesia-Poland (36 boys, 44 girls) of mean age 12,95±2,65, mean IDDM duration 4,77±3,15 years, mean HbA1c 8,52±1,9%. We found: (1) 78,85% of all IDDM children carry something to treat or prevent hypoglicaemia, more girls than boys, and more American than Polish ones ($p<0,01$). (2) Only 59,92% IDDM children carry "soluble sugars" (no statistically significant differences between sexes and nationalities). (3) No correlation between "carrying sugars" and age, metabolic control, number of glycaemia measurements, forgetting insulin injections and kind of insulintherapy. (4) Duration of diabetes was not correlated with carrying "sugars" whereas was correlated with carrying "other food" ($p<0,05$). (5) 53,4% children never forget insulin injections. (6) Forgetting insulin shots was correlated with IDDM duration in boys group, with increasing level of HbA1c in all children group, with the intensive insulin therapy in girls and Polish groups ($p<0,01$ in all cases). Conclusions: (1) Young diabetics more often forget their insulin injections than carrying "sugars". (2) Older girls with longer IDDM duration and worse metabolic control check their glycaemia less frequently and forget the insulin injections more often.

2516

QUALITY OF LIFE FOLLOWING CHANGE IN DIABETES THERAPY

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Twenty-eight subsequently recruited diabetic persons who were switched from oral hypoglycemic agents (OHA) to insulin therapy (Group 1), were compared with 28 persons who remained on OHA (Group 2) with respect to quality of life issues. The samples were matched according to sex (13 males and 15 females), age (63.3 ± 7.83 in Group 1 vs 60.8 ± 10.1 yrs in Group 2), duration of disease (11.5 ± 6.2 vs 9.4 ± 6.1 yrs), education and family status. The World Health Organisation Quality of Life Questionnaire (WHOQOL) was used to assess the individual quality of life initially and after a two-month follow-up period. T-tests were used to compare the two groups' quality of life indicators. Initially, Group 2 gave higher ratings, indicating better quality of life with respect to overall quality of life (t= -1.945 p=0.05), physical health (t= -2.412 p=0.02) and level of independence (t= -2.939 p=0.05), while no differences were found with respect to the psychological, social and personal beliefs domain. After the follow-up period the groups were comparable in all quality of life aspects excepting the level of independence which remained higher in patients who continued with oral therapy (t= -3.469 p=0.01). Observing changes inside the groups, no differences were registered in Group 2 during the follow-up period, while patients who changed their therapy to insulin significantly improved some psychological quality of life determinants (Negative feelings; t=-2.77 p=0.01) and improvement in the whole psychological domain was almost significant (t=-1.87 p=0.07).
From the holistic point of view, assessing quality of life when undertaking therapeutic interventions is justified.

2517

PREDICTORS OF DIABETES-RELATED QUALITY OF LIFE.

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The present study is aimed at evaluation of the impact of demographic and clinical features on diabetes-related quality of life. A consecutive series of 480 diabetic patients (81 IDDM, 399 NIDDM; 250 F, 230 M), aged 18-70 yrs (m±sd 53.1±13.3), with a duration of diabetes of 10.2±8.7 yrs, Body Mass Index 27.2±4.9 kg/m^2, HbA_1c 7.4±1.6 %, was studied. Quality of life was measured using a recently developed questionnaire, the Well-being Enquiry for Diabetics (WED), investigating four different domains: physical symptoms (WED-S), diabetes-related psychological distress (WED-D), mental health (WED-M), and practical impact (WED-I). Multivariate analysis was used to detect the specific contribution of each variable to quality of life. Type of diabetes did not appear to interfere with total and subscale WED scores. Among patients with NIDDM, those receiving insulin treatment showed lower WED scores (meaning worse quality of life), particularly in the WED-S and WED-I subscales. Both in NIDDM and IDDM, female patients resulted to have a worse diabetes-related quality of life ($p<0.05$) than males. An inverse correlation was observed between Body Mass Index and WED-D ($p<0.01$), WED-I ($p<0.05$), and WED total ($p<0.05$) scores. Age and duration of diabetes were directly correlated ($p<0.01$ and $p<0.05$, respectively) to WED-S. At multivariate analysis, an inverse correlation was found between WED-S scores and HbA_1c. In conclusion, female sex, insulin treatment (in NIDDM only), higher age, and longer duration of disease are all predictors of worse diabetes-related quality of life. Overweight is also capable of affecting quality of life, determining a greater diabetes-related physiscal impairment (WED-I) and psychological distress (WED-D). Finally, HbA_1c does not appear to correlate with overall quality of life (WED total), but is significantly affected by mental health (WED-S).

2518

WHAT SIGNIFICATION HAS THE DIABETES DISEASE FOR THE CHILDREN AND THEIR PARENTS ?

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The Pediatric Clinic in Borås has a group center for diabetic children and adolescents. The intention is that one patient group with the same diagnosis shall meet. In may - 95 an evaluation of the activity started and has been carried out as one pedagogic part and one psychological, which the latest is described here.
Method: Material is collected by interviews with children,adolescents and parents that regulary participate in the group center. Totally 30 randomised interviews have been carried out.**Results:** The moment of falling ill has a vital importance for how the child and the parent react on the disease initially. The factors that contribute to this is the chronological age, individuell mature, develepment phase and life- and familysituation. The same factors contribute that the signification of the disease vary. Parents and the adolescents with diabetes have private admissions about what has cause the disease. Especially among the mothers of the diabetic children there are guilt problems connected to this, that is experienced as all from manageable to seriously handicapping. In normal crise processes the mothers have used the group center in a terapeutic way. The value of the group for the smallest children appears to be learning by imitating. This has in many cases had a great practical importance in their own handling of injection.
Conclusion: The group center appears to have a subjective appreciated terapeutic effect on the mothers. Since the study confirms what previous research has presented, that the mothers of children with diabetes show different symtoms of anxiety, should this form of parental treatment be a good indirect help for the children. Differential diagnostics should however be applied more in an early stage to separate children and parents in normal crise processes from those who need more individual treatment. As cronics often have an unnecessarily large identity of illness, the pediatrics should act for not giving the children this to early. For that reason the group center should primarly have an practical approach to the child before puberty. That goal is reached in the effect of learning by imitating.

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PSYCHIATRIC MORBIDITY AND METABOLIC CONTROL IN DIABETIC PATIENTS

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Psychiatric morbidity and especially depression is believed to be an important aspect of Diabetes Mellitus (DM).The prevalence of depression in DM is reported to vary between 8.5% to 27.5%.Thirty-nine patients (20 males and 19 females) suffering from DM entered the study. Their age was 44.41±15.7 years (range 18-70), BMI values were 25.55±3.56 (range 17.91±32.46), and the duration of illness 8.58±7.56 years (range 1.5-33). Sixty-Four percent of them were overweight, 60.6% were insulin dependent and 68.57% were well controled. Their HbA1c was 7.7%±1.72% (range 3.7±-11%) and their glucose level was 204.31±88.55mg% (range 81.454). GHQ -28 and Beck Depression Inventory (BDI) were used for psychometric evaluation. Stepwise Multiple Linear Regression Analysis (SMLRA) was used for the analysis of data. The least squares method was used to draw the Regression line in bivariate scatterplots, to search for non- linear relationships. From SMLRA results it seems that C dimension of GHQ correlates with HbA1c, D dimension of GHQ correlates with glucose levels and management of DM ane BDI correlates with HbA1c, managenent of DM and BMI. Bivariate scatterplots, however showed a non-linear relationship between depression and DM. Depression, measured either by BDI or D dimension of GHQ seems to correlate weakly with management of DM when HbA1c values are under 10% or glucose levels under 250mg% . However, beyond these values the increase in depression is impressive

2520

A WALKING GROUP - ITS ROLE IN THE MANAGEMENT OF NIDDM

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The benefits of regular exercise for people with NIDDM is well documented. However, motivating individuals with NIDDM to do so can be a challenge particularly as they are often older and may have other conditions interfering with their ability to exercise. To address these factors a supervised, weekly walking group was set up. The aims of the group were: 1) to provide a safe, supportive environment where people with diabetes may enjoy the benefits of exercise and 2) to encourage and equip the individual with the skills to incorporate exercise into their diabetes management plan. The route chosen took approximately 45 minutes to complete. Random blood glucose tests (RBG) were performed and feet inspected prior to walking and on return. Morning tea, a popular part of the program, was provided after the walk. The group consisted 10 males and 6 females. Mean age was 65 years (range 45-76) and duration of diabetes was 5.3 years (range 1-20). 8 subjects were treated with diet alone, 8 with oral hypoglycaemic agents. Mean individual attendance rate over 12 months was 77%. Mean weight at commencement was 85.7kg. Weightloss occurred with 13 subjects (range 0.3 - 5.2kg), weight gain occurred with 3 subjects (range 0.3 - 2.9 kg).Mean weightloss for the group was 1.2kg. Mean HbA1c fell from 7.6% to 7.3% with 75% showing an improvement. Blood glucose results (mmols/L) are summarised below.

Pre Walk	RBG Range	Mean Pre walk	Mean Post walk	Mean Drop	Hypos
N = 37	3.6 - 5.4	4.7	4.2	0.5	4
191	5.5 - 9.9	7 .7	4.8	2.9	21
92	10.0 - 14.9	11.5	7.0	4.5	3
17	>15.0	16.7	13.1	3.6	0

In conclusion, the benefit of walking, as a group exercise, was demonstrated by improvement in short term blood glucose and positive trends for HbA1c and weight control. The incidence of hypoglycaemia (10%) reinforces the need to test RBG before and after exercise. High attendance rates and increasing membership highlight the importance of social interaction as a motivating factor for incorporating exercise into a diabetes management plan.

2521

PSYCO AND SOCIAL PROFILE OF THE ELDERLY DIABETIC.

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The purpose of this study was to raise the psyco and social profile of the type I and II elderly diabetic belonging to the Brazilian middle class and from 60 to 65 ears old. To accomplish this an assessment containing 45 objective questions, which after proper validation, whose applied to 83 individuals of both sexes. The data were computed trying to verify the percentage of incidence of each one of the raised itens. The middle class elderly diabetic profile revealed, in the great majority, a person that, in accordance to medical literature, accepts badly the modifications imposed by diabetic treatment (57%). On the other hand, does not present difficulty in learning (94%), more physical problems that make him/here incapable (chewing-92%; hearing-57%; seeing-80% or seringe use-91,5%) cares for his personal independence, does not feel abandoned by his family(86%), lives, most the time, with his consort(56%) and the treatment does not substancially compromises his monthy butget (53%).

2522

EFFECT OF ACUTE TEMPERATURE INCREASE ON HYPOGLYCEMIC EPISODES

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In this study, metabolic parameters of 31 IDDM patients (F/M, 12/19,mean age: 14.1 ±2.4) used to living in warmer temperatures(20-23°C) and joined to a diabetes camp held at a higher environment (36-39°C) were observed for 10 days and compared with 13 IDDM camp attenders (F/M, 6/7,mean age: 15.6 ±4.0) originally living in this hot area. Although similar isocaloric diet and same exercise-insulin program were applied before and during the camp, there was a significant difference in number of hypoglycemic episodes between patients from warmer and living in hot regions; 78/14 (p=0.02) . The difference was particulary remarkable in first 48hrs;64/14 (p=0.00). Patients from warmer regions were evaluated in two groups based on ages: Gr.I; <15yrs old (n:16) and Gr. II; ≥15yrs old (n:15). When number of hypoglycemic episodes observed in first 48hrs (20/3, p =0.00) and following days(44/11, p=0.00) were compared, we found that attacks in the first 48 hrs were high in both groups. Evalution of insulin requirements have shown that in Gr.I precamp insulin dose $0.77IU.kg^{-1}.day^{-1}$ decreased in first 48hrs;0.63 (p=0.01) and reached approx. to initial value at the end;0.74. However, in Gr.II insulin requirement decreased from 0.73 at initial to 0.54 at 48hrs (p= 0.00) and to 0.49 at the end. Although pre and postcamp BMI were closed to each other in both groups; findings of acute increase in incidence of hypoglycemic episodes along with rapid decrease in insulin requirements in first 48hrs have suggested that the main reason for this among patients from warmer regions was rapid insulin action due to acute increase in temperature during camp. Furthermore, we concluded that smaller children adapted to the environment easier than older ones.

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COMPREHENSIVE EVALUATION OF THE FACTORS RESPONSIBLE FOR SELF-MANAGEMENT OF DIABETES.

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Sucessful self-management of diabetes is dependent on a wide range of beliefs, attitudes and, to a lesser extent, basic knowledge and/or demographic factors. All require assessment if educational needs of patients are to be determined and met. A well validated questionnaire, (41 questions) previously shown to assess these factors in insulin treated subjects and one newly developed for non-insulin treated subjects (54 questions; Cronbach alpha 0.78} were applied to consecutive attendees (253 insulin treated, age 18-74; 203 non-isulin treated, age 28-75) at routine diabetes clinics over a six week period. Factor analysis was performed on the responses of 202 (insulin treated) and 139 (non-insulin treated) subjects and regression coefficients of individual questionnaire and factor scores against HbA_1 determined. Nine factors (Eigen values 5.72 - 1.18), namely effect on lifestyle, self efficacy, weight concerns, benefits of treatment, targets, confidence/coping, general emotional adjustment, food concerns, and barriers to treatment in insulin treated and five factors (Eigen values 10.2 - 1.87), lifestyle, benefits to treatment, barriers to treatment, self efficacy and targets in non-insulin treated subjects emerged from this analysis. Weight concerns ($p<0.05$), targets ($p<0.001$), general emotional adjustment ($p<0.05$), barriers to treatment ($p<0.01$) and total questionnaire score ($p<0.05$} in insulin treated and lifestyle ($p<0.05$) in non-insulin treated subjects were significantly correlated with HbA_1. The variation in number of subjects (5 - 55%) scoring low for separate factors indicate that the questionnaire may be able to distinguish individuals well adapted to certain factors, though not to others. The questionnaire scores provide an overall estimate of the educational process and should enable education to be directed to meet specific needs of individuals and provide explanation of reasons for failing to meet glycaemic targets.

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PSYCHOSOCIAL ASSESSMENT GUIDELINES FOR PEOPLE WITH DIABETES IN THE PRIMARY CARE SETTING.

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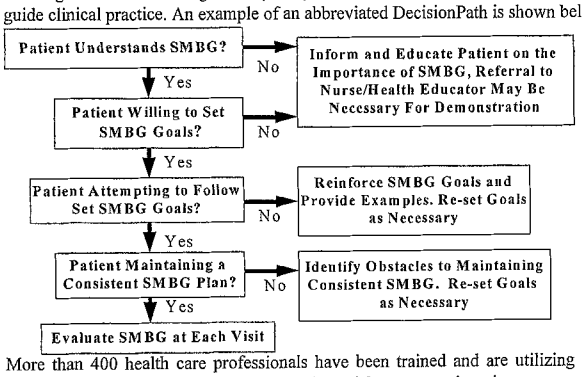
A survey of primary care practices showed that adherence issues are frequently noted in medical charts as an obstacle to adequate diabetes care. This is documented as patient refuses treatment, SMBG, dietary plan or exercise. This is explained by patient confusion about diabetes and its management, provider limited time and a general lack of intervention strategies available in a primary care setting. In this project, guidelines for adherence assessment and improvement were developed using the Staged Diabetes Management (SDM) format which relies on DecisionPaths to guide clinical practice. An example of an abbreviated DecisionPath is shown below.



More than 400 health care professionals have been trained and are utilizing these DecisionPaths. Recognizing limited psychosocial resources in primary care, this approach focuses on four readily attainable diabetes management variables: medication, food plan, SMBG and exercise. It encourages patient behavior change by re-education, setting specific targets with the patient, frequent re-assessment of goals (with re-setting goals as needed) and close follow-up.

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Quality Management

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DETERMINING DIABETIC TREATMENT REGIMENS BASED UPON SOCIOCULTURAL HABITS OF KENYANS

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In order to develop therapeutic regimens compatible with the Kenyan lifestyle, the lifestyle had to be identified and the insulins and oral agents placed appropriately to mealtimes. Eighty diabetic and nondiabetic patients waiting at the Eye Unit clinic were interviewed to determine food choices and quantities with frequency and timing of meals. Combining the action, peak, and duration of insulins and oral agents, we developed treatment regimens that were compatible with the Kenyan socio-cultural habits. Fitting the treatment modalities into the lifestyle has made adherence easier and maintaing good control more possible.

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EFFECTS OF ETHNICITY ON METABOLIC CONTROL DURING THE FIRST SIX YEARS OF NIDDM. TME Davis, CA Cull, RR Holman and RC Turner for the UKPDS Study Group, Diabetes Research Laboratories, Oxford, UK.

Changes in metabolic indices were evaluated in 3,319 patients with NIDDM recruited to the UK Prospective Diabetes Study (UKPDS) at diagnosis and followed for 6 years. All patients had 3 months' dietary therapy initially and then other treatments were randomly allocated. 2,720 (82%) were White Caucasian (WC), 331 (10%) were Indian-Asian (IA) and 268 (8%) were Afro-Caribbean (AC). After adjusting for age, BMI and HbA_{1c} at randomisation, there were no significant ethnic differences in the change in HbA_{1c} over 6 years (ΔHbA_{1c}; P=0.1). However, in those who were obese (>120% ideal body weight) at randomisation, ΔHbA_{1c} was greater in IA and AC than WC patients (means 0.59%, 0.68% and 0.27% respectively; P=0.02). Significant interactions between ethnicity and allocated treatment were present in ΔHbA_{1c} in obese patients, with metformin-treated AC patients having the greatest improvement in ΔHbA_{1c} (-0.72%) compared to the other 15 ethnicity-treatment combinations (range -0.20% to 1.38%; P=0.03). Significant differences in serum lipids were also found in AC patients who had the greatest increase in ΔHDL-cholesterol adjusted for age, BMI and fasting serum HDL-cholesterol at randomisation (0.13 vs 0.03 and -0.03 mmol/L in WC and IA respectively; P<0.0001). In summary, these data suggest that obese AC and IA patients have greater progression in glycaemia than WC. However, obese AC patients treated with metformin show the best glycaemic response to any allocated treatment in the three major ethnic groups in the UKPDS. AC patients have the most favourable change in serum lipids over 6 years of NIDDM.

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STANDARDS OF QUALITY OF HEALTH CARE IN DIABETES IN NORTH SPAIN: A DIABCARE-BASED ANALYSIS OF 3646 PATIENTS.

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Several reports have proven that the incidence and progression of diabetic complications may be reduced by early surveillance and treatment and, on the other hand, by risk factor control.The aim of the study was to evaluate and compare the quality of diabetic care (QC)between the different outpatient specialized diabetic units in Cruces Hospital Health Area in the Community of Pais Vasco, Spain through DiabCare-based analysis of 3646 diabetic patients. This health area have about 400.000 inhabitants, and include 1 main hospital, and 4 specialized diabetic clinics (7 endocrinologists provide the whole specializad diabetic care for the community in these centers). Data collection was in DiabCare Basic Information Sheet, made in 1995 in diabetic patients older than 14 years. Differences in QC between hospital treated or outpatient clinics treated patients were analyzed with the Chi-square test and Mantel-Haenzelt test for trends. Data are expresed as means+/-SD and range. Results: There were 620 patients (17%) with type 1, and 3026 (83%), with type 2 diabetes mellitus. Mean age was 58,6±13 years. Female / male ratio was 52,5 / 47,5%.

Process of care (%):	GLOBAL	HOSPITAL	OUTPATIENT CLINICS	P
Hb A1C	91	98,6	87,2 (83,8-95,7)	<0,0005
Educational activities	34,4	71,9	19,5 (11,3-41)	<0,0005
Glycemic control	52	81,5	41,5 (36-47)	<0,0005
Ophtalmoscopy	85,5	99,4	78,5 (74,1-87,1)	<0,0005
UAE analysis	68,3	92,5	56,1 (17-88,5)	<0,0005
Blood pressure	97,9	99	97,4 (91,6-100)	<0.0005
Lipids	87,8	97,4	82,9 (70,9-98,2)	<0,0005
Smoke inquiry	76,6	76,1	76,9 (60,3-94,2)	NS

High blood pressure was known in 27%(10-44), hypercholesterolemia in 25%(17-37), hypertriglicerdemia in 20,5%(13-35), and 20,3%(7-35) smoked. Poor glycemic control (Hba1c A1c>7,5%) was present in 64%(62-69), diabetic nephropathy in 32,3%(26-55), and rethinopathy in 41,7%(39-45). Blindness affected 3,3%, stroke 5,3%, limb amputations 2,3%, renal failure 0,8%, and ischaemic heart disease 8,9%. We conclude that specialized diabetic care can be ameliorated, although there are great differences between the diverse outpatient units and hospital practice. Nevertheless, prevalence of cardiovascular risk factors was lower than expected.

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MANAGEMENT OF DIABETES IN INNER-CITY PATIENTS: THE GRADY MEMORIAL HOSPITAL EXPERIENCE.

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African-Americans have increased prevalence of diabetes, and increased risk of complications; inner-city patients face additional problems due to limited resources. The Grady Diabetes Unit provides high-volume care (~1,000 new patients a year) for patients who are predominantly African-American (87%) and poor (54% have incomes below U.S. Federal Poverty Levels), with low health literacy skills (inadequate in ~50%). In 4,886 new patients, 88% had NIDDM, and 73% were obese; 34% of 578 patients had renal damage at first presentation. Our management utilizes a team approach (nurse-providers, dietitians, podiatrists, and physicians), with stepped intensification of care (diet, oral agents, insulin). In 563 patients who returned for visits at 2, 4, and 6 months, HbA1c fell from 9.3% to 7.5% ($p<0.001$). Moreover, use of pharmacologic agents decreased: in 1,156 obese patients, 71% were using such therapy at presentation, but only 54% at 6 months. Since our patients report difficulty in understanding food exchanges, we initiated a randomized controlled comparison between an exchange diet (ADA) and a "healthy choice" diet (HC) which focuses on food choices and moderation of intake without emphasis on portion sizes or weight loss. The 311 ADA and 375 HC patients were indistinguishable in weight, obesity, gender, duration of diabetes, and HbA1c. The groups exhibited comparable weight loss (36% of ADA lost at least 5 lb, vs. 30% of HC; p=NS), and fall in HbA1c at 6 months (from 9.9% to 7.9% in ADA, vs. 9.3% to 7.6% with HC; p=NS); there was also no difference in lipids or BP, or use of pharmacologic agents.

Conclusions: Stepped care with a team approach constitutes an effective paradigm for management of inner-city patients; a healthy choice diet is easier to teach than an exhange diet, and metabolic outcomes are similar. Flexibility in therapy can help overcome barriers to effective care.

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CSII-THERAPY: IMPROVEMENT OF GLYCEMIC CONTROL WITHOUT A LONGTERM INCREASE OF HYPOGLYCEMIA

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Objective: The DCCT observed 62 hypoglycemic episodes (assistence required) and 16 episodes of coma or seizure per 100 patient-years in the intensive therapy group. We tested if CSII-therapy would reduce the frequency of hypoglycemia compared with DCCT results. **Methods:** 250 IDDM-patients were requested to answer a standardized questionnaire for quality-management 15 months after the beginning of CSII-therapy. This questionnaire includes questions about hypoglycemia-frequency und insulin dosage. Furthermore the patients were invited to return a blood sample for determination of glycemic control (Ghb, affinity-chromatography, mean of non-diabetics 5,5 ± 0,5%). 148 patients (age 37,8 ± 12,9 years, diabetes duration 16,9 ± 9,2 years) returned questionnaires and blood samples (drop out-rate 40,8%). **Results:** Glycemic control improved considerably from Ghb 10,7% before CSII - therapy to Ghb 7,9%. The daily insulin dosage was reduced from 56,5 ± 23,4 insulin units to 49 ± 17 insulin units. Overall 32,8 hypoglycemic episodes (assistence required) and 12,3 comas per 100 patient-years occured. Within the observation time the patients reported a decline in the frequency of hypoglycemia. In the first three months 70,2 hypoglycemic episodes and 29,7 comas per 100 patient-years occured; in the following three months there was a reduction to 67,5 and 18,9 events respectively, and in the 9 months there after this rate dropped to 17 hypoglycemic episodes and 5 comas per 100 patient-years. 20 patients reported hospitalisation caused by hypoglycemic episodes. **Conclusion:** In spite of a marked improvement in glycemic control and reduction of insulin-dosage there was overall an unexpected low incidence of hypoglycemia compared with the intensive-therapy-group of DCCT. After the initial adaptation to the new therapy conditions, the relativly high incidence of hypoglycemia in the first six months dropped markly. As a long term perspective CSII-therapy will not inevitably increase negative side effects like hypoglycemia.The incidence of severe hypoclycemia is even less than DCCT-data indicate.

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THE METABOLIC CONTROL IN CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES IN KRASNOYARSK REGION.

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We examined 142 patients with type 1 diabetes, aged 2-19 years, with a diabetes duration from 1 to 12 years. The degree of glycemic control was estimated with the help of glycosylated hemoglobin (HbA1c). HbA1c was measured by a HPLC method (normal range 4,5-7,0 %). 57 patients (40%) were in better control (HbA1c < 8,5%), with significantly lower diabetes duration ($p<0,001$), more frequent daily blood glucose monitoring ($p<0,02$) and more injections/day in comparison to children and adolescents (n=85, 60%) in poorer control (HbA1c >8,5%). In group with low diabetic control complications were revealed more frequently. After 5 years 34,5% patients had microalbuminuria, retinopathy was diagnosed in 38%. The education and intensive treatment diabetes allowed to achive higer degree metabolic control.

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THE EFFECT OF EVALUATION BY PATIENTS ON THE PROCESS OF ANNUAL REVIEWS IN DIABETIC CARE.
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Objective To assess the effect of evaluation carried out by patients on the process of annual reviews in diabetic care and to measure the degree of patient satisfaction. Design Patients attending the clinic for annual reviews were given a leaflet explaining what to expect at the consultation. They were invited to complete an evaluation questionnaire anonymously at the end of the consultation. The process of annual reviews carried out was audited during a four week period. The results were compared with an audit of 100 conseecutive annual reviews before the onset of the study. Main outcome measures - Indicators in the process of annual reviews and patient satisfaction ratings. Results Indicators relating to the examination of blood pressure, feet, eyes and metabolic control showed a significant improvement as a result of evaluation by patients. Indicators for clinic urine tests, ser um cholesterol and microalbuminuria did not reach an adequate standard. Conclusion Audit by patients improves the quality of annual reviews and it is also a tangible expression of patient empowerment

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BLACKSEADIAB: REGIONAL EUROPEAN INFORMATION NETWORK AIMING AT A BETTER DIABETES CARE IN THE COUNTRIES OF THE BLACK SEA REGION. E. Stanciu, C. Ionescu-Tirgoviste, S. Pruna, S. Bejan, G. Rachiteanu, I. Cazacu and I. Mincu. Clinic of Diabetes, Institute "N.C. Paulescu", Software ITC, Bucharest, Romania.

The **BlackSeaDiab Working Group** was created in view to promote in the 12 countries surrounding Black Sea the objectives of the St. Vincent Declaration and of the Acropolis Affirmation. Ethnical, cultural and economic heterogeneity gives a unique opportunity to know the specific problems related to diabetes care in the participating countries. The project will provide the informational support required by the **BlackSeaDiab Action Project**. The solution sought had to facilitate an extension of the network from a minimal level up to a top level, with the smallest costs. A two stage solution has been adopted. In the present stage, an INTERNET compatible network (protocols, application interfaces) is realised using existent physical communication channels, with limited INTERNET access. In the second stage, the network will be developed to use high speed communication channels, with total INTERNET access from any point of the network. The architecture of the system is a *modular* and *open* one. This means that the architecture is compatible with and can be easily integrated into existing information systems and networking environments. Each module is in fact a **National BlackSeaDiab network Section** and is composed of a central node connected by different types of links with a number of local nodes. The access to all these nodes will be accomplished from access points. The scope of the project ranges from routine diabetes care to diabetes research. A wide range of software applications will be developed such as: patient monitoring, programs for the assistance of the clinical research regarding various aspects of diabetes mellitus, epidemiology, education and treatment, statistical analysis of information recorded in data bases, etc. In view to attend such objectives the main instrument is creation in each participating country a **BlackSeaDiab Registry of Diabetes**, which will allow to obtain: accurate prevalence and incidence of both IDDM and NIDDM patients; continuos up-date regarding the presence of chronic complications; mortality data; main demographic and socio-economic date related to the quality of diabetes care.

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EVALUATION OF A DIABETES ADVISORY SYSTEM IN ADOLESCENTS

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The aim of this pilot study was to evaluate insulin adjustment advice by a Diabetes Advisory System in adolescents. 19 unselected adolescents, 12-16 years of age with diabetes duration > 1 year, were randomised into 2 groups, matched according to sex, age and HbA_{1c}. The study period was 3 months with intensified contact in the first month and a follow up period of 2 months. In the first, second and fourth week all patients collected data on meals, insulin doses and 4 daily blood glucose measurements for 3 consecutive days. The patients had insulin doses adjusted in the first, second and fourth week. In the target group the insulin adjustments were exclusively based on the advice from the system. Adjustments in the control group were based on the departments routine principles. HbA_{1c} was measured in the first week, just prior to the initial data collection, and after 3 months. 6 patients were excluded for different reasons and the study ended up with 5 patients in the target group and 8 patients in the control group. When comparing HbA_{1c} at the beginning and at the end of the study, no significant difference was seen neither in the target group (HbA_{1c} 9.6% to 9.0%) nor in the control group (HbA_{1c} 9.4% to 9.1%). However, in the same period a marginally significant mean reduction in insulin dosage was seen in the target group (0.82 to 0.72 U/kg, $p = 0.06$), but not in the control group (0.98 to 0.97 U/kg). This reduction in insulin dosage in the target group was reflected in a marked reduction ($p = 0.09$) in number of measured hypoglycaemias (< 3.5 mmol/l) from 0.41 to 0.07 attacks per day. The pilot study suggests the Diabetes Advisory System being a safe tool for insulin dose advice in adolescents obtaining as least as good a result as the traditional setting.

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EXPERIMENTING WITH THE SYSTEM OF THE QUALITY CARE CONTROL IN POLAND

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Exploiting the fact, that diabetes care in Poland is based on a uniform structured system, the network of 11 regional and 1 central centers for quality control was inbuilt into it. Each regional center was formed as a group of 3-4 spontaneously interested in quality control diabetologists. Such centers was equipped with standardised hardware, Polish version of the Diabcare chart, statistical programmes and modest operational budget. Central center function as the organizer, scientific planner, statistical analyst. The main goal of this system were: measurement of the St. Vincent Declaration targets for whole country, production of objective data for regional comparisons in the relation to local potential and investments. The system was able to produce the respective data in the randomly selected representative group of 1271 of IDDM, which were validated and analysed accordingly to the Diabcare chart 12 areas of interest. The clinical characteristics of the studied population, the biochemical parameters including HbA1c, the parameters assessing the life style, morbidity due to chronic complications, educational activities and performance of diabetes care providers were determinated as aggregated outcomes for the whole country and separately for 11 regions. The average age at the group was 34.0 ± 10.3 years, diabetes mellitus duration 13.0 ± 9.35 years, BMI 23.6 ± 3.04 kg/m^2, systolic blood pressure 126 ± 17.2 mm Hg, diastolic 78.5 ± 9.12 mm Hg, HbA1c 7.99 ± 1.72%, total cholesterol 191 ± 41.5 mg/dl, triglycerides 108 ± 73.7 mg/dl; percentages of smokers was 21.2%, alcohol users 4.2%, morbidity due to retinopathy 37%. The differences between regions were very significant and were connected with the local quality of care indexes. Conclusions. The system used revealed the average level of quality and strong regional differences calling for action and investments. It also proved to be useful for measuring the distance from the St. Vincent targets.

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IMPLEMENTATION OF SPECIALIST RECOMMENDATIONS BY PRIMARY CARE PHYSICIANS

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Specialist annual review clinics have been developed to assist general practitioners (GPs) to care for their diabetic patients. This study was undertaken to determine the implementation by GPs of recommendations made as a result of attendance at our Diabetes Complication Screening Clinic (DCSC) which is part of the Shared Care Program conducted with the local GP Division. Follow up and implementation by GPs of recommendations made at the DCSC were reviewed in patients reattending after 1 year. Written recommendations on management of diabetes, complications and atherosclerosis risk factors, are made by an endocrinologist, diabetes educator and ophthalmologist. 134 patients attending over a 10 month period who had previously attended the DCSC one year previously were assessed for implementation of recommendations. In addition, benefits/deterioration from implementation/lack of implementation of glycaemic and dyslipidaemia management recommendations were made, based on comparisons of initial and follow up HbA_{1c} and lipid profile results. A total of 128 recommendations were made in 71 of the 134 patients related to diabetes control, dyslipidaemia, hypertension, obesity, microalbuminuria, renal impairment and referral to diabetes educator, dietician, podiatrist, ophthalmologist & vascular surgeon. The annual frequency of GP diabetes-specific visits ranged from 0 to 10, with a mean of 3.9. 62.5% of recommendations made at the DCSC were implemented. 73.9% of the recommendations for glycaemic control were implemented with a benefit in 35.3% and deterioration in 50% where recommendations had not been implemented. 77.8% of recommendations for management of dyslipidaemia were implemented with a benefit in 28.6% and deterioration in 25% of those where implementation had not occurred. The lowest implementation rates were recorded for referrals to other diabetes health professions: diabetes educator,dietician & podiatrist. Overall implementation of recommendations made to GPs from DCSC is satisfactory but could be improved. To address the 37.5% failure to implement recommendations, greater patient and GP participation and education about the service and recommendations are being trialed. Written recommendations are being provided and explained to patients and GPs are being encouraged to undertake a clinical attachment at the DCSC.

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EVALUATION OF A DIABETES ADVISORY SYSTEM

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A double blind clinical trial was conducted to evaluate advice on insulin dose adjustment, generated with the help of a decision support system. The study was conducted on 12 IDDM patients admitted consecutively to Sønderborg Hospital due to poor metabolic control. On admission, the patients were maintained for about 4 days on an intensive regimen with 8 daily measurements of blood glucose and 8 daily injections of soluble insulin. Typically on the fourth day they were switched to a regimen with 3 daily injections of soluble insulin and one injection at night of intermediate-acting insulin. After a further 4 days they were discharged with this regimen. 6 patients were assigned randomly to the target group and 6 to the control group. The advice on insulin doses during the period with 4 daily insulin injections was generated by an experienced diabetologist at Odense University Hospital for the patients in the control group and by the diabetes advisory system for the patients in the target group. The clinician in charge of the patients at Sønderborg Hospital did not know the source of the advice. 2 month after the patients were discharged, HbA1c was measured. For the patients in the target group HbA1c was improved by 1.9% ± 2.9% (mean ± sd), from 9.4 ± 1.4% to 7.5 ± 1.0%. For the patients in the control group HbA1c was improved by 0.9% ± 1.4% (mean ± sd), from 8.1 ± 1.4% to 7.2 ± 0.9%. Although the patients in the target group thus seemed to achieve larger improvements in HbA1c than the patients in the control group this was not statistically significant due to the small sample size (p=0.24). In conclusion, advice generated by the system seems to be safe and of a quality comparable to advice from an experienced diabetologist.

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COMPLICATIONS IN TYPE 1 DIABETICS ARE CAUSED BY ELEVATED BLOOD GLUCOSE LEVEL: RESULTS FROM AN AUSTRIAN CENTER.

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Aim: The aim of the study is to estimate the risk of the development of complications, depending on the metabolic balance (measured by HbA1c ≤ 6.1), kind of treatment and duration of the illness.

Methods: 177 type 1 diabetics treated in our center were selected at random. 72 were treated from the beginning, 105 started 7.1 a (sd 10.5) after the onset. The 177 pat. (1405.3 pat. years), age of onset 27.0 a (sd 13.0), duration of illness 14.9 a (sd 11.7), present age 41.8 a (sd 14.8); 45% have conventional (KT), 55% intensive therapie (IT). HbA1c: KT = 7.8%, IT = 7.9%, severe hypoglyc. KT = 4.7/100 pat. years, IT = 11.7/100 pat. years; 37.8% have HbA1c < 7%; 53.3% have no microvascular complications. In the group treated from the beginning n = 72, duration of illness = 8.1 a(sd 5.6), HbA1c KT = 7.2%, IT = 7.2%; 46% have Hba1c < 7%; 78% have no complications. All of them took part in our training and attended the outpatient clinic at least 4 times yearly (BG self-control, therapy adjustment)

Results: After 1/2 year with a level of HbA1c > 7.0% the risk for complications rises to 2.4 times as opposed to a level < 7%. Depending on the duration of the illness 0.67 is the risk for the first 5 years, 2.45 between 5-10 years, 3.12 after 15 years. 0.85 / 5.53 / 2.57 for retinopathy respectivly. 1.04 / 7.58 / 3.12 for neuropathy. Only 13% have nephropathy (too little data for statististical analysis). For patients treated in our center from the onset the equivalent risk is only half.

Conclusion: This study shows like many other studies (DCCT, Kroc, SDIS, Steno, Oslo etc.) that the long term complications are directly related to the elevated blood glucose level. HbA1c level of 15% above the normal seems to have little risk for developing late complications together with a rate of hypoglyc. as low as possible. It is very important to start at the onset with an optimal therapy. This is the most important point of the study. The duration of illness plays only a role in cases of poor control of the metabolic balance. Not the type of therapy but the results are important.

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FEASIBILITY OF AN INTRANET-SOLUTION FOR DIABETES DISEASE MANAGEMENT IN A ROUTINE HEALTH CARE ENVIRONMENT

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Progress in achieving higher levels of primary health care quality depends crucially on the implementation of structured care procedures on the basis of standardised documentation and guidelines. This applies as well to organisational as to medical quality. Aim of the project is to demonstrate the feasibility of an intranet solution respecting the aforementioned features thus providing the health care team with useful and effective support in the management of diabetes. The disease management module is organised cascade-like where the user starts from a general top level proceeding to levels of greater details. Documentation according to standardised data sets (DiabCare Diabetes Data Set, Care Card Diabetes) is imbedded into structured routine procedures which may be tailored to the individual needs of a specific health care setting. Whenever necessary the user may request reference to generally accepted guidelines and knowledge bases via easy to use hypertext links. Documented data are transferred in anonymised form to a central data base where evaluations are carried out with respect to watchdog rules, patient centred benchmarking, and the DiabCare quality indicators. This intranet solution is realised in standard html-technique and therefore widely independent of the users technical prerequisites. It could be demonstrated that the described system is technically feasible and faces wide acceptance among health care practitioners because of its easy and intuitive way of use, its flexibility, as well as its referral to commonly accepted standards and guidelines. A disease management module implemented as an intranet solution contributes to the structuring of routine health care procedures according to standards and guidelines thus providing the basis of quality management in primary health care.

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GENDER EQUITY IN SWEDISH DIABETES CARE: A REVIEW

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Gender differences in the utilization of health services may be explained by differences in the epidemiology of disease and accompanying need of services or by differences in the expected safety, effectiveness and cost-effectiveness of medical interventions. However, gender differences may also reflect care providers' knowledge and attitudes or differences in the accessibility of services to males and females. Such differences may indicate gender inequity in health care and may adversely affect the quality of care. In 1995 the Swedish government appointed an expert committee to analyse gender equity in health care, and diabetes care was selected as one of the fields for review. The aim of the review was to explore the performance of diabetes care in Sweden from a gender perspective. The review was based on published data on health care utilization, metabolic control, patient satisfaction, quality of life, and mortality.

The registered level of use of inpatient care with the diagnosis diabetes mellitus is identical for the sexes before the age of twenty, while males use more inpatient care between twenty and seventy-five years of age. Young and middle-aged diabetic females receive more outpatient care than diabetic males, which is mainly explained by females' use of services at gynaecology clinics and community health centres. Data from two counties show no gender differences in metabolic control, but no comparisons are available on a national level. Studies of patient satisfaction indicate less satisfaction among diabetic females. Females also have reported poorer quality of life than males. Results from the Diabetic Incidence Study in Sweden indicate higher excess mortality in young and middle-aged diabetic males than females. A follow-up study of individuals identified in the Surveys of Living Conditions show that diabetic females with low socioeconomic status have higher excess mortality than diabetic females with high socioeconomic status, while the social class gradient is less prominent among males. - More research is needed to explain gender differences in patient satisfaction, quality of life, and mortality. Monitoring the impact of gender should become an integrated part of quality management in diabetes care.

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The therapeutic reality of type II diabetes

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In spite of all improvements that have been made in diagnosis, drug therapy and education, the therapeutic results obtained with many type II diabetics are lamentably poor in the light of the St. Vincent Declaration. Against this background, it appeared useful to examine the problem using an integrative research approach, i.e. qualitative group interviews validated by individual interviews. All the professional categories involved in therapy were interviewed: 200 general practitioners, 90 hospital-based doctors, 110 diabetes advisors and nurses, 15 care-givers from the out-patient services and 15 from old retirement homes and 20 old age type II diabetes patients.

- The most significant problem in diabetic management is the inability of health-care professionals to motivate the diabetic patient to take responsibility for his disease and act in a way that promotes his own health. (None of the people interviewed was familiar with any motivation theory or its practical application).
- All those interviewed agreed that the full potential of somatological therapy cannot be realised as long as diabetic patients cannot be motivated and as long as therapy is so poorly integrated into the everyday situation of the patient.
- Interviewers were asked to estimate the potential therapeutic success; general practitioners said 0 to 15 per cent, hospital-based doctors said 20 to 60 per cent!
- 75 per cent of the general practitioners interviewed, but only 25 per cent of the hospital-based doctors, asked whether purely somatological therapy goals are truly significant targets and quality parameters for patients.
- Only 10 per cent of those asked were familiar with the NIDDM Policy Group recommendations.
- The perception of health-care professionals is coloured by frustration, resignation and underrating.
- Nursing staff in old retirement homes and out-patient services complained about the lack of interest on the part of general practitioners in coordinating the care they give diabetic patients with the care given by the nursing services. Also, neither general practitioners nor hospital-doctors are familiar with the early demential deterioration processes that occur not uncommonly in elderly diabetic patients.
- Diabetes advisors and nurses agreed that it is ultimately neither possible nor sensible to attempt weight reduction through diet or any form of nutritional intervention in chronically obese elderly diabetics.
- Patients do not feel that diabetes is one of their really important problems in life.

The study identified three factors which cause diabetic therapy of the elderly to fail:

1. The fundamentally reduced ability of the diabetic patient to manage his disease competently and on his own responsibility. This could be due to early demential processes which makes both patient education and self-management impossible.
2. The amount of freedom to make changes that elderly type II diabetics allow to those treating them. Care-givers generally tend to assume that the diabetic wants to improve his diabetes. This is the basis on which they offer therapeutic help. Care-givers almost never try to establish a real dialogue with the patient as an equal and respected individual as a framework for deciding what the patient actually wants to do about his diabetes and what degree of change he thinks could be possible.
3. The various institutions that care for diabetics on a day-to-day basis often have completely different ideas about therapy. In general, none of the parties concerned tries to coordinate therapy with the care given by another party. There is often a serious lack of understanding of other treatment systems, and this makes appropriate and sensible cooperation between the systems possible.

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AN AUDIT OF STRUCTURE, PROCESS AND OUTCOME OF CARE OF THE DIABETIC CLINIC NATIONAL HOSPITAL SRI LANKA.

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We audited the structure, process and outcome of care at the Diabetic clinic National Hospital, Sri Lanka. A previously validated MCQ paper of 10 questions which assessed knowledge of diabetes on insulin therapy, dietary management, management during acute illness and management of emergencies was administered to all patients. The function of the clinic was assessed using previously validated audit care record forms. Diabetes knowledge among patients, waiting times, bypassing of local institutions, availability of diagnostic equipment, screening activities and time spent per consultation was assessed. The clinic had a daily attendance of 186 patients who were seen between 0800 to 1200 hours. A single medical officer spent 2.14 minutes per patient. No screening was performed. There were no facilities to examine patients or for patients to sit while consulting the doctor. The diabetes knowledge score was 15.06SD 3 from a maximum of 40. 43% had bypassed a local institution. Reasons for bypass included non availability of drugs and the expectation of quality care at NHSL. Patients spent a mean of 1.5 SD 0.7 hours travelling to the clinic and waited a mean 1.56 SD 0.4 hours to see the doctor and 1.3 SD 0.12 hours to obtain drugs. We concluded that the services of the diabetic clinic do not meet the standards expected of a clinic.

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DIABETES EDUCATION SERVICES STANDARDS: VALIDATION OF RECOGNITION PROGRAM.

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Standards are always the first step in any recognition or accreditation process. They are the benchmarks towards which to strive. Yet, the development of this vital criteria is often flawed, providing a weak foundation for the systems built upon them. This presentation describes the extensive process used to develop Canadian Diabetes Education Service Standards and a Canadian Recognition Program. The Standards, (Outcome Process and Structure Standards) were developed through extensive participation of all stakeholders over a two year consensus building process. Workshops, randomized Focus Groups and two rounds of Delphi Survey enabled all stakeholders (educators, consumers, physicians, organization) to participate. Attention to research methodology throughout added to stakeholders commitment and belief in the Standards. The development of the voluntary Recognition Program was likewise both rigorous and participative involving four Pilot sites, two Annual Meeting presentations and a coordinating Task Force. A two part program was developed consisting of a self-assessment through Portfolio Development and an external review by a National Review Panel. The Portfolio is a collection of prospective data gathering instruments that demonstrate how the service meets the Standards. Each instrument was examined for construct and expert validity with the Standards. Strictly quantitative standardized testing was rejected in favor of more qualitative measures that are appropriate in diverse settings, systems and locations across Canada and congruent with the Outcome Standards for Diabetes Education. The Recognition Program was launched in 1996. The Canadian Standards for Diabetes Education Services are currently being considered by the Diabetes Education Consultative Section of the IDF for International use.

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DIABETIC KNOWLEDGE AND SELF-CARE BEHAVIOR IN DIABETIC COMPLICATIONS.

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To improve diabetes care in the management of patients with NIDDM, we have initiated a special clinic for screening chronic diabetic complications. Patients at risk for developing diabetic foot, neuropathy, nephropathy and retinopathy need special educational program for better management. We investigated the basic diabetic knowledge and self-care behavior and correlated with the diabetic control and complications. 71 male and 49 female patients with NIDDM were recruited from July to November, 1996. Their mean (SD) age was 58.4 (11.9) and the duration of NIDDM was 10.7 (9.2) yrs. The perentage of patients who were illiterate, semiliterate, graduates of elementary school, junior high, high school, and college or higher were 12.5%, 6.7%, 20.8%, 5.8%, 25%, and 29.2%, respectively. The score of better self-care behavior correlated significantly ($p<0.05$) with lower serum cholesterol and triglyceride levels, lower incidence of retinopathy and hypertension in simple regression analysis, and correlated with lower triglyceride and blood pressure by multiple regression analyses. Higher knowledge of diabetes correlated significantly ($p<0.05$) with younger age and higher educational levels in both simple and multiple regression analyses. Better self-care behavior seemed to be beneficial for management of diabetic complication. Lack of knowledge about the diabetic complication might prevent better self-care. Therefore, diabetic education specifically designed for prevention or better care of diabetic complication might be very useful and deserves implemented in the screening clinic for complications.

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DIABCARE Q-NET NL, ST. VINCENT GOALS INTO PRACTICE

G. Storms, D. Vermey, CBO, Utrecht, The Netherlands

The European project DiabCare Q-net under the umbrella of the St. Vincent Declaration, aiming at quality management in diabetes care, is put into practice in the Netherlands with a bottom up approach, as a quality management system located at the CBO, the Dutch National institution for quality management in clinical practice. The software is provided by Medinet: DiabData Pro, client software to fill and sent the Basic Information Sheet to a server by modem, and server software, to administer the received data and provide benchmarks to the clinical end-users. Users are general practitioners, paediatricians and internists/diabetologists. They were contacted for their interest for diabetes care and not by their scientific institutions. Together they form the usergroup that "owns " the data on the server. The system is intended to monitor the clinical performance of health care teams by comparison of the clinical care to the standards as set by the expert guidelines defined by the different working groups of St. Vincent (indicators). These indicators are compared in a ranked order between the treatment sites (benchmarking). If a treatment site decides that the outcome of an indicator has to be improved, they start a Quality Circle, a structured improvement process for the health care team plus the end-users, being the people with diabetes. The complete functionality of the system including the data available and the outcome of a quality circle will be demonstrated during the presentation.

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IMPROVEMENT IN QUALITY OF DIABETES CARE IN PRIMARY HEALTH CENTERS .J.F.Cano P.Tomás, M.Berenguer, R.M.Gimbert, J. LLussa, X.Mundet, M.Birulés and the GedapS Group.Catalan Family and Community Medicine Society. Barcelona (Spain).

Aims: To assess changes in quality of care on type 2 Diabetes, attended in Primary Health Care (PH), after an intervention programme based on St. Vincent Declaration.**Methods:** Cross-sectional study carried out in 1993 and 1995 in 50 PHC Centres (34% rural) attending 1.256.193 adults. Cases were selected by systematic sampling from 31.050 registered NIDDM. Intervention was based on publication and delivery of Diabetes Guidelines adapted to primary health care and the performance of Workshops in different geographical areas, stressing in educational interventions, early detection of complications and foot care **Results**:Patients evaluated were 2.595 in 1993 and 2250 in 1995, without differences in gender, age or average time of diabetes evolution. The impact on Process Indicators was:

	1993	1995		1993	1995
Visits patient/year			*Presence in clinical*	%	%
Nurse	5.1	4.7	*records of:*		
Medical	3.8	2.8	Tobacco smoking		
for Education	1.61	2.3	Blood Pressure	92	92
Education/Visits ratio	17,9 %	30 %	HbA1c	73	75
B.GlucoseSelf-Monitor.			Cholesterol	78	80
Total			Microalbuminuria	34	50
Insulin-treated	31%	36%	Funduscopy	55	53
	62%	68%	Foot Examination	50	57

Intermediate result outcomes: We found an increase in percentage of diabetics with good/acceptable control of HbA1c (+2%), and a decrease of % of patientes with HbA1c > 10. The acceptable control of cholesterl increased (5%), and 3% decrease in foot ulcers/ lesions. **Conclusions**.- Results seem to confirm the effectiveness of our programme, specially to promote educational activities by PH teams, early detection of chronic complications

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ROLE OF A DIABETIC CLINIC FOR SCREENING OF CHRONIC DIABETIC COMPLICATIONS. EXPERIENCE FROM A MEDICAL CENTER IN TAIWAN.

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Many animal experiments and recent clinical trials have all demonstrated the importance of tight glycemic control in the prevention of chronic complications of diabetes. Even under the best of circumstances, it is not easy to keep all the patients under strict glycemic controls all the time, and since the onset of many complications is insidious, we set up a special clinic to screen for diabetic complications. From July to November 1996, we screened 120, 71 males and 49 females, for microangiopathies and peripheral vascular disease. Their mean (SD) age was 58.4 (11.9) and the duration of NIDDM was 10.7 (9.2) yrs. Among them, only 11 (9.2%) had been examined by an ophthalmologist before entry. With a non-mydriatic fundus camera, we found diabetic retinopathies in 18 (16.5%) of the remaining patients. Thirty-four (28.3%) patients were known to be proteinuric. However, 31 (36%) of the remaining patients showed presumptive evidence of microalbuminuria by a semiquantitative method (Micral test, Boehringer Mannheim). Eighteen (15%) of the patients had received nerve conduction velocity study before entry. In the remaining patients, 38 (37%) had at least one abnormality in tests including current perception threshold at 5, 250 and 2000 HZ, Semmes-Weinstein (#5.07) monofilament test, and vibration test with Riedel-Seiffert tuning fork. None of the patients had been examined for foot problems by Doppler ultrasound. Four (3.4%) of the patients showed abnormal low ankle-brachial indices by Doppler exam. Our preliminary data strongly suggested the needs for implementation of a screening exam for complications into our daily practice. Undiagnosed chronic complications may be uncovered earlier for prompt attention and intervention.

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QUALITY IMPROVEMENT SYSTEM IN FRANCE : A REGULAR ASSESSMENT. RESULTS AFTER 4 YEARS.

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Since 1993 information on the quality of care is annually gathered from diabetic centres over a one-month period in October. Data collection is voluntary and anonymous. A form in triplicate (the European BIS) is systematically used, and one copy is given to the patient as part of the improvement system itself (awareness of the SVD, late complications, prevention and self monitoring, improved communication with primary care team). Most of the data entry is made centrally and included in a pedagogical project. Each centre receives after a few weeks a confidential analysis of his own results and anonymous bench-marking figures. After 4 years, through regular campaigning each year, the network has succeeded in covering all French regions and the number of centres has increased from 71 to 255. Different structures are now involved : 45 university hospitals, 163 others hospitals, 92 diabetologists in private practise . 80 % of the initial membres are still active in the network. The quality of data is improving (ex : missing HbA1c 10,6 % in 93, 6,2 % in 95). Intermediate outcomes have a tendancy to improve (ex : % of patients with HbA1c < 8 % improved from 34 % in 93 to 41 % in 95) but these results need to bo confirmed. Analysis of the 4 th campaign is now being done. The centres have gained a clearer picture of local hospital care. The constant weight of recent SVD complications (6 %) over this period shows the necessity of long term strategies to achieve SVD targets.

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DIABCARE HUNGARY: IMPLEMENTATION OF A TOOL FOR CONTINUOUS QUALITY IMPROVEMENT IN DIABETES CARE

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To reach targets of the St Vincent Declaration, the ***DiabCare programme*** of the WHO/IDF-Euro, a monitoring device for continuous quality improvement of diabetes care was implemented in 1994 in Hungary as a part of the National Programme. **20 'centres'** (including 2 GPs) sent **3975 aggregated**, anonymised **records** (data of 3835 diabetic patients; 140 cases repeatedly; age, gender, type, duration and form of treatment of diabetes mellitus [completeness: 93%], other therapy, last lab values of previous 12 months, complications, St Vincent targets, pregnancy) for central evaluation.

				age		*duration*		
	n	**male**	**IDDM**	**<35yrs**	**56-75 yrs**	**6-15 yrs**	**>15 yrs**	**on insulin**
1994	2085	41%	29%	21%	48%	38%	34%	59%
1995	771	44%	44%	21%	36%	39%	38%	73%
1996	1119	43%	25%	12%	49%	38%	30%	60%
'94-96	3835	43%	29%	18%	47%	39%	33%	61%

Results for the whole period: self monitoring: 52%, severe hypoglycaemia: 7%. Late complications: any retinopathy 62% (7% proliferative, 7% laser therapy in the previous year), blindness: 4%. Microalbuminuria (MAU; >30 mg/day; completeness of measurement: 41%): 29%, renal failure 2%. Hypertension rate: 8%. HbA1(c) was measured in 61% of the cases (>8%: 43%). With increasing number of reported cases (more NIDDM and/or diabetes with shorter duration) a drop in the percentage of smokers (20 vs 11%), alcoholics (20 vs 11%), in the number of cases with MAU (32 vs 26%) and with any retinopathy (78 vs 39%) between '94 and '96 could be observed with a decrease in frequency of severe hypo- and hyperglycaemias. Comparison of data of participating centres with country data in quality circles (diabetes team, administrators and GPs) helped in continuous quality improvement of diabetes care.

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Primary Care Provider (PCP) Non-Compliance: Lack of Action for Type II Diabetes Related Abnormalities.

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We hypothesized that poor diabetic outcomes may be due to lack of provider action in accordance with published guidelines. A prospective cohort of 1136 diabetic patients in 8 indigent care clinics will be followed for 2 years, collecting both medical care process data, intermediate outcomes, and patient interviews. First year chart data are presented. Action taken by PCP is defined as one of the following: start or change in pharmacological treatment (RX), recommendation of lifestyle change (LS) such as diet or exercise, or early follow-up (FU) visit to reassess value before implementing new intervention. The cohort was 67% female, with a mean age of 53±12 and diabetes duration of 6.9±8 years. The average # of visits to their PCP in the past year related to diabetes was 4.3. For patients whose glucose was ≥140 milligrams/decilitre or HbA_{1c} >7, 35% of the visits to PCP contained no documentation of actions to decrease blood glucose levels. For RX actions taken, insulin was either begun or increased in 36% of visits or oral agents were either begun or increased in 35%. Among non-pharmacological actions, counseling on diet or exercise occurred in 36%. Similarly, for systolic blood pressure ≥140 mmHG or diastolic blood pressure ≥ 90 mmHG, in 66.6 % of visits no action was taken by the PCP; for cholesterol ≥200, HDL ≤35, LDL ≥130 or triglycerides ≥250, 67.6% of visits had no action taken; and, for albuminuria at random > 30 milligrams/decilitre, 83.4% (dipstick method). A lower proportion of visits with foot abnormalities had no actions: 31.1%. We conclude that, despite seeing patients every 2-3 months, PCPs do not necessarily follow published recommendations for action on metabolic, microvascular, or cardiovascular abnormalities. Potential explanations include PCP lack of awareness of guidelines, poor chart documentation, or inability to deal with multiple abnormalities in 10-15 minute visit lengths.

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QUALITY OF DIABETES MEDICAL CARE FOR PATIENTS WITH ACUTE CEREBROVASCULAR DISEASE, SALVADOR, BRAZIL

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Diabetes Mellitus (DM) is the most underestimated disease of the adult population, even when it deals with acute and severe cardiovascular co-morbidity. An undiagnosed and/or untreated DM in an acute case of Cerebrovascular disease (CbVD) worsens its prognoses making it possible unnecessary death.The aim of this study was to analyse the professional commitment in order to identify, to diagnose and to treat DM in patients with acute CbVD. All medical records of hospitalized CbVD patients, in the 1994 year, Salvador, were reviewed and it were measured the frequencies of: questoning history of DM, previous DM treatment, lab tests for glycemia and DM treatment during the present hospitalization among CbVD patients. The results were compared for the 3 medical assistance sectors· Unique Health System (UHS), Private Health Insurance + private medical assistance (I+P) and "Convênios" ("C"). Frequency ratios or association proportion teste were used in the analysis. The expected frequencies for questioning DM history, lab test and treatment for DM were ≅ 100%. Results and conclusions. From the 1227 CbVD patients, 1048 were under SUS assistance, 124 I+P and 55 in "C". a) questioning history for DM =32,4%(31,7 to 56,0%); b) self-reported DM =16,1% (14,6 to 34,5%); c) comproved DM among the questioned patients= 47,1% (46,1 to 61,3%); d) lab test performed for DM=36,9% (33,2 to 72,7%); e) diagnosed DM by glycemia= 51,4% (50 to 65%); f) treatment for DM patients=91,0%(76,9 to 93,%); g) unknown history and no lab tests performed=65,8% (43,7 to 68,3%). The lowest results (in brackets) for items **a** to **e** were seen in the UHS (the owner governamental hospital and those private paid by UHS) as well as the greatest value for item g. ($p<0,01$ or $< 0,05$) The lowest frequency of treatment and unknown data were observed in "C" The results pointed out the bad quality of medical assistance for both, DM and CbVD patients in Salvador. It is worthy to notice that the included variables in this analysis were the most simple and the least expensive ones for DM diagnosis. It is obvious the contribution of health services for DM/CbVD complication/death, since most diabetic patients stayed undiagnosed. This results may be helpful to improve attitudes, behaviors, programs and quality of medical assistance

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HALF CENTURY TRENDS IN MORTALITY AMONG INSULIN DEPENDENT DIABETES MELLITUS PATIENTS WITH ONSET BEFORE 15 YEARS OF AGE. C.Ionescu-Tirgoviste, E. Farcasiu, I. Cazacu, and R Florea. Clinic of Diabetes, Institute "N.C. Paulescu" Bucharest, Romania.

Mortality is the most significant single indicator of the quality of diabetes care. The mortality status as at 31 December 1994 was determined in Bucharest Diabetes Centre for all IDDM patients with onset before 15 years of age between 1942 and 1994. No data were available for the death in severe ketoacidosis occurring before 1974. After this date, 14 patients in this age group died during the first admission , 9 in the decade 1975-1984 and 5 in the decade 1985-1994. Of the 467 registered cases (241M/226F) 36 patients relocated from Bucharest and 78 cases (16.7%) died. The median age at the time of diagnosis of these cases was 9 years (range 8mo.-14 yrs). Median duration of diabetes was 13 yrs. (range 1-46). The distribution of cases ending in death along the decades is given in the following table:

Decade*	No.of cases	Mean age at onset	Mean duration of diabetes	Mean age at death
1942-1950	3	9	2	11
1951-1960	9	9	9	18
1961-1970	9	11	10	21
1971-1980	19	8	18	26
1981-1990	30	10	20	30
1991-1994	7	3	35	38

*In the last year of each decade, the no. of children with newly diagnosed diabetes was: 1950-14 1960-29 1970-73 1980-139; 1990-229; 1994-403

The causes of death as obtained from various sources were: end-stage renal failure 37.5%; cardiovascular complications 21.5%; severe infections 15.1%; severe ketoacidosis 8.6%; miscellaneous 17.3%. The 14 casses which died in "innaugural" diabetic ketoacidosis were not included in these percentages.

Conclusions (1) Our population based analysis shows that the prognosis of childhood onset IDDM had improved considerably in the period 1942-1994 with a constant increase in the life expectancy. (2) An improvement in health care is expected to lead to a decrease in the number of cases dying in ketoacidosis and end-stage renal disease.

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ASSESSMENT OF QUALITY OF CARE FOR TYPE II DIABETES IN RURAL PRIMARY CARE SETTINGS.

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Twenty-two rural primary care clinics in Minnesota, serving 10% of the population, were selected in order to assess current diabetes practices. Each site had a minimum of five general or family physicians, one registered nurse and one general dietitian available for patient services. No site had an endocrinologist, nor routinely referred to a diabetes specialist for management of type II diabetes. All clinics were located in communities with a population of between 5,000 and 10,000. At each site, 30 individuals with type II diabetes were randomly selected and their charts were reviewed by a multidisciplinary diabetes team. Along with standard demographic data, ten sentinel measures were ascertained: HbA1c (value and frequency), SMBG, eye examination, screening for microalbumin, foot inspection, diabetes education, nutrition education, type of treatment, stage of therapy (start, adjust or maintain) and number of months since change of treatment. Six hundred and sixty patients participated in the study (55% female and 45% male) with an average age of 62 ± 12. Because there was no significant difference between sites, all data were pooled for this analysis. HbA1c values were found for 442 (70%) of the subjects of whom 44% were <8% (normal range 5-6.5%), 32% were between 8 and 9.5% and 24% had HbA1c's of >9.5%. The overall average HbA1c was 8.8%. Food plan alone was prescribed in 14% and sulfonylurea therapy in 52% of the sample. Twenty-six percent of the patients were treated with insulin: 1 injection (1%), 2 injections of split-mixed insulin (24%) and 3 injections using bedtime NPH (1%). Five percent of the patients were on combination insulin and oral agent therapy and no documentation of treatment was found in 2% of the cases. Diabetes and nutrition education occurred in 35% of the cases. Surveillance for foot complications was found in 16% of the patients. Eyes were examined in 23% of the cases and screening for microalbumin occurred in 2% of the subjects. Although 67% of the patients were rated in poor control, only 17% had a change in therapy during the previous month and 24% during the previous 3 months. Overall assessment of diabetes care in this region showed that more than two-thirds of patients are at high risk for the development of microvascular disease.

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THE DIABCARE QUALITY NETWORK: THE ITALIAN EXPERIENCE BASED ON AN ORIGINAL TECHNICAL SOLUTION

M Massi Benedetti, R Norgiolini, F Capani, Q. Carta, M.E. De Feo, C. Taboga, G. Tognoni, M C Tardioli, M. Bruni, M Leandri, M. Orsini Federici, A. Nicolucci, G. Vespasiani - on behalf of the SID-AMD Study Group for the Implementation of the St. Vincent Declaration (SVD)

The DiabCare Q-Net Italy is a project for a national implementation of the European Q-Net principles. It is the result of a collaboration among the WHO Collaborating Centre for the Improvement of Quality of Care in Diabetes, the SID-AMD Study Group for the implementation of the SVD, the Mario Negri Sud Consortium with technical support of Medimatica s.r.l.. The Italian project aims at: a)evaluating the quality of diabetes care, b)creating an operative instrument for immediate data recording and aggregation, c)facilitating a rapid feed-back of information for a multidisciplinary approach in diabetes care (quality circles), d)implementing intervention plans and e)evaluating the impact on the quality of care.The DiabCare Q-Net Italy is based on an original system for data transmission which consists of: a)the Basic Information Sheet (BIS) modified for data transmission via fax, b)a central unit for data recording and aggregation (without manual input) using a software which transfers data into files, c)three update sheets, d)two personal cards for the complete anonymization of information The entire process of data aggregation and feed back of the information is activated and driven by the peripheral user. To date 29 Centres for diabetes nationwide have activated the system and 126480 data related to 1527 diabetic patients have been collected. Data are aggregated at national and macroregional (North, Centre, South) level. Some of the information of one Centre vs regional and national data are reported as an example. The mean age and HbA1c of the selected patients was respectively: 40.7 (national), 35 (macroregion) and 37.7 yrs (centre) and 7 8% (national), 7.7% (macroregion) and 7.3% (centre). The availability of information - related to the process indicators - resulted to be homogeneous for blood pressure, HbA1c, eye examination, weight, height; vice versa the availability of information related to foot inspection, serum creatinine, macroproteinuria, triglycerides and cholesterol resulted to be highly variable throughout the country. The availability of information related to outcome indicators is quite similar at different levels regarding myocardial infarction, blindness and proliferative retinopathy, while it is highly variable when nephropathy and ictus cerebri are considered. The overall results indicate that the DiabCare Q-Net is a valid instrument for external comparison of data, to support the continuous quality improvement in diabetes care.

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ASSESSMENT OF METABOLIC CONTROL IN ETHIOPIAN DIABETIC PATIENTS BY GLYCOSYLATED HAEMOGLOBIN. SEYOUM B, ABDULKADIR J, WORKU Y, BERHAN B, FELEKE Y, MENGISTU Z, AYANA G, ADDIS ABABA UNIVERSITY, ADDIS ABABA, ETHIOPIA.

Glycosylated haemoglobin (HgIc) was determined in a total of 302 diabetic patients regularly attending at the Tikur Anbessa Hospital Dabetic Clinic. The mean age was 41.1 years (range 14-85). There were 160 males (53%) and 142 females (47%). One hundred forty patients (46.4%) were type I and 162 (53.6%) were type II. The mean duration of diabetes was 9.4 years (range 1-34 years). The mean HgA1c was 10.4% (range 5.9-17.4%). 42 (13.8%) patients had excellent (<7.9%), 37(12.3%) patients good (8.0-8.9%), 49 (16.2%) patients fair (9.0-9.9%) and 174 (57.6%) patients had poor control (≥ 10%). On the day of the examination the mean fasting blood glucose (FBG) and random blood glucose (RBG) were 195.5mg% and 273.1mg% respectively. HgA1c correlated negatively with age r = 0.27 (95% CI -0.39 to -0.18) and positively with FBS and RBS, r=0.45(95%CI 0.36 to 0.54) and r=0.49 (95%CI 0.40 to 0.57) respectively, but HgAlc had no correlation with sex, duration of diabetes, serum lipid level or retinopathy. HgAlc was significantly higher in Type I as compared to Type II patients (11.1% versus 9.8%, p<0.001). In conclusion, the overall metabolic control of our diabetic patients is not satisfactory. This is related possibly to inadequate care as well as adverse socioeconomic factors precluding home blood glucose monitoring, periodic measurements of HgAlc and strict dietary control all of which would be difficult to improve in the short term.

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EVALUATION OF THE BEDSIDE HEMOCUE BLOOD GLUCOSE DATA MANAGEMENT ANALYZER

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Bedside testing of blood glucose done with glucose meters meant for patient self monitoring often requires additional measurements in the clinical laboratory. The blood glucose analyzer Hemocue® (Mallinckrodt Medical GmbH, Germany) is based upon a photometer, which is automatically regulated after each measurement. The optical measuring microcuvette contains all reagents prefilled and takes 5 µl of blood. The glucose reaction is a modified glucose dehydrogenase method with the additional formation of a colored formazan. End-point measurement is done at λ 660 nm and 840 nm. The Hemocue analyzer was tested in comparison to Kodak Ektachem Clinical Chemistry Slide® (Ektachem 950IRC System, Johnson & Johnson Clinical Diagnostics), Gluco-quant Glucose® (Hitachi 717, Boehringer Mannheim) and Ebio 6666 (Eppendorf) in a routine central laboratory setting. For this first part of the evaluation 322 blood samples (NaF, 28 to 400 mg/dL) were analyzed at the same time on all four instruments and data were evaluated by the Passing/Bablock procedures. The intra-assay coefficients of variation in a blood glucose range from 40 to 309 mg/dL (n=20 each) were determined as 1.0 to 1.8% (Hemocue), 1.0 to 1.7% (Ebio), 1.4 to 1.9 % (H 717) and 0.6 to 0.9 % (Ektachem). The inter-assay coefficients of variation (n=15) were determined as 1.7 to 4.7 % (Hemocue) and 2.7 to 4 0 % (Ebio). The Hemocue system (y) shows good correlation with both Ektachem (a=-5.7;b=1.02;r=0.991) and H717 (a=-3.4;b=1.12;r=0.991) by the Passing/Bablock procedure. The correlation is less good in the case of Ebio (a=12.3;b=0.95;r=0.988), but also the correlation between Ebio and either H717 a=15.6;b=0.84;r=0.994) and Ektachem a=17.9;b=0.92;r=0.993) is not as good as with the three systems Hemocue, H717 and Ektachem. The shown analytical performance together with the quality testing program meets the requirements for medical laboratory settings. Under the supervision of the clinical laboratory personnel the HemoCue system could be used for glucose bedside testing with all its known advantages for the diabetic patient, but without additional measurements in the central laboratory.

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DIABCARE QUALITY NETWORK IN EUROPE

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The major target of the project "DIABCARE Quality Network in Europe" (DIABCARE Q-Net) is the implementation of a continuous quality development system for diabetes care across Europe as a pilot study. The project is mainly funded by the European Community and is part of the Telematics Applications Programme, Health Care section. DIABCARE Q-Net is linked to the St. Vincent Declaration of 1989. The core of this declaration is to substantially reduce the complications of diabetes: blindness, kidney failure, amputation, coronary heart disease and pregnancy hazards. The tools for this pilot study, i.e. for data collection, electronic transfer, aggregation, evaluation of quality indicators, comparison, feedback and local quality improvement were developed during a previous project of the European Community between 1992 and 1995. One main part of these tools is the Basic Information Sheet (BIS), which contains those data items necessary to monitor the quality of care of an individual patient. This tool is now also available as a Windows program for data entry, retrieval, evaluation and automated data transfer. The result of this project will consist of a network of service centers evaluating, benchmarking and improving their outcome through the routine use of a telematic framework. All participating centers (GPs and clinics in Europe) will get rapid feedback by standardized benchmarking. First installations of such service centers for benchmarking feedback are already in use in Netherlands and Portugal. Additional solutions by data entry via fax were also developed. Such a solution including a service centre is now in use in Italy. The best performers can be identified for consultation, referral, or training of the other partners. This presentation will give a description of the project and an overview of the progress of the ongoing implementation process in Europe.

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DESIGN OF A COMPUTER PROGRAM TO EVALUATE RISK FACTORS FOR LOWER-EXTREMITY AMPUTATION (LEAs).

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We designed a computer program in order to determine specific risk status for LEAs (neuropathy, peripheral vascular disease and foot deformity) and inadequate practices for foot care. This computer program uses Microsoft Foxpro and Windows 3.0. Files are .DBF(dbase and clipper compatible). This program was applied to 59 diabetic subjects (Group A) who underwent LEAs between the 1st of January 1994 and 31st of December 1995 and 250 consecutive diabetic patients (Group B) without LEA of similar age, sex, duration of disease and Hba1c level. Despite a greater neuropathy, peripheral vascular disease and foot deformity risk-score in patients from group A, at least one inadequate practice in relation to foot care was found in all diabetic patients. Proportion of subjects with eyesight and mobility selfcapacity to inspect the feet was scarce and similar in both diabetic population, living alone more than 75% of subjects. Despite this, only 2.7% from group A and 60% from group B visited to the chiropodist at least one time each two months. In conclusions, the program used in this study is efficacy in order to classify diabetic patients by risk status for LEA and can be applied in an ordinary medical office setting.Taking this in mind, a reduction in LEAs would be achieved if subjects with diabetes mellitus could have a satisfactory access to chiropodists.

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GLYCOHEMOGLOBIN A1c MEASUREMENT: MAY HPLC WORK IN THE ROUTINE WAY?

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Clinical usefulness of glycated hemoglobin (HbA1c) crucially depends on the accuracy and precision of its assay. Whilst we compared an immunological bench-top analyzer (DCA 2000, Bayer Diagnostici, Milano) to the HPLC reference method used in the hospital laboratory (Diamat and Fast Diamat, Bio-Rad Lab., Milano) by assaying multiple control sera, we found so many sources of systematical analytical errors in the routine use of HPLC to compromise between-assay precision. DCA 2000 showed intra- and inter-assay coefficients of variation (CV) of 1.1 and 2.3% with the normal standard serum, 1.0 and 4.2% with the pathological one; Diamat yielded CVs of 1.3 and 7.0%, 1.3 and 5.7%, respectively. Although the measurement of 161 blood samples evidenced that Diamat usually overestimated HbA1c (paired t test, $p<0.001$), a great variability of Diamat performance became evident when the relationship Diamat vs DCA was evaluated day by day over 17 days of observation (Anova, $p<0.001$). Intra- and inter-assay CVs of Fast Diamat, that initially (new instrument still on approval) resulted 0.6 and 2.5% (normal standard serum), 0.3 and 1.9% (high standard serum), yet after a 6 month-use in the routine laboratory became 3.1 and 3.2%, 1 and 12.3%, respectively. Main sources of error were: inaccurate autodilution, unsuitable parameter settings, disregard of the maintenance schedule. We conclude that a routine use of HPLC by non skilled personnel implies the loss of HbA1c value in the clinical practice.

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***TELE-DIAB*: SENDING ELECTRONIC PATIENT RECORDS VIA E-MAIL**

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One advantage of being hooked up to the INTERNET is that we can now send software and data files via e-mail. This allows us to send files including laboratory data, photographs, and biosignals to our centres or to our colleagues in the districts in a matter of minutes. Since this is new to most medical doctors the aim of this research was to develop a few standard instructions for the storage and transfer medical information and health care data between the national district centres and the transnational centres in the Black Sea area. For assemble the data file(s) or software programs to be sent via e-mail we used the DIABCARE for Epi-Info database, provided by WHO/Europe and a software developed in our laboratory, written in Turbo Pascal programming language and compiled into an executable form, which has the potential to record simultaneously the ECG and the pulse blood flow signal and real-time analysing of the signals. The software programs or data are in binary form, whereas e-mail is in text form. All that was required was a ZIP file utility (such as PKZIP), a MIME-capable e-mail program (such as Eudora) and a connection to the INTERNET by dial-up via ordinary phone lines. In the first step all of the software and data files were archived (compressed) into one file using a ZIP file utility. In the second step was composed an e-mail message, in the body of the message was written a brief summary of the medical problem and then from the e-mail program was attached the ZIP archive created. The binary files get attached to the e-mail and do not appear in the body of the message but the header of the message will list the attachment(s). Because errors are possible in the body of the message should be listed the files that are sending and their sizes, in bytes. The e-mail program will convert the binary files to a text-like form before sending it and the receiving program will convert it back to the binary. The recipients must check that the files arrived with the correct sizes. We have been tested this system in a pilot study on five Romanian centres and on two Black Sea area countries: Moldavia and Ukraine. In conclusion, the preliminary results shows that the transfer using the e-mail, via ordinary phone lines, have 100 % succesful worked for more than 300 patient records in Diabcare database and for more than 100 biosignals recorded with the software developed in our laboratory.

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THE APPLICATION OF A CHIP CARD BASED INFORMATION SYSTEM FOR MULTIDISCIPLINARY CARE OF PATIENTS WITH DIABETES

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We have developed and tested a chip card based portable electronic patient record for the documentation,follow up and quality assessment of patients with diabetes.The system architecture is composed of the chip (smart) card,the processor,the data structure,security functions,the operating system on the chip card and the interface between chip card and application The software is tailored to present screens based on an enriched data set version of the «Saint Vincent meetings» basic information sheet.
The workstations,composed of office computers with card readers,were located at the outpatient's department,the dieticians office,the endocrine laboratory.A mobile workstation was also added by using a portable computer connected to a card reader for the home visit by the diabetic nurse.50 patients (47 NIDDM/3 IDDM) participated in the study each carrying his/her own chip card when visiting the hospital.Three issues were tested.1.Patient and health personnel acceptability of the system.2 The effectiveness of the system in communicating information among the health care team members and 3.Security aspects related to the chip card use.The methods used were based mainly on specially designed questionnaires, assessment of time spent updating the chip card and the central data base in comparison with keeping paper records and personal interviews.The acceptantance of the system was high in both patients (76%) and health providers (82%).Patient documentation and exchange of information, following initial adjustment, improved although the time spent with the card was 34% more that using paper records due mainly to software imperfections.Finally the security proved to be satisfactory as no one could have access to the data on the card without knowing the card's PIN.

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INTERNET EXPERIENCE WITH A FREEWARE INTERACTIVE EDUCATIONAL DIABETES SIMULATOR

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Diabetic patients performing self-monitoring blood glucose (SMBG) measurements can often be uncertain about how to interpret the data. SMBG data are made available to health-care professionals at clinic/general practice visits and therapy is adjusted accordingly. However, educating patients to "close the loop" and act on their own SMBG data between clinic visits can be problematical. An interactive educational simulator of glucose-insulin interaction in type 1 diabetes mellitus has been developed for standard IBM compatible PCs. Patients and their relatives, students, nurses and clinicians are the intended users. They can select case scenarios on-line and interactively simulate or demonstrate the glycaemic effects of changes in insulin therapy and/or diet. An infinite number of changes can be made - ranging from adjusting the times, preparations, doses and number of insulin injections to changing the carbohydrate content or times of meals. If patients/ students are unsure what to try simulating next, and require guidance, a knowledge-based system can provide suggestions - increasing the educational utility of the software. The system was recently refined and now incorporates a new, dedicated data entry screen and case scenario database. It caters for a wide variety of commonly used insulin preparations, including premixed (biphasic) preparations. As a consequence of its ease of use, after some extensive beta-testing by diabetic patients and health-care professionals around the world (all Internet users), the software was formally released in June 1996 on the World Wide Web as a non-commercial contribution to continuing diabetes education. It can be downloaded *gratis* from the Diabetes UK Internet site (*http://www.diabetic.org.uk/aida.htm*). Thus far over 2900 people have visited the site and over 1500 copies of the software have been downloaded. It is hoped that such interactive teaching aids may in time help to safely improve patient self-management between clinic visits. The software is also available without charge directly from the authors.

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THIS ABSTRACT HAS BEEN WITHDRAWN BY THE AUTHOR.

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I.T. FACILITATED WHOLE POPULATION IMPROVEMENTS IN INTERMEDIATE OUTCOMES OF DIABETES CARE.

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In 1992 a district diabetes information system (DDIS) was introduced to an existing (commenced 1988) population based diabetes care programme (urban population 230,000; 2% diabetes, 872 Type 1, 3746 Type 2). DDIS supports service administration and guideline implementation, and feeds back "clinical performance" to primary and secondary health care professionals. During 1992 principally demographic data was entered but since 1993 "process" and "outcome" data has been routinely recorded. The impact of DDIS on the "intermediate outcomes" of diabetes care has been evaluated.

	Type 1		Type 2	
	1993 (%)	1996 (%)	1993 (%)	1996 (%)
Systolic BP <160 mmHg	79.5	81.0	61.5	71.9[a]
Diastolic BP < 95mmHg	92.8	91.3	86.9	88.1
HbA_1c < 7.0%	51.4	66.8[a]	66.7	74.4[a]
Cholesterol <5.5mmol/l	40.7	47.6	26.2	37.8[a]
HDL >1.0mmol/l	77.1	85.1[b]	58.2	62.5
BMI <25kg/m²	42.8	45.0	22.4	23.9

[a] $p<0.001$, [b] $p<0.05$ compared with 1993

We conclude that the introduction of DDIS has been associated with improved glycaemic control (Type 1 and Type 2) and coronary risk factors (Type 2 > Type 1), namely dyslipidaemia and systolic hypertension. In this care setting introducing DDIS has been associated with a substantial improvement in the outcomes of diabetes care.

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EVALUATION OF OPTICAL MEMORY CARD SYSTEM TO CONSTRUCT MEDICAL INFORMATION NET WORK FOR DIABETIC PATIENTS

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To prevent the occurrence of diabetic complications, it is important to improve clinical care of diabetic patients at any place he may live or work or travel. In order to do so, it is necessary to construct medical information managing network among medical centers for smooth exchange of present and previous clinical data, such as laboratory data and prescriptions. In this study, we have tried to develop and evaluate the medical optical memory card system to construct medical information network.

[Method and Material]

Medical optical memory card system were constructed with 1) optical memory card {size: 54x85.5mm, WORM type (write once read many), maximum memory size: 4.1 megabytes}, 2) card reader / writer (LC-304, Conlux, Japan) , 3) personal computer, 4) color image scanner, and 5) printer. Various laboratory data including laboratory data and imaging data (X-ray, CT, MRI, RI), stored in the host computer in the hospital or clinics, were able to send directly by LAN (local area network) or transfer to the card system by recording device using text file.

[Results and Conclusions]

Optical memory card system is useful for diabetic patient's medical information managing due to tight security of recorded data, large capacity of memory size, and high-speed reading and recording capacity.

Although further study may be necessary to improve handling of imaging data, we could construct the medical information managing network by this medical optical memory card system.

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I.T. FACILITATED WHOLE POPULATION IMPROVEMENTS IN THE PROVISION OF DIABETES CARE.

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Minimising the adverse outcomes of diabetes depends on regular effective preventative care for everyone with diabetes. Since 1988 in our urban district (population 230,000; 2% diabetes, 872 Type 1, 3746 Type 2) family practitioners and the hospital diabetes centre have been collaborating in a structured preventative care programme. In 1992 a District Diabetes Information System (DDIS) was introduced; it supports care delivery, collects the UK Dataset as a by product of care, and feeds back 'performance measures' to all diabetes care providers. The impact of a DDIS on the district annual performance of routine preventative care processes has been evaluated.

	1993	1995	p (Chi^2)
Annual review	61%	73%	<0.0001
Never reviewed	31%	15%	<0.0001
Key processes completed during annual review examination			
Weight	93%	94%	0.12
Blood Pressure	96%	98%	<0.0001
Eye examination	83%	93%	<0.0001
Foot examination	88%	93%	<0.0001
Renal check	44%	69%	<0.0001
Glycaemic check	65%	85%	<0.0001
Lipid check	59%	69%	<0.0001

We conclude that the introduction of the DDIS has been associated with a marked improvement in preventative care performance.

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The Impact of a Diabetes Electronic Medical Record (DEMR) on the Process of Care for Patients with Diabetes/S Smith, M Murphy, T Huschka, S Dinneen, C Gorman, B Zimmerman, R Rizza, J Naessens/Mayo Clinic Rochester, Rochester, MN, USA **Objective:** To compare the compliance with diabetes care performance indicators for those providers and their patients who utilized a DEMR compared to those who did not. The DEMR is a computerized system for facilitating the management and documentation of care for patients with diabetes. **Population:** As part of the pilot study for Provider Recognition by the American Diabetes Association (ADA); adults (16 years or older) seen in the Metabolic Clinic (MC), Mayo Clinic Rochester during the first quarter of 1996, with a diagnosis of Insulin-dependent or Noninsulin-dependent diabetes mellitus were identified electronically by ICD-9 codes: 250.00-250.93, 362.01, 362.02, 366.41, and 357.2 (total 1235 patients). To be eligible for study, the patient must have had a diagnosis of diabetes for a minimum of one year prior to the MC visit date. They must also have had a visit in the MC coded for diabetes in the prior 12-24 months (238 qualified patients). **Design:** A random sampleof 82 patients were selected for medical record review for the ADA performance indicators. Comparisons between patients whose providers use the DEMR (39) and those who used the paper record only (43) were based on Chisquare tests for percents and Wilcoxon rank-sum tests for ordinal and continuous variables. **Results:** Number of foot exams, blood pressures, and of glycated hemoglobins were significantly better in those providers using the DEMR. **Conclusions:** Process of care and/or documentation for patients with diabetes followed in a subspecialty clinic were significantly enhanced by the use of a DEMR. Translation of these results into general practice needs to be confirmed.

Performance Indicator	DEMR	No DEMR	p Value
Tobacco status and advice to quit	97.4%	95.3%	0.615
Blood Pressures per patient per year	3.87	3.14	0.024
Dilated eye exam in the last year	97.4%	95.3%	0.615
Foot exams per patient per year	3.21	2.07	<0.001
<4 Glycated hemoglobins per year	5.1%	32.6%	0.002
Lipid profile in the last year	84.6%	69.8%	0.112
Urinalysis, if negative microalbumin in last year	53.8%	48.8%	0.650
Mean glycated hemoglobin value (most recent)	9.64%	10.25%	0.192

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MULTILINGUAL COMPUTER ASSISTED HISTORY SOFTWARE FOR DIABETES PATIENTS.

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The ability of physicians and nursing personnel to obtain a detailed medical history from a diabetic patient is often compromised by the lack of interpreters for patients speaking languages other than that of their physician. This problem is especially acute in clinics which provide care for many new immigrants. In order to improve communication in this setting, I have developed software MediSpeak which allows a physician to obtain a detailed medical history from a patient who speaks a different language. Over seven hundred questions in English have been arranged by medical topic and translated into Spanish, Vietnamese, and Russian. By using digitized sound files the computer will "speak" each question in the patient's native tongue. 200 questions apply specifically to patients with diabetes mellitus. All questions are in a Yes-No format or require simple gestures by the patient to indicate location of symptoms. The program has been written in both PC and MacIntosh versions and can be used on most labtop computers at the bedside. A written record of the questions and answers in both languages is available to the physician at the end of the session. This program is presently being used in a medical clinic in the U.S. with a large number of Spanish speaking patients, and has the potential to assist all physicians who treat diabetic patients who speak different languages.

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INSULIN STORAGE BY DIABETIC PATIENTS IN ETHIOPIA.

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A total of 140 Type 1 diabetic patients were interviewed to determine how they store their insulin at home. The mean age of the patients was 31.0±10.2 years. The majority 84 (60%) patients were males & the rest 56 (45%) patients wre females. The mean duration of Diabetes mellitus was 8.3±5.4 years (range 1-27 years). About 20% of the patients have stopped their insulin for some days to weeks because of various reasons. Only 26.4% of the patients store their insulin in refrigerator, the rest they store it in clay pots filled with water and sand 17.8%, in empty tins 17.1%, in cupbords 17.1%, on floors 15.7% and in glasses 3.6%. The mean glycosylated hemoglobin was 11.1±2.3% (range 6.5±17.4%),. The educational status, the income and the glycosylated hemoglobin is not statistically different in the different groups ($P>0.05$), where as hyperglycaemic symptomes are significantly less in patients who store their insulin in the refrigerator ($P<0.01$). In the majority of our patients because of the poor socioeconomic status, The site of storage of insulin is likely to be hot, which might be associated with significant problems of tempreature related deterioration and thereby poor metabolic control.

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CLINICAL PROFILE OF DIABETIC POPULATIONS IN ANDHRA PRADESH : A PILOT STUDY.

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Clinical characteristics of diabetics managed at different hospitals were compiled from a questionnaire based data from 24 diabetologists of Andhra Pradesh state. Majority (72.6 ± 30.8, 0-98 %) diabetics were diagnosed after 30 years age. Among them, 71.5 ± 24.4, 0-95 % were on oral hypoglycemics, 14.3 ± 12.9, 0-44% were non-ketotic yet requiring insulin, 7.7 ± 5.5, 1-18 % were ketosis-prone requiring insulin and 1.1 ± 1.6, 0-5 % were also ketosis-prone but never on insulin. Obesity defined by BMI>24 kg/m^2 was noted in about half (48.8 ± 26.0, 0-90 %) of all adult diabetics. One-third of the diabetics were diagnosed before 30 years age. Among them, 50.4 ± 29.3, 0-90 % were ketosis-prone on insulin, 6.5 ± 9.4, 0-30 % were also ketosis-prone but treated with oral hypoglycemics only, 26.4 ± 19.4, 0-70 % never had ketosis and managed with oral hypoglycemics alone and 14.4 ± 13.1, 1-50 % also had ketosis-resistance but required insulin. BMI was below 19 kg/m^2 in 10.8 ± 10.7, 0-37 % of all young diabetics. Among these young and lean, 10.9 ± 9.7%, 0-30 % were ketosis-resistant with evidence for pancreatic calcification, 38.1 ± 25.9, 0-70 % had ketosis-resistance alone, 39.7 ± 29.4, 0-90 % were ketosis-prone and further 6.6 ± 6.5, 0-20 % had pancreatic calcification along with evidence for ketosis. Clinical types of diabetes and therapy modalities practiced by diabetic care providers in a state were largely unknown due to lack of population based studies and diabetes registries. Data as reported in this pilot study was essential for understanding specificities of diabetes in a region and for appropriate health care planning.

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INTERNATIONAL GUIDELINES FOR DIABETES CARE: A MULTI-CENTER, MULTI-NATIONAL STUDY

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The purpose of this study was to determine whether a single set of guidelines could be adapted for multiple international sites. Ten countries participated in the development and implementation of international- guidelines for the detection and treatment of diabetes and its complications. Using Staged Diabetes Management (SDM), a protocol-based approach to guidelines and clinical pathways for decision-making, diabetes experts in Japan, Poland, Mexico, Brazil, France, Germany, Russia, Canada, Australia and the United States took part in a four step approach to the adaptation of SDM for their country.

Step I: In each country expert committees composed of diabetes specialists and primary care practitioners were formed to coordinate the customization of SDM.

Step II: Each committee customized SDM guidelines for diagnosis, treatment options, therapeutic goals, monitoring and follow-up to reflect available resources and current system of diabetes care. These customized guidelines were supported by Master DecisionPaths (algorithm) for each type of diabetes which provide a roadmap for sequencing treatment options, as well as Specific DecisionPaths to guide starting and adjusting treatments, complications surveillience and management, medical follow-up, patient education, nutritional and exercise interventions and psychosocial assessment and therapy.

Step III: Once consensus was reached, the customized version of SDM was translated into the native language of each country.

Step IV: After review by the International Diabetes Center, implementation models for each country were developed. The implementation process included baseline chart audit, orientation and training, and outcome evaluation. Three different international strategies emerged: diffusion from a single expert center, diffusion through regional centers, and diffusion through individual facitilites or national healthcare providers. In the United States, this approach has already been shown to reduce inconsistency in treatment and to significantly improve glycemic control with average reduction in HbA1c by 1.8%. Complications surveillance has increased by 60% for eye and peripheral vascular disease.

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A practical program of insulin distribution in Zaire.

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The Centre Médical Evangelique (CME) is a large referral hospital in Northeast Zaire. Diabetic care at CME has for many years been severely hampered by the expense of available insulin with a month's treatment costing approximately one person's entire monthly wage. The results of this included apathy about diabetes on the part of medical and nursing staff and the inadequate treatment of many insulin-dependent patients. Since October 1995 CME has received regular supplies of insulin along with some blood glucose testing sticks and insulin syringes from the Insulin Distribution Program of the International Diabetes Institute (IDI), Australia. A total of 3100 vial equivalents has been donated up to December 1996. Only transport and associated costs are paid for by CME. This supply of quality, cheap insulin has altered many aspects of diabetic care at CME. Patients can now afford appropriate long-term treatment so their diabetic control has improved. Lives that previously would have been lost due to an inability to pay for insulin have been saved The numbers of diabetic patients attending at CME has increased approximately 150%. The hospital has gained an enthusiasm for treating diabetes with increasing interest shown by both nurses and doctors. A new diabetic clinic has opened in a nearby town to encourage attendance. Other hospitals in the region are now benefiting from the program and research into diabetes has been stimulated. Scientific data shows the long life of appropriately stored insulin and some vials have been used that are past their nominal 'use-by' date. The large number of different types of insulin received has caused some confusion amongst staff. The future of the project is good but effort is needed to ensure that insulin is as widely available as possible in the region The Insulin Distribution Program which involves in Zaire a partnership between the IDI and CME has been the key to stimulating interest in diabetes in Northeast Zaire Large quantities of insulin are available form various sources around the world but much is being destroyed. We encourage the distribution of this life-saving drug to the many regions of the world where insulin shortage is a reality.

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DIABETES HEALTH PROFESSIONAL STAFFING IN NEW ZEALAND P.L. Drury and R. Cutfield. New Zealand Society for the Study of Diabetes. Auckland, New Zealand.

There is little data on the staffing of specialist diabetic public health facilities in New Zealand (NZ) since a survey 10 years ago. In late 1995 we mailed such a survey to respondents in all 23 Crown Health Enterprises (CHEs) enquiring about nursing/ educational, dietetic, medical and podiatry time devoted to diabetes. Replies were received from all 23 CHEs including a total of 26 individual areas of provision. Results are calculated as whole time equivalents (1 WTE = 40 hrs per week) per 100,000 population served (Total NZ population = 3.54 million).

	Nursing	*Dietetic*	*Medical*	*Podiatry*
Mean WTE	1.86	0.60	0.37	0.14
Maximum	5.00	0.76	0.91	0.32
Minimum	1.02	0.15	0.02	0

Few areas lacked significant nursing or dietetic input, though there were wide variations in provision, not obviously related to local diabetes prevalence or geography - NZ Maori, Pacific Island and Asian communities have very high prevalences of diabetes. Several districts, including some populations > 100,000, still have only token medical time allocated with no trained diabetologist, while 5 districts had no public podiatry service. We conclude that: (1) There are major deficiencies in provision of specialist services for diabetes in most parts of NZ, especially in medical and podiatry time; (2) Staffing levels compare most unfavourably with other countries with much lower prevalences of diabetes; (3) Any attempt to improve the care and outcome of diabetes will currently probably be limited by lack of skilled personnel.

2573

HOW DO INDIVIDUALS WITH DIABETES USE THE ACCIDENT AND EMERGENCY DEPARTMENT?

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Previous studies suggest diabetes is not a risk factor for accident and emergency (A&E) department use, despite being a major risk factor for admission and outpatient attendance. The aim of this historical cohort study was to determine whether individuals with diabetes have a different pattern of A&E department use to the general population. Service use by 696 individuals with diabetes over an eleven year period was compared with use by 696 non-diabetic individuals. The two cohorts were matched on age, sex and general practitioner. The geographical distributions of their addresses were similar. More visits were made by the diabetic cohort (1002 vs 706, p=0.0001). 121 visits by the diabetic cohort were directly related to diabetes, including 52 for hypoglycaemia. The diabetic cohort also had more visits for medical illness unrelated to diabetes (357 vs 231, p=0.0001). The number of visits for injuries, including selfharm, was not significantly different (524 vs 475, p=0.3). Individuals with diabetes were not significantly more likely to be referred by a general practitioner (14% vs 16%) or admitted (20% vs 17%). They were more likely to have arrived by ambulance (36% vs 26%, p=0.02), even for injury related visits (24% vs 15%, p=0.05). Individuals with diabetes make more frequent visits than the general population to the A&E department. These excess visits were for both diabetes and other medical illnesses. There was no excess of visits for injuries. The difference is therefore probably due to greater morbidity rather than a lower threshold for attendance.

2574

NORMAL POPLATION RANGES AND AGE RELATED CHANGES IN HbA1c MEASURED USING THE DCA 2000

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The Bayer Diagnostics DCA 2000 analyser a rapid outpatient HbA1c method is becoming increasingly widely used in diabetes clinics to provide same visit results. Recently a new cartridge which reduces the analysis time to 6mins was introduced, but normal population ranges have yet to be established with this method. The aim of this study was to establish such a reference range in a typical caucausian UK population. Samples were obtained from 102 volunteers with no history of diabetes or serious medical problems. Volunteers were obtained from general practice "well persons" clinics, well visitors to the hospital and hospital staff (age 18-82yr, mean 46.2yr; 54 female and 48 male). There was a clear age related increase in HbA1c, $y=4.75 \pm 0.13x$, $p<0.001$. The mean for this population was 5.2%. Results were not normally distributed hence the normal range was determined using 95% confidence limits. For the whole population the reference range was 4.5% to 6.2%. This reference range is very close to that in the Diabetes Control and Complications (<6.05%) study allowing direct comparisons to be made with that study.

2575

INSULIN STORED IN MATKA (EARTHEN PITCHER) WITH WATER FOR 60 DAYS DOES NOT REDUCE IN BIO-ACTIVTY.

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Background: Several insulin requiring diabetics in India do not have a refrigerator at home. Moreover, supply of electricity is not regular in several parts of India. With an attempt to reduce possible damage to insulin several diabetics store their insulin in *Matka* (an earthen pitcher with water) in a steel box / plastic bag floating in water.

Aim: to ascertain whether insulin storage in *Matka* would lead to reduction in bio-activity, and, to study whether there is any difference between human and bovine insulin preparations in this regard.

Design: Randomised trial. *Comparison groups*: human and bovine regular and lente insulin *Exposure:* storage in Matka for 30 and 60 days in peak Delhi summer (6 samples each for both exposures: total 48 samples). *Outcome variable:* mouse blood glucose bioassay units (total 600 animals, reused thrice: total experiments: 1800) (British Pharmacopoeia 1980)

Results: There was no deterioration of insulin bio-activity on storage up to 60 days. (neither bovine/ human; nor regular/ lente; nor 30 days/ 60 days)

Conclusions: insulin stored in *Matka* does not lose its potency when compared to appropriately stored insulin/ standard insulin crystals. Insulin treated patients in tropics can reliably store insulin in *Matka*.

Acknowledgements: Novo Nordisk (India) Pvt Ltd., Knoll Pharmaceuticals, Torrent Pharmaceuticals.

2576

FUNDIABETES: AN INTEGRALL MODEL OF DIABETES EDUCATION.

N.Sanz, and A.Tami, M.Natera. FUNDIABETES, Fundación de Atención al Diabético, Caracas, Venezuela.

FUNDIABETES, Fundación de Atención al Diabético (Foundation for the care of diabetic patients) was officially established in 1989. Since its initial operation, the foundation has carried out a series of activities aimed at providing the community with Health Education and Prevention Programs. Such activities include health fairs, scientific conferences, press conferences, publication of educational material and patient education and orientation at the foundation's facilities. The institution also provides the diabetic community with free medication or discount rates. Health Fair: The health fair is a one day public event in which the foundation tries to create awareness, among community members, about the complications and consequences of diabetes, obesity, high blood pressure, chronic coronary heart disease and high cholesterol levels. This is done through educational speeches and workshops and by providing immediate on-site health care (blood tests to measure glucose and cholesterol levels, urinetests, blood pressure, etc.). Health fairs are carried out nationwide on a quarterly basis and up until now 14 fairs have taken place. Scientific Conferences: The objective of such conferences is to keep health professionals, linked to the area of diabetes, up to date on latest tendencies and treatment therapies in such field; therefore, the scientific conferences count on the participation of reknown national and international specialists, nurses, educators and students. To add to the conferences, FUNDIABETES holds specialized courses and supervises and participates in research projects (under and graduate thesis). In addition, the foundation has taken part in the following events: I Congreso Bolivariano de Educación Diabetológica; Latin-American Diabetes Association's consensus on " Prevention, Control, and Treatment of NIDDM" as a work group member; a Multicentric Study on Diabetes in Venezuela carried out together with the Joslin Diabetes Center of Boston, DESG and and will participate in the epidimiology study of the WHO already initiated in European countries under the name DIABCARE.

2577

GLYCEMIC CONTROL IN CHINESE DIABETICS MANAGED BY HOSPITAL CLINICS

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There are about 20 million people with diabetes in China, of which only a small minority is treated with insulin. The aim of this study was to assess the glycemic control in insulin-treated Chinese diabetics, managed by diabetes clinics in major hospitals. The data is baseline data collected from clinics, which participated in a study on transfer from locally-produced animal insulin to human insulin. A total of 630 patients from 43 clinics were assessed.

Results (years, median values and span):

Age: 53 (6 - 88); Duration of diabetes: 8 (0 - 41)

Duration of insulin treatment: 2 (0 - 30);

fasting blood glucose: 9.1% (2.9 - 30); HbA_{1c}: 10.9 mmol/l (1.4 - 41)

A total of 81 severe hypoglycemic events over 3 months were reported, corresponding to 0.53 events/patients/year.

HbA_{1c}	% of patients	Glycemic Control*
< 7	9.7%	optimal
7 - 9	39.5%	fair
> 9	50.8%	poor

** Asia Pacific Guideline on management of NIDDM 1995.*

Conclusion

Although the patients represent a mix of IDDM and insulin- requiring NIDDM, we have used the targets for glycemic control defined by the Asia-Pacific Guideline for NIDDM as reference. According to these targets, half of the patients were in poor control, and only 10% could be characterised as in optimal control. This result calls for more detailed investigations on the diabetes management of the world's largest population of diabetics.

2578

"DIRTY" VS "PURE" INSULINS : RELEVANCE, RECOMMENDATIONS AND REQUESTS FROM A DEVELOPING COUNTRY

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Both medical and socio-economic factors dictate the use/ choice/ prescription of bovine-BI (conventional, "dirty"*) versus porcine-PI/ human-HI (monocomponent, "pure"*) insulin, especially in a developing country, without universal health insurance/ reimbursement (* terms projected by the drug industry). [India: (estimates in US$) Annual insulin costs - 14000 units BI = 35, PI = 120, HI = 200; 1990 per capita income = 160, diabetes health care costs (excluding hospital visits) 80-320]. Absolute medical indications for PI/HI being systemic BI allergy and severe insulin resistance, economic affordability appears more to be a social indication. In an attempt towards a medically rational and socially justifiable consensus regarding insulin prescription, we evaluated data on 232 insulin users at our center (fee for service). Among 82 patients initially on BI: (a) during a total of 640 patients years of follow up, only 4 developed systemic insulin allergy necessitating change to HI; (b) 22 changed to HI for social reasons (rich or reimbursement); (c) the remaining 56 patients have continued to use BI, without any problems. Among 150 patients initially put on (elsewhere) or opted for (at our center) PI/HI, 11 patients subsequently sought change over to BI, due to unaffordability. Thus currently (1996), 29% of insulin requiring patients (67 out of 232) at our center are using BI without adverse effects; out of 3 patients who reported local allergy, one opted for immediate change to HI, whereas the other 2 have continued on BI, with disappearance of local allergic symptoms. Current mean insulin doses/ day of BI versus PI/HI users are 34 and 28 U, respectively. Conclusion: In the current economic scenario, we recommend an educated "cafeteria" approach, allowing the patients to choose the type of insulin, but for one of the absolute medical indications for HI. We pray for "HUMAN INSULIN FOR ALL BY 2000 AD", AT AN AFFORDABLE PRICE.

2579

THE EMPHASIS ON CHANGING LIFE-STYLE IN THE TREATMENT OF DIABETIC PATIENTS.

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In a secondary community diabetic clinic, the treatment of diabetics was based on changing the life-style of patients in order to improve glucose control and decrease risk factors for cardio vascular disease.

Methods: Working with a multi-disciplinary team, 90 patients were followed for a period of 6-42 months. The treatment was based on 4 factors:-nutritional education, encouragement to exercise regularly, medication as required and attention was given to the psychological aspects of this chronic disease.

Results:- By working as a team, emphasizing changing life-style, a number of factors were improved in the patients. 1) Decrease in Hb A1c 2) Performance of regular exercise 3) Improved lipid profiles 4) Improved blood pressure control. The percentage of patients with very poor glucose control (HbA1c>10% Normal <5.8%) decreased from 38% to 13%. The percentage of patients with reasonable control (Hb A1c <8%) increased from 34% to 59%. The percentage of patients with marked hypertriglyceridemia >200mg%, decreased from 37% to 27%. The percentage of patients with hypercholesterolemia (LDL-chol.>160mg%) decreased from 23% to 13%. Twenty seven percent of the patients had uncontrolled hypertension at the beginning of the study and only 9% of patients still had uncontrolled blood pressure at the end of the study. The percentage of smokers decreased from 7-4% and the percentage of patients who performed regular exercise increased from 24% to 63%.

Conclusions:- The effective treatment of diabetics depends largely on patient compliance to the treatment. In a multi-disciplinary clinic where the emphasis is on changing patient life-style, along side medication, a significant improvement in glucose control can be achieved, as well as decreased risk factors for cardiovascular disease. These results are facilitated by increased compliance, obtained by a holistic approach to the patients.

2580

DIABETES NIGHT CLINIC - A PROMISSING CARE SYSTEM FOR DIABETELOGISTS AND TEAM

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The target for diabetes management is a good metabolic control, no acute and no late complications by social integration and normal lifestyle.Insulin treated diabetics are more frequent in hospitals than nondiabetics.For this reason we changed in 1985 the hospitalisation procedure into a Diabetic Nightclinic – mix from out- and inpatient management. At 4 p.m.the patient joins the clinic, professional nurses and laboratory assistents note the daily events, blood sugar self monitoring results and the daily dietary specials.Afterwards the patiens have dinner together.Between 8 and 9.30p.m.teaching classes are hold by physicians in groups or be trained individual .Over the night nurses control continously the metabolic parameter.The patients take the breakfast individual and leave the clinic for the day either for work or personal routine. The costs of the Nightclinic are much cheaper than traditional clinics.Our results from 1990-1995: **Basic data:** n = 3421 diabetics, n=1864 female (54,5%) and n=1557 (45,5%), type 1 n= 1779 (52), type 2 n=1613 (47,2),diabetes duration(years) 12,4 ±9,3 (0 - 66,age (years) 45,8±17,6 (9-88),HbA1 10,8 ±2,3% (5,2-20,4%).Insulin treated n=2495 (72,9%), n = 926 (27,1%) oral drugs or other kinds of treatment. Indications for the clinic: poor metabolic control - no efford under outpatients conditions, gestational diabetics diabetes manifestation, acute decompensation without coma,CSII contraindications: mental and physical handicap to leave the clinic for the day. **Results:** average hospitalisation(in nights) 9±4,1(1-30),able to work during inpatient time 77,3 % (n=2644),unable to work 1,1 ±2,8 days (min 0, max 22).By good metabolic control in more than 75% no second hospitalisation was necessary up to two years.Conclusion: The principles of the nigth clinic are good instruments to treat diabetics with poor metabolic control.Costs can be demonstrated.This is a system for the future, but it needs a specialised medical stuff.

2581

QUALITY OF CARE IN NIDDM PATIENTS WITH HYPERTENSION IN MOSCOW.

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To evaluate a quality of primary care in NIDDM patients with hypertension we examined 144 patients who participated in educational programme for Type II non-insulin-requiring diabetic patients. 46 patients from this group (19 male, 27 female) had hypertension. Mean age of these patients was 52,9±1,5 years, diabetes duration 6,2±1,0 years, hypertension duration 12,2±2,0 years, BMI 29,7±1,2 kg/m^2, total cholesterol 6,3±0,2 mmol/l, triglycerides 3,0±0,4 mmol/l. After education diabetes control was improved (HbA1c decrease from 9,9±0,3 to 8,3±0,4%, $p<0,01$, weight loss 3,6±0,5 kg, $p<0,05$). Blood pressure control remained unsatisfactory (systolic blood pressure 160±4 mm Hg, diastolic blood pressure 97±2 mm Hg). We found that 26% have no antihypertensive treatment, 4% use beta-blockers, 17% - angiotensinconverting enzyme inhibitors, 13% - calcium channel blokers, 9% - reserpin, 31% were on combined therapy. 57% of this patients never performed blood pressure self-monitoring, about 50% were taking antihypertensive drugs periodically. Correction of dyslipidaemia was not done in anybody. These results have pointed out great needs for improvement of care in NIDDM patients with cardiovascular risk factors. One of such approaches could be implementation of special educational programme for Type II diabetic patients with hypertension which was initiated in Moscow.

2582

DIABETES CARE IN NAVAJO-SPEAKING PATIENTS

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The Navajo Nation, the largest American Indian tribe living on a reservation in the southwestern US, has experienced rapidly increasing rates of diabetes in recent years with rates 3 times those of the general US population. Many older diabetic patients speak only their native language and require skilled interpreters on health care visits. To examine the impact of language skill on tertiary preventive care for non-English speaking Navajos with diabetes, the 1994 and 1995 Diabetes Quality Care Audits (QA) were supplemented by a special study. Random samples of charts were drawn for the routine QA review of diabetes care practices each year (n=193) from the ongoing diabetes registry at the Shiprock Indian Health Service Hospital. The registry presently includes over 3000 patients who receive care at this regional medical center in the northeast section of the reservation.. In 1995 these charts were reviewed again for notations about interpreters and providers were interviewed about the need for interpreters for each patient. 23 patients were excluded for whom language or blood sugar data were unavailable. 46% (78/170) needed interpreters. Navajo-speaking patients were older with mean age 63 years compared to 52 for those who spoke English adequately ($p<0.001$) and had longer duration of diagnosed diabetes 8.6 years vs 7.3, but the difference was not statistically significant ($p=.18$). Both groups received screening laboratory tests, immunizations and education at similar rates. Non-English speaking patients received more comprehensive foot exams (47% vs 35%, $p=.09$) and fewer eye exams (39% vs 53%, $p=.07$). After adjusting for age, proteinuria, and serum creatinine, the mean of three blood sugars during the preceding year was 211 mg/dl in the patients who needed interpreters compared to 191mg/dl in those who did not ($p=.16$). Although evidence of urinary tract infection was found more commonly on the charts of non-English speaking patients, after adjusting for age, the difference disappeared. With the availablilty of skilled interpreters tertiary preventive care can be given to American Indians speaking their native language. Further research is needed to determine the best ways to improve metabolic control, patient self-care practices, and the basic understanding of diabetes among Navajo people.

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PROSIT-PROJECT: SCREENING AND INTERVENTION FOR DIABETIC NEPHROPATHY

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In order to reduce new cases of end-stage renal disease and to meet one of the targets of the St. Vincent Declaration, a structured screening and intervention programme for diabetic nephropathy has been implemented in Munich, Germany. The screening was performed in offices of 58 randomly selected primary care physicians. In 586 diabetic patients independent of age, sex, duration, type and therapy of diabetes self-screening has been performed using the qualitative dipstick test Micral-Test® S. The urine samples were retested by medical staff using the semiquantitative Micral-Test®. Patients identified with micro- or macroalbuminuria (n=206; 35%) were offered to participate in an intervention programme based on optimization of blood glucose, blood pressure and protein restriction. Physicians were provided with therapeutic recommendations and were asked to retest every 3 months. To support these efforts a special quality circle was founded. In addition training of patients, nurses and doctors was started. After one year no further increase of micoalbuminuria was observed in the patients with microalbuminuria. Preliminary data show that the use of antihypertensives increased markedly with the consequence of a significant lowering of diastolic blood pressure. At the present the PROSIT-Project will be implemented throughout the country of Bavaria as well as in the urban areas of Hannover and Hamburg.

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DETERMINANTS OF ATTENDANCE AND GLYCAEMIC CONTROL IN PUBLIC SECTOR PRIMARY CARE DIABETIC PATIENTS IN CAPE TOWN, SOUTH AFRICA.

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The goal of this study is to identify intervenable risk factors for poor attendance or poor health outcome in diabetic patients in order to design programmes to improve their health. METHODS: A cross-sectional analytic study based on data from patient interview, retrospective record review and HbA1 estimation was undertaken in the three largest ambulatory primary care diabetes clinics in African residential areas in Cape Town. 243 patients were randomly selected from those attending in the last 6 months of 1992. Cross tabulations were used to identify variables associated with attendance of appropriate frequency and regularity and/or HbA1 <10%. Multivariate analysis was used to estimate the strength of the independent associations. RESULTS: **Attendance**: age, pensioner status, sex, knowledge of disease, patients perceptions of care or of staff attitudes, distance from clinic and waiting time were not significantly associated with attendance. Only self perceived health positively predicted attendance (OR 3.69, CI 1.06-12.06). **Outcome**: No predictors of outcome were found; neither attendance, nor the factors listed above. CONCLUSION: No intervenable risk factors could be identified for either attendance or outcome. This failure is unlikely to be explained by instrument or data quality and suggests that the investigators models of causal associations with attendance and outcome are inadequate. Alternatively, the complex web connecting demographics, patient attitude, service interaction and attendance and outcome is highly-variable, consequently far larger samples are required to detect associations in what are likely to be small subgroups. More qualitative research on patient perception of the determinants of attendance is required and interventions based on these should undergo trial.

2585

GLYCEMIC CONTROL IN A PRIMARY CARE HMO PRACTICE WITH DISEASE AND CASE MANAGEMENT.

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This study is designed to determine if there is improved glycemic control using a proven disease management system, ***Staged Diabetes Management*** (SDM) in a primary care practice of a group model HMO. SDM stresses attention to timelines and glycemic targets, with rapid modification of therapies until an effective therapy is achieved. To test this system, 131 patients (predominantly with Type II diabetes) and sub-optimal glycemic control were selected. In this 1 year study, diabetes care is being provided by a diabetes nurse clinician, internal medicine physicians, clinical pharmacists, and registered nurses, all following the SDM system. In the first three months of the study the average hemoglobin A1c (HbA1c) declined from 9.00 ± 1.4 at baseline to 8.38 ± 1.16 ($p < 0.001$). At the beginning of the study 13% of patients had HbA1c < 7.5, whereas after 3 months on SDM this had increased to 30%. Our research indicates that the most important factors in improved care are: decreased practice variation by providers due to universal implementation of ***Staged Diabetes Management***, practical knowledge-based case management which was stratified to reflect the complexity of the patient's medical condition, and improved teamwork in the management of this stratification. Thus, data from the first three months on this system demonstrate the effectiveness of a systematic, comprehensive data and time-sensitive approach to blood glucose control. Trained case managers can be empowered to treat most patients with diabetes.

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DIABETES NURSING-DEFINING THE PRACTICE

A. Nettles, D. Fleming, M. Funnell, D. Guthrie, D. Hentzen, D. Kruger, C. Trapp, E. Walker, and J. Williams.

Advanced Practice Nurses are making an effort to promote better use of diabetes nurses in the health care system and to increase awareness of their contribution to people with diabetes. They are currently finalizing a description of the scope and standards of practice of the diabetes nurse educator and the advanced practice diabetes nurse in the US. Definitions from the American Nurses Association were used as a framework. This presentation will include a description of the process, methods and resulting document. Two levels of specialization will be presented. This document will be used to influence curricula in schools of nursing and develop levels of continuing education. A national advanced certification examination is being considered. Much of diabetes nursing care is unpublished, under-reported, and varies from country to country. Nurses outside of the US may find this document helpful for developing a description of their own. Where such descriptions already exist, new opportunities may develop for international consensus on practice commonalties resulting in collaborative research, policy development, and international educational opportunities.

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DIABETES DISEASE STATE MANAGEMENT BY DIABETES EDUCATORS IN MANAGED CARE.

L Blonde, R Guthrie, JL Parkes, and BH Ginsberg, The Ochsner Clinic, New Orleans, LA, USA

Staged Diabetes Management (SDM) is a program that is designed to change the practice behavior of health care providers (HCP). It contains algorithms and practice guidelines for primary care physician (PCP) and other HCPs to assist them in delivering better and more consistent diabetes care to their patients with diabetes. We have set up a 12 month study of SDM in which diabetes educators are managing 130 patients under the supervision of an endocrinologist. Control subjects are being treated according to the usual practice of Ochsner clinic by primary care physicians. The primary end point is hemoglobin A1c (HbA1c), but the full analysis includes process data, and quality of life analysis. At this time, the interim, HbA1c results (and number of subjects) are:

Group	At Entry	Delta At 3 months	Delta At 6 months
SDM	8.6±1.6 (123)	1.3 (94)	1.8 (29)
Control	8.2±1.7 (99)		0.5 (26)

The SDM group had a 1.8 point fall in HbA1c at 6 months $p<0.001$), whereas the small decrease in the control population was not significant. In addition, 70% of patients in the SDM group achieved a HbA1c within 1.5 of normal compared to 30% in the control group. We conclude that SDM is effective in a managed care setting when performed by diabetes educators and results in substantially lower hemoglobin A1c, values which should eventually lead to lower incidence of diabetes complications. This may allow less expensive, more appropriate care for patients with diabetes

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TRIVANDRUM MODEL OF RURAL DIABETES CARE FOR DEVELOPING COUNTRIES - A 5 YEAR EXPERIENCE.

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It was planned in the year 1990 to care for the rural diabetic population of Trivandrum district of Kerala State in South India. As a first step many one day diabetes camps were organised in rural areas. Later on 24 permanent monthly diabetes clinics were started in such ideal locations as to cater for the diabetics detected at the above camps. A main diabetes centre was set up in Trivandrum City to care for the urban diabetic population and also to co-ordinate the functioning of these rural monthly clinics. A team consisting of a diabetologist, laboratory technician, pharmacist and dietician visits these clinics every month to impart free diabetes care. During each visit to these clinics, diabetes education is given by the diabetologist and dietary advice by the dietician. Blood sugar estimation is done by means of Glucometer and their treatment updated. Each of these rural clinics is attended by 150 to 200 diabetic patients every month. Our experience during the last 5 years shows that 90% of these diabetics are controlled by this method and this model of rural diabetes care seems to an ideal for developing countries.

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PRACTICE AND PROFESSIONAL CONCERNS AMONG MEMBERS OF THE AMERICAN ASSOCIATION OF DIABETES EDUCATORS (AADE).

M. Graff, Longmont Clinic, Longmont, K.Ernst, Emory Clinic, Atlanta, J. Norman, Department of Health, Olympia, C.Tobin, Conrtol Diabetes Services, Atlanta, C. Steil, Samford University, Birmingham, and R. Rubin, Johns Hopkins, Baltimore, USA.

This study assessed practice and professional concerns among a representative sample of the membership of the American Association of Diabetes Educators (AADE). Areas assessed included practice responsibility and concerns, national certification as diabetes educators, advanced practice credentialing, and continuing education. A random sample of 731 AADE members, of whom 66% were nurses and 25% dieticians, completed a mailed survey. Responses were reliable to within 3.7% at 95% confidence. Respondents reported a high level of practice responsibility: 43% practice as a diabetes treatment team leader or case manager; 54% serve patients without supervision in problem solving and in assessment. When asked what they considered the most important issue facing diabetes educators, the largest number of respondents (33%) said third-party reimbursement for their services. Sixty-eight per cent of respondents said they were certified by The National Certification Board for Diabetes Educators (NCBDE), originally founded with support from the AADE A majority of respondents said they believed that an advanced practice credential was necessary to enhance the specialty of diabetes education among physicians (59%), and third party payers (50%). Forty per cent of respondents said they had attended an AADE Annual Meeting, and 42% said it was their leading source of continuing education. In addition, 36% own the Core Curriculum in Diabetes Education, published by the AADE, and a majority devote at least 30 minutes to reading the monthly AADE journal, *The Diabetes Educator*. The results of this study show that members of the AADE have a high level of practice responsibility, and seek opportunities offered by certification, the potential of advanced practice status, and the continuing educational offerings of their Association to further their professional development. An organization such as the AADE significantly enhances the professional practice of its members

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AMBULATORY PATIENT GROUPS (APG) AND DIAGNOSTIC RELATED GROUPS (DRG): A BASIS FOR FUNDING CALCULATION

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Due to the high cost of inpatient care, combined with reduction in hospital bed availability, many institutions have moved away from the inpatient model of diabetes management to an ambulatory care model. While DRGs have been created to monitor throughput and resource use of inpatient services, APGs for diabetes have not been well developed. The aim of this study was to use a computerised database to monitor the resource utilisation of a large ambulatory diabetes centre. Using computerised records on 2994 patients, caseload profiles were generated for different services. The cost of providing each service was calculated based on the hours spent on direct patient care + apportioned indirect cost, then converted to a bed day equivalent (BDE).

Service	No. of Encounters *	Total hrs *	BDE
Stabilisation †			
IDDM	6.4	2.4	0.6
NIDDM	5.6	2.3	0.6
Commencement of insulin †			
IDDM	15.6	9.2	2.4
NIDDM	8.7	5.9	1.6
GDM ‡			
Diet alone	11.6	2.4	0.7
Insulin required	11.8	4.0	1.2
Management of neuropathic ulcer †	7.9	10.1	2.6
Education §	1.0	1.0	0.2

* including telephone contact, † 3 month figure, ‡ average follow up 13.3 weeks, § per hour

This study showed that adapting work practice to incorporate systematic collection of data facilitates monitoring of the human and fiscal resources required for diabetes ambulatory care. It also served to highlight a broader perspective of the roles of computerised databases. In turn, this information can form the basis of APGs which, in addition to DRGs, can be used for funding calculation.

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SPECIALIST DIABETES NURSES ARE EFFECTIVE IN THE CARE OF DIABETIC IN HONG KONG

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Background. It is advocated that Nurse Specialists trained in diabetes care, with adequate support from specialist physicians and other health care resources, should contribute substantially in the care of diabetic patients. **Aim.** We aimed to study the effectiveness of Specialist Diabetes Nurses in caring for diabetic in Hong Kong. **Subjects and Methods** In this non-comparative study, we recruited 30 newly admitted, uncomplicated diabetic patients who had no active concomitant medical illnesses. They were first jointly assessed by specialist physicians and Specialist Diabetes Nurses. Then they were followed up by Specialist Diabetes Nurses for 6 months. During this period, the Specialist Diabetes Nurses provided education, performed complication screening, monitored the diabetic condition, ordered investigations, recommended self-care methods, prescribed drugs and adjusted drug dosage. The Specialist Diabetes Nurses also coordinated the other health care disciplines to take care of the patients. **Results.** At the end of 6 months, 28 diabetic patients aged 28-76, duration of diabetes 0-11years, were available for analysis. Twenty-four patients were on oral hypoglycemic drugs, 2 patients were on insulin and 2 patients were on combination treatment. Body weight decreased from 64.5±12.0kg to 63.4±11.5kg ($p=0.053$). Fasting blood sugar reduced 19% from 10.2±2.7mmol/L to 8.3±1.9mmol/L ($p=0.005$). HbA1C reduced 22% from 8.6±2.3% to 6.7±1.1% ($p<0.001$). Cholesterol decreased from 5.6±1.0mmol/L to 5.2±0.9mmol/L ($p=0.022$). Triglyceride decreased from 2.3±2.7mmol/L to 1.6±1.4mmol/L ($p=0.1$). No severe hypoglycaemia occurred. The mean number of nurse consultation was 8.3. In addition, no hospital admission was required and all patients were satisfied with this health care model. **Conclusion.** We conclude that Specialist Diabetes Nurses are effective in providing care to diabetic patients in Hong Kong.

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CURRENT STATUS OF DIABETES CARE IN CHINA.

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Diabetes is a major health problem in China as more than 20 million people have it. To assess the current status of diabetes care in China, we conducted a situational analysis focusing on the various stakeholders in the health care system. Twelve tertiary care hospitals in 5 cities (Shanghai, Hangzhou, Chengdu, Xian & Beijing) were surveyed. We also met with officials from the Ministry of Health. The analysis showed that the majority of patients with diabetes are cared for at the district and community hospital level, where diabetes care is rudimentary and there are no diabetes trained physicians. Patient education was mainly conducted by physicians with nursing staff playing a limited role. Greater nursing input was seen as important; however, this was hindered by a shortage of nursing staff at both the endocrine ward and hospital level. Dietitians are almost non-existent in the Diabetes Team. Even at the tertiary care level, there is a shortage of physicians trained in diabetes to provide care. Some departments had as many as 4000 patient visits/year cared for by one or two physicians. Patients with diabetes are often admitted for treatment of diabetic complications, glycemic control and initiation of insulin therapy. The average length of stay is ~28 days. Barriers to care identified by those interviewed include the high cost of therapy, an inadequate understanding that diabetes is a life-long and serious disease by both patients and administrators, inappropriate health beliefs, and the absence of a team approach to diabetes management. These findings indicates that (1) diabetes patient education is in its infancy in China; (2) more health professionals should be trained in diabetes care; and (3) a social marketing program is needed to convey the message to patients, healthcare professionals and administrators that diabetes is a common, growing, serious health problem in China but one that can be controlled with appropriate care.

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COMPUTERIZATION OF DIABETES DISEASE STATE MANAGEMENT

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Diabetes Disease State Management programs, such as Staged Diabetes Management (SDM) provide methods to translate important clinical research like the DCCT into widespread practice by primary care physicians. Computerization of these programs will allow better access and usage of these important tools. SDM, designed to change the way we deal with patients with diabetes, is based upon 5 principles: 1) Community involvement in setting diabetes care guidelines, 2) Negotiation of therapeutic goals with patients; 3) Appropriate timelines for therapeutic success; 4) Use flowcharts for medical decisions; and 5) Evaluation and appropriate alteration of the program. This program is being implemented in countries throughout the world. Previous studies have shown that this program lowers hemoglobin A1c by about 2 points over 6-12 months. Since it is a flowchart-based system it lends itself to computerization. The computer program uses a split screen. One part contains the flowchart, while the other has hypertext information screens giving detailed practical clinical and therapeutic information specifically related to the selected section of the flowchart. As the health care worker progresses along the flowchart they are informed about important aspects of diagnosis and treatment and the steps that they take are documented by the computer. The computer program also contains algorithms to help with diagnosis and a database that is compatible with DiabCare. Currently the program is written in C++, but is being rewritten in HTML to be used with the Internet and Intranets. Use of computer programs such as this by primary care health care workers should result in better diabetes care.

2594

STAGED DIABETES MANAGEMENT: THE PILOT STUDY IN MEXICO
J. Rodríguez, Barry Ginsberg, Patricia Sosa, Reyna Núñez, LE Ortiz, CSU 8 Prados, SSA, Tultitlán Mex. In order to assess the level of feasibility, implementation and effectiveness of Staged Diabetes Management in Mexico, a pilot study was started in 1995. Ten centers from various institutions (public and private) were enrrolled, with the goal to achieve improvements in compliance, metabolic control and reduction in morbidity as primary outcomes. The first participant facility is a health center located in a suburban area. Patients treated come from a low socioeconomic status, have no access to social security and have very limited resources. Training of a general practitioner, provision of printed materials for the doctor and patients was started in October 1995. After one year of follow-up, 74 patients have been treated with this program, 21 males and 53 females; 2 patients are insulin-dependent and 72 non insulin-dependent. The majority of the patients had been diagnosed from less than 5 years: 38 had history of hypertension, 55 are obese and 6 had previous cardiovascular events. By comparison to initial blood glucose levels, patients receiving medical care with Staged Diabetes Management showed a progressive improvement: initial mean fasting glucose levels are 219.5 mg/dl; consecutive levels decrease to 1661.1, 147 and 156 mg/dl respectively ($p < 0.001$). Compliance with medical indications has been high, concurrent morbidity has been low, and the rate of patients in good control has increased from 20% (initial) to 55% (fourth visit). In conclusion, Staged Diabetes Management is a feasible, economic and effective program for outpatient management by general Mexican doctors.

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STAGED DIABETES MANAGEMENT: WORLDWIDE DIABETES DISEASE STATE MANAGEMENT
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To help provide the best possible control of patients with diabetes, we have produced a disease state management system for diabetes, called "Staged Diabetes Management"(SDM) and are implementing it worldwide. SDM, designed to change the way we deal with patients with diabetes, is based upon 5 principles: 1) Community involvement in setting care guidelines, 2) Negotiation of therapeutic goals with patients; 3) Appropriate timelines for therapeutic success; 4) Use flowcharts for medical decisions; and 5) Evaluation and appropriate alteration of the program. SDM is designed to be altered by a country to meet its needs and resources. It helps primary care physicians deliver better care using a team approach and to co-manage patients with specialists when appropriate. It has a complete set of materials for the community, the individual health care provider and the patient. Models of SDM have been developed and tested in both developed areas of the world and areas with emerging health care systems. Country-specific models of SDM are being implemented in the USA, Canada, Mexico, Brazil, Poland and Japan. A meta-analysis of 7 clinical trials with over 450 patients has shown an average fall in hemoglobin A1c of 1.8 points (equivalent to a drop in mean blood glucose of about 3.5 mM or 65 mg/dL). Preliminary pharmacoeconomic analysis demonstrates a lifetime cost saving of over $27,000 per patient. In summary, the diabetes health care team needs to work with primary care providers to prevent the complications of diabetes in their patients. Staged Diabetes Management provides the tools for this task

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PRIMARY CARE MANAGEMENT OF HYPERTENSION AND DYSLIPIDEMIA IN INDIVIDUALS WITH DIABETES MELLITUS
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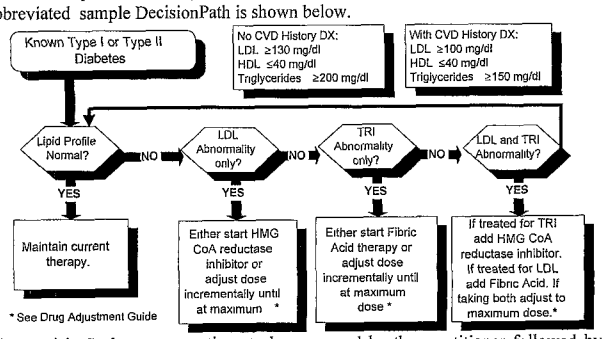
The current management of hypertension and dyslipidemia in individuals with diabetes varies widely in the primary care setting. In order to reduce this variation, specific practice guidelines and DecisionPaths (algorithms) for the management of these diseases have been incorporated into Staged Diabetes Management (SDM), a comprehensive set of guidelines and clinical pathways for the management of diabetes in the primary care setting. The practice guideline define the blood pressure (130/80 mmHg) and lipid level (see figure) for diagnosis. Treatment options and targets are highlighted as well as appropriate times for monitoring and follow-up. Specific DecisionPaths, with algorithms for guiding clinical decision-making have been developed for lifestyle modifications and pharmacologic therapies. An abbreviated sample DecisionPath is shown below.



The DecisionPath poses questions to be answered by the practitioner followed by suggested course of action. Results show implementation of these guidelines have lowered both diastolic and systolic hypertension by an average of 5 mmHg and have improved overall consistency in management of hypertension and dyslipidemia in several primary care settings.

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QUALITY OF DIABETES CARE IN SELECTION-FREE POPULATIONS (JEVIN-ZEUVIN) - DO WE NEED DIABETOLOGISTS?
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In 1990 in the former Eastern Germany the health care system was decentralized. Increasingly, patients were treated no longer by diabetologists, but by non-specialized doctors. In 1994/95 90% of all insulin treated patients (IDDM/NIDDM n = 127/117), aged 16-60 years, living in the city of Jena (100,247 inhabitants) were examined by a physician (JEVIN). In Jena the diabetes care unit still exists. 37% of IDDM and 32% of NIDDM patients were regularly seen as out-patients. For comparison, in a district without out-patient unit (28,000 inhabitants, all patients were treated by non-specialized doctors), 81% of insulin treated patients (IDDM/NIDDM n = 25/33) were examined (ZEUVIN).**Results:** HbA1c (mean normal 5%, HPLC, Diamat^{R}) in IDDM: ZEUVIN 9.1 ± 1.6% vs JEVIN: Patients treated by diabetologists 7.7 ± 1,4% ($p = 0.0002$), patients treated by non-specialized doctors 8.6 ± 1.9% ($p = 0.17$). HbA1c in NIDDM: ZEUVIN 9.3 ± 1.9% vs JEVIN: Patients treated by diabetologists 8.3 ± 1.9% ($p = 0.04$), patients treated by non-specialized doctors 9.0 ± 2.0% ($p = 0.54$). There were no differences ($p < 0.05$) between the groups in respect of age, diabetes duration, BMI, incidence of severe hypoglycaemia, ketoacidoses and quality of life. Since decentralization of the health care systems there are significant differences in quality of diabetes care between patients treated by diabetologists and those treated by non-specialized doctors. Following recommendations of the St. Vincent Declaration and to prevent costly diabetes complications maybe resulting from a lower quality of care, specialized diabetes treatment must be available for all patients. In particular in rural areas out-patient units and diabetologists seems to be mandatory.

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MICHIGAN DIABETES OUTREACH NETWORK MODEL: A SYSTEM OF SUCCESSFUL INTERVENTIONS AND OUTCOMES

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The Michigan Diabetes Outreach Network Model is essentially a nurse case management system which focuses on standardizing the care and education provided by agencies to persons with diabetes. Various studies indicate strong efficacy of the Network model. For its initial four years of operation, UPDON demonstrated the following results for its partner agency participants compared to non-participants in the Upper Peninsula of Michigan: **Hospitalizations were 45% lower, mortality 27% less, and amputations 31% lower.** Types of agencies involved include home nursing care, outpatient rural health clinics, Indian health centers, and health departments. The presentation will describe the model and its care requirements in the areas of lower extremity assessment, hypertension, eye disease and nutrition. The data collection system will be described and discussed. Certified Diabetes Educators provide professional education programs to the staff of participating agencies across the region including self-paced, self-instructional learning modules for nurses which are approved for continuing education credits. The Network serves as a diabetes resource center for the public, people at risk for diabetes, and those with diabetes. The Network model has been expanded to cover the entire state of Michigan. The presentation will provide specific guidelines for implementing this successful model in other regions.

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TITLE: DIABETES HOME EDUCATION BY COMMUNITY HEALTH WORKERS IN INDIGENT MEXICAN AMERICANS

AUTHORS: B.W. Roberts, N. Alvarado and A. Garza, STOP Diabetes, San Antonio, Texas, USA

Home Outreach Prevention Education by Community Health Workers (CHW) for Type II diabetes in Mexican Americans (MA) was done over a four year period in a low income area of San Antonio, Texas, sponsored by the Texas Diabetes Council. Over four years there was transient, but no statistical improvement in elevated hemoglobin A1C (HgbA1C) and lipid levels. Many of the 65 target families received financial support, completed less than secondary school education, reported physical and substance abuse, lack of family support and mental health issues to deal with in their lives. The CHWs were trained over a three month period by a certified diabetes educator and paid at minimum wage ($5.00/hour US). They visited each client family at home weekly for one hour, held weekly screenings in the target community, and held weekly client classes for another 35 diabetics not visited at home (control group). Over the 4 years there was no improvement in HgbA1C and lipids in this group. The CHWs intervened in the social and economic issues of the families 5.3 x monthly and 68% had a positive health outcome. Half of the referrals were to hospitals, specifically for diabetes, or to a community service agency. Only 4 client families refused to follow-up with their referral. We conclude that indigent MA Type II diabetics can benefit from home outreach education, but have many serious psychosocial and economic problems that must first be addressed in order to have successful diabetes care.

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„Treatment patients with NIDDM according to SDM Standards in Łódź"

The aim of the study was to assess the metabolic control of patients with type II diabetes mellitus treated after the introduction of Staged Diabetes Management (SDM). All the patients were referred to our department; previously they were unsuccessfully treated in outpatient clinics in the Łódź district.

The subject were 41 randomly selected patients aged 52-71 years (mean 61.9 years), including 24 women and 17 men. The duration of diabetes was 2 to 21 years (mean 9.9). Most of them were overweight: BMI 28-37 (mean 31 kg/m^2).

Nine had late complications such as retinopathy, eleven polyneuropathy and two nephropathy. Hypertension was present in eleven cases.

Six months after SDM, metabolic control was assessed as the levels of HBA_{1c} and mean blood glucose (MBG) - fasting and postprandial. HBA_{1c} decreased from 9.0 ± 0.75 % to 8.25 ± 0.7 % ($p<0.001$), fasting MBG 140.5 ± 24.2 mg/dl to 119.2 ± 17.3 mg/dl ($p<0.0001$) and postprandial MBG 184.4 ± 19.4 mg/dl to 161.2 ± 14.9 mg/dl ($p<0.0001$).

During the period of evaluation no severe hypoglycemia was observed. Reduction of weight was obtained: mean BMI decreased from 31 to 29.8 kg/m^2 ($p<0.05$). There was no weight gain.

CONCLUSION: The introduction of the SDM program, along with the inevitable „study effect", led to significant reduction of HbA_{1c}, fasting MBG and postprandial MBG without hypoglycemia or weight gain in a representative group of NIDDM patients.

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DECREASE OF AMPUTATION RATE AFTER INTRODUCTION OF FIRST POLISH INTERDISCIPLINARY DIABETIC FOOT CARE-TEAM MODEL

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It is estimated that the rate of amputation in diabetic foot patients rangers from 30 to 50%. According to the recommendations of the St. Vincent declaration the first interdisciplinary team of diabetic foot care consisting of a diabetologist, ortopedist, general and vascular surgeon, kinesitherapist, educator and shoe-maker was established at the Department of Metabolic Diseases. The team makes collaborative decisions about diabetic foot patients, including the decisions about amputations. Over 2,5 years of activities 196 patients have received treatment, including 114 patients with ulcerations. Of this group 100 patients were hospitalized, 14 received out patient treatment consisting of guided antibiotics, wound managment, intensive insulin therapy, immobilization accompanied by isometric exercises. Among these 114 patients amputation above the knee was decided upon in 2 (2,3%) patients, and sparing amputation in 4 (3,7%) patients. An overall amputation rate was 6%. **Conclusions:**The low rate of amputations in patients with foot ulcerations indicates the usefulness of this therapeutic model. The efficacy of treating diabetic foot may be improved by collaborative assessment therapy and recommendation with no extra costs.

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NURSES' CLINIC: A NECESSARY ASPECT OF DIABETIC CARE.

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Saint-Louis hospital's Diabetology nursing staff inside the hospital's project, planned out the nursing care project for the department, according to the medical team project.Seventy-two percent of inpatients suffer from diabetes, the nursing and medical staff felt that the creation of a Nurses' Clinic had become a necessity. Several staff members define :1/The objectives :- verifying patient knowledge regarding self-test and injecting equipment as well as patient glycemic control diary completion;- nursing follow-up planning.2/ The organization :- in terms of material resources (a consultation room);- in terms of human resources (ten nurses carry out on consultation). 3/ The daily considerations :- one clinic open on every weekday;- by appointment; - using a common follow-up tool: the patient's file. This project was endorsed by the department's general staff meetings. The nurses ' Clinic opened on April 25th. As of October 1996: 399 weekday clinics were planned; 368 clinics were effectively held; 1285 patients made an appointment and 1103 patients came to their appointment (85%).After two and a half years, we were able to observe the following: - clinic nurses remain satisfied with their work; - their competence is well-recognized by physicians, patients and their relatives;- objectives are more clear ;- patients are discharged with a follow-up summary.On the other hand : - many patients are now referred to the Clinic because of lower-extremities lesions, even though that was not one of the nurses 'initial objective.The nurses and Head Nurses perform a follow-up evaluation of the Clinic on a twice yearly basis in order to examine and solve organization and attendance issues. Malfunctions are evaluated and corrected.

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„TREATMENT PATIENTS WITH IDDM ACCORDING TO SDM STANDARDS IN ŁÓDŹ"

The aim of the study was to assess the metabolic control of patients with IDDM treated with insulin according to the principles of Staged Diabetes Management (SDM).

The subjects were 46 randomly selected patients, aged 18-58 years (mean 34.2 years): 27 women and 19 men with IDDM of duration from 3 months to 33 years (mean 11.5 years), BMI 18 - 30 (mean 23.2 kg/m^2).

Twenty three patients had no complications, four had retinopathy (two with proliferative retinopathy), one with nephropathy, four with retino- and neuropathy (one with proliferative retinopathy), two presented retino- and nephropathy and twelve had retino-, nephro- and neuropathy.

After six months of treatment following the introduction of SDM, metabolic control was assessed by levels of HbA_{1c} and mean blood glucose levels (MBG), both in fasting and postprandial. Significant decreases of HbA_{1c} and MBG values after treatment were found. HbA_{1c} fell from 8.25 ± 0.83 to 7.50 ± 0.74 % ($p<0.0001$), fasting MBG from 156.8 ± 48.9 mg/dl to 123.1 ± 14.4 mg/dl ($p<0.0001$) and postprandial MBG 195.5 ± 42.1 to 157.4 ± 21.2 mg/dl ($p<0.001$).

Unlike the DCCT, neither severe nor clinical significant hypoglycemia was observed during the treatment. The average BMI in the group was not changed ($p>0.05$).

CONCLUSION: The introduction of the SDM program, along with the inevitable „study effect", led to significant reduction of HbA_{1c} and fasting and postprandial MBG without hypoglycemia in a representative group of IDDM patients.

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Unnecessary costs provoked by prescription of drugs for the treatment of diabetes related long-term complications in East-Germany

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Introduction: In Germany, services of the health care system have to follow the rule of economic efficiency (§ 12 SGB V). However, the prescription of non-effective drugs is a common problem in Germany [Heise et al., Dt Ärztebl 1995; 92: A3549]. The aim of this study was to estimate the costs that arise in East Germany due to the prescription of drugs with unproven efficacy for the treatment of diabetes related long-term complications. Patients and Methods: Data of every patient with IDDM or NIDDM (n = 459; age 55,8 ± 18,1 [12-93] years) that was treated in the in-patient diabetes care unit of Jena in 1994 has been recorded consecutively with the aim of analysing the drug therapy. The following drugs have been classified as not suitable for the adjuvant treatment of diabetes related complications: alpha-lipoic acid (ALA), calcium dobesilate (CD), ginkgo biloba (GB), "agents for improvement of vene function" (IVF), vitamin B combinations (VBC), pentoxyphylline (PE) and naftidrofuryl (NA). Since controlled clinical trials could not prove any relevant therapeutical benefit of these drugs, they are not listed in international treatment recommendations. Results: 16,8 % of the patients were treated with one or more of the above mentioned drugs. The most frequently prescribed drug was ALA (45,5 %), followed by CD (33,8 %), GB (20,8 %), IVF (16,8 %), VBC (13,0 %), PE (11,7 %) and NA (5,2 %). These drugs caused costs of about 960 DM per patient and year (drug prices in 1994). Conclusion: Presuming a diabetes prevalence of 5 % and a prescription of drugs similar to the one in this study, drugs with unproven efficacy would cause costs of more than 120 million DM per year in East Germany (15 million inhabitants) and an estimated 640 million DM in total Germany (80 million inhabitants). These findings show that there is a huge potential for saving costs in the German health care system.

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EVALUATION OF PHONE CALLS AS A LINK BETWEEN DIABETIC PATIENTS AND A DIABETOLOGY DEPARTMENT.

ML. COTTEZ, F. GRONDIN, JF. GAUTIER, Endocrinology Department, Saint-Louis Hospital, Paris, France.

The aim of our study was to perform a quantitative and qualitative evaluation of the phone calls we receive from diabetic patients. We designed a standardized telephone message form to register each call. The yearly number of calls were: 206 in 1992; 377 in 1994; 559 in 1995; 472 as of Oct. 1996. The forms are placed on the nurses' desk close to the phone. A standardized set of questions and answers was designed by the team to homogenize nurses' questions and answers. **Results:** the various motives for calling were comparable in 1992 and 1994. These were: 1/ adaptation of insulin doses, hypoglycemia, hyperglycemia 43,% ; 2/ technical problems 22%, 3/ other 17,9%, infections and intercurrent diseases 15%, travel across time zones 1,3%. During the first ten months of 1996, the motives were: 1/ adaptation of insulin doses, hypoglycemia, hyperglycemia 51,9%. 2/ infections and intercurrent diseases 19,1%. 3/ technical problems 15,5%, other 12,3%, travel across time zones 0,6%. The decrease in technical problems could be explained by the creation of the department's nurse clinic for outpatients in April, 1994. **Conclusion** : Resorting to a standardized message form for each call is useful because it leaves a trace of incoming calls, the name of the patients as well as the nurses, a homogeneous response, increased knowledge of patients' difficulties and an improved link between patients, nurses and doctors.

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STRATEGY TO DECREASE THE COST OF DIABETES CARE IN DEVELOPPING NATIONS

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Diabetes represents a socio-economic problem in the third world where the gross National Product (GNP) can be under $500 per capita in some developping countries like Haiti. In these countries only a small prosperous minority can afford the medical treatment of diabetes which costs the patient up to $900 yearly for ambulatory care. To overcome that difficulty, the Fondation Haitienne de Diabète et de Maladies Cardio-Vasculaires (FHADIMAC) has developped the following strategy: most part of the fees paid by members (over 2000) is used to buy generic drugs and insulin at a preferential rate. Drugs are sold at low cost to members of Fhadimac and insulin is given free to IDDM patients under 30 years of age. With this strategy, diabetics members at the Fhadimac spend between $30 to $147 (including drugs) for the management of their disease which represents a decrease 6 to 30 times less than for patients outside Fhadimac. Young Insulin Dependant Diabetics are treated for a symbolic charge. Conclusion. In developping nations where the large majority of patients can not afford the cost of treatment of diabetes and where the government are unable to meet the health needs of the population, diabetes association in using such strategy can allow access to quality care for the needy.

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USING PATIENT CHARACTERISTICS TO DETERMINE THE COST OF CARE: IS NEAR ENOUGH GOOD ENOUGH?

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The Diagnosis Related Groups (DRGs) classification, originally developed as a quality assurance tool, has been widely adopted as a basis for funding health services. The DRG classification is intended to assign acute inpatient episodes to classes which are clinicaly similar and resource used homogeneous. Funding is then allocated prospectively according to projected patient loads. The purpose of this study was to determine the extend to which this objective was met in a sample of acute inpatients with one or more diagnoses related to diabetes. The sample comprised all discharges (n=2094) with one or more diabetes diagnoses from acute care hospitals in the Illawarra Area Health Service in 1993-1994. A subsample of 386 records was selected for a more detailed analysis by chart audit. There were 3 major findings. First, the discharges were distributed among many DRGs in a way which was neither clinically coherent or resource use homogeneous. Second, there were many data errors which resulted in assignment to a DRG that did not reflect the clinical state of the patient or the resources used in management. Third, the DRG logic appears to ignore or underestimate the costs associated with treating diabetes as a secondary diagnosis. Diabetes diagnoses have little influence because DRGs generally fail to take account of resources used to treat conditions other than the principle diagnosis. DRGs use LOS as the primary predictor of cost, therefore in an ideal classification, DRG assignment would explain 100% of variations in LOS. Data analysis in this study showed that the DRG assignment explained only 28% of variation in LOS by the sample. Results of this study indicate that hospitals will not be reimbursed for the complete cost of care under the DRG system.

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BUILDING A SHORT-TERM COST-EFFECTIVENESS MODEL FOR ORAL ANTI-DIABETIC DRUGS IN THE U.K.

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A short-term (6 months) cost-effectiveness model has been built to simulate current medical practice and disease progression in type 2 diabetes patients who have not responded to diet and exercise alone. Current medications are associated with short-term side-effects, such as gastrointestinal upset and hypoglycaemia, which may lead to poor compliance, treatment cessation, and therapy switching. These outcomes, in turn, may contribute to both suboptimal control and increased healthcare resource utilisation. As the study is designed from the healthcare payer's perspective it includes only direct medical costs. The model framework was developed using treatment guidelines and expert opinion on treating type 2 diabetic patients. Main data sources for the model were published literature, and IMS Mediplus and EPIC GP databases. Mediplus yielded data on the probability of treatment cessation, reasons for stopping treatment, replacement medication and concomitant agents taken for treating any side-effects. Data was collected for first and second-line therapies after diet. EPIC provided detailed data on healthcare consumption related to therapy switches. Final outcomes are measured in cost per symptom-free day. From the oral antidiabetic agents available in 1996 (biguanides, sulphonylureas and acarbose) it is found that metformin is 3 times more cost-effective in the short-term than an acarbose strategy. Sulphonylureas were almost twice as cost-effective as acarbose. Analysis by subgroups (obese versus non-obese; elderly versus young) did not change these findings. Current databases are an excellent source for building medical practice and disease progression simulation models. This model can serve as a framework for assessing the cost-effectiveness of new interventions.

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POTENTIAL COST SAVING ASSOCIATED TO EXCESS AMPUTATIONS IN DIABETIC SUBJECTS IN AREA 7 MADRID.

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We estimated the direct cost of nontraumatic lower extremity amputations (LEAs) in diabetic (DMP) and non-diabetic (NDMP) population between January 1st, 1994 and December 31st, 1995 in area 7 of Madrid, Spain, taking in consideration the duration of the hospital stay, the duration of rehabilitation in the out-patient clinic after discharge, and the use of artificial limbs and their maintenance. The average cost of a LEA was 4 million pts. Total direct costs per year were 74.654.510 pts in the DMP and 40.918.603 in the NDMP. The total cost estimated, if the incidence of LEAs in DMP were identical to the one found in NDMP, was 2.574.292 pts per year. Bearing this in mind, potential cost saving associated to excess amputations in diabetic population was more than 72 million of pts per year, 12.6 millons of pts per 100000 inh. This study reveals that potential cost saving could be estimated and would be achieved if subjects with diabetes mellitus could have a satisfactory foot care system.

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National Diabetes Programs

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ONE YEAR FOLLOW-UP OF PATIENTS WITH TYPE II DIABETES AFTER IMPLEMENTATION OF STAGED DIABETES MANAGEMENT.

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Six hundred and twenty-one patients with type II diabetes treated at 27 clinics by primary care physicians were randomly selected in order to evaluate their care prior to the introduction of an innovative community-based program designed to implement standardized care protocols modified by community practitioners. From this population 3 clinics were randomly selected for close follow-up. The clinics did not differ significantly on any of the key study variables (size, population served and composition of health care professionals). For each clinic, a detailed audit of diabetes practices prior to and one year following program implementation was ascertained by chart audit and interview for 62 randomly selected patients. These patients did not differ significantly from the 621 patients at entry for age (62 ±12), sex (46% male, 54% female), type of treatment (11% food, 45% oral agent, 5% combination and 39% insulin), complications rate (55% hypertension, 5% kidney disease, dyslipidemia 10% and eye disease 10%) or level of glycemic control (52% HbA1c > 8%). Forty-five professionals (physicians, nurses and dietitians) at these 3 clinics participated in the training program in which criteria for screening, diagnosis, treatment and follow-up were modified based on standard protocols from Staged Diabetes Management (SDM). At entry, HbA1c was 8.72 ± 2.18% (normal range 4.1-6.4%). One year following implementation of SDM average HbA1c reduced significantly ($p<.001$) to 7.51 ± 1.43%. As part of SDM protocols, patients in poor metabolic control (HbA1c >8%) are rapidly moved to alternative therapies. The health professionals at these sites selected specific criteria for starting and changing therapies: diet only therapy was begun when fasting plasma glucose (FPG) at diagnosis or during treatment was <200 mg/dl, oral agents (sulfonylureas or biguanides) were initiated when FPG was between 200-300 mg/dl and insulin (multiple injections) was begun when FPG was >300 mg/dl. If the HbA1c did not decrease by 0.5-1%/month on the initial therapy, the practitioners were expected to increase the dose or change the therapy. In this study, changes in therapy occurred in 61% of the patients. Finally, significant improvement in glycemic control occurred without severe hypoglycemia, or adverse acute complications.

2611

Problem in Diabetes Awareness -Using Bahrain as an Example from the Arab Region

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The main aim of this paper is to identify problems in diabetes awareness - using Bahrain as an example from the Arab Region.

Problems in diabetes awareness occurs when the individual is unable to assess positive, negative influences on personal health and cannot evaluate their susceptibility to various illnesses. The individual also cannot be engaged in self - assessment; not aware of health values, nor decision making and lacks responsibility for health and self-care.

The problems of awareness may be from the diabetic individual who may not comply and set barriers (due to level of education, age; attitude; cultural influences; religions belief and health practices; and lack of accepting the problem); the health professional (not updated with diabetes knowledge and skill; not aware; shortage of manpower and load). health care facilities (not within the reach, no budget, no community link or diabetic education); media (not much or weak media advertisements; no link with health resources; lack of continuity).

In Bahrain inspite of small population (half million), free health care and within the reach, yet problems of diabetes awareness exists. The shortage of manpower, that cannot be freed for the specialized diabetic care. However, there is no actual structured diabetic education. There is no budget and at the ministerial level diabetes care is not visualized as a specific but as something that must be integrated within the general care even at the Primary Level. Besides the diabetic client is faith controlled surrendered to the health care professionals. There is no decision or participation in planning of self-care.

The word "diabetes" is sensitive, few talk freely about it, also causes social pressures and media is inadequate. Therefore such a barriers are blockers to awareness and those who are diabetic advocates are battling diplomatically.

2612

THE WILL FOR PROPHYLATIC TREATMENT OF MEN AT RISK TO GET ADULT DIABETES.T.Virtanen.Satakunta Polyth.Inst.Pori.FINLA

The study searched for a group of people at risk to get adult DM from among the employees of three offices of the town of Pori,Finland.The main aim was to find out their will to action which would keep up their health.The men were 40-60 years old and the risk to get adult DM was based on their own risk factors as well as those occurring in their families. In the second stage men got questions about their knowledge,attitudes and motives for selftreat ment of adult diabetes. They were also on an information meeting in order to get motivation for selftreatment. The found group at risk was 166 persons from 349 men.The risk of getting adult DM was widely known but the subjects were not aware of their own predisposition to getting it even a great number of risk was found ih their families.Those who answered questions of nutrition seemed to have a good knowledge on the general level (77/121 answers) but only half of them was aware of the risk of fat.The main motives for exercise were the prevention of illness,becoming physically fit and feeling good after the exercise.Those who completed the questionnaire found it positive to its focus on health and personal interaction.One of the objectives of the project was also reached when its follow-up study was started.

2613

QUALITY OF DIABETES CARE IN A SELECTION-FREE POPULATION OF DIABETIC PATIENTS FIVE YEARS AFTER DECENTRALIZATION OF THE HEALTH CARE SYSTEM (JEVIN)

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In 1989/90 a population-based, selection-free trial of insulin treated diabetic patients aged 16-60 years and living in the city of Jena (ca. 100,000 inhabitants) was started. In total 83% of the target population (IDDM/NIDDM n = 131/59) was examined in 1989/90. At the follow-up examination in 1994/95, 5 years after the decentralization of the health care system, 83% of these patients (IDDM/NIDDM n = 87/44) were reexamined.

Results: For IDDM patients under specialized care HbA1c/mean normal was 1994/95 similar to 1989/90 (1.6 ± 0.28 vs 1.55 ± 0.34, p = 0.52, n = 32), it was higher for IDDM patients under non-specialized care (1.73 ± 0.35 vs 1.52 ± 0.34, p = 0.002, n = 55). For NIDDM patients there were no differences 1994/95 vs 1989/90 (1.8 ± 0.36 vs 1.74 ± 0.36, p = 0.4). The incidence of acute complications (severe hypoglycaemia with need of glucose or glucagon injection, ketoacidosis) remained stable. The prevalence of nephropathy and peripheral neuropathy (assessed according to Young et al.) remained stable too, but the prevalence of retinopathy increased in both IDDM and NIDDM (71/79% vs 45/20%, p = 0.001/0.001).

In order to achieve optimal quality of diabetes care and to prevent diabetes long-term complications, specialized diabetes treatment must be available for all patients.

2614

PREVENTION OF DIABETIC FOOT.

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The aim of this manual is to teach primary health care professionals, a clinical based method for screening of thehigh risk foot diabetic patients. A screening sheet was designed for annually examination of all diabetic patients. This evaluation assigm a proportion of risk to the following risk factors: 1. Previus ulcer or amputation (30%), 2. Clinical Neuropathy (20%), 3. Clinical Vascular periferic disease (15%) 4. Foot deformities (15%), 5. Irresponsability or Alcoholism (5%), 6. Established Nephropathy (5%), 7. BLindness (5%), 8. Diabetes longer than 10 years, living alone, retinopathy, low education and male sex (1% each one). Patients who get less than 20%, are considered at low risk, and are annually reevaluated. Patients who achieve 20% or more are considered at high risk, and are specially educated to prevent ulceration; they also receive podologist care, and a more frecuent medical control.

2615

A NOVEL NATIONAL DIABETES PROGRAMME USING A DISEASE MANAGEMENT APPROACH.

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The progressive rise in morbidity and mortality from non-communicable diseases poses an increasing problem for health care providers in developing countries. A novel approach is being undertaken in Ghana through a national diabetes disease management programme. The objective is to provide a measurable improvement in delivered diabetes health care using patient outcomes (acute/chronic complications and mortality). The five year programme has three major components: an epidemiological survey, a nation-wide training programme for health care professionals and the establishment of a national register. The epidemiological survey is being performed using World Health Organisation guidelines and will study 5000 individuals. The prevalence of diabetes complications will also be examined. The training programme has been initially developed with an external centre of expertise and is now managed entirely locally. A "train the trainer" approach is taken and local resources utilised wherever possible. Selected multidisciplinary teams from existing centres underwent training covering all aspects of diabetes management as applicable to local conditions, and the programme is now expanding to first regional, then district level. The Ministry of Health is the main provider of health care in Ghana, and governmental involvement is ensured by representation on the newly formed National Diabetes Advisory Board which oversees the entire project. Throughout the project period data will be collected to establish a national register and monitor diabetes outcomes. Cost of treatment data will also be collected. The disease management multidisciplinary approach has typically been considered for developed countries. It is anticipated that this project will not only provide hitherto largely unavailable information concerning the prevalence and cost of diabetes in sub-Saharan Africa but, if successful, will demonstrate the effective use of available Western partnerships and provide a template for national diabetes health care delivery in developing countries which is proven to be both effective, sustainable, and cost efficient.

2616

THE AUSTRALIAN NATIONAL ASSOCIATION OF DIABETES CENTRES: NEW ORGANISATION, NEW HORIZONS

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Despite strong professional and consumer organisations for diabetes there were no organisations specifically catering for the needs of specialist diabetes services. To address this deficiency, the Australian Diabetes Society and Australian Diabetes Educators Association jointly established a National Association of Diabetes Centres (NADC) - possibly the first of its kind. The NADC links Australian Diabetes Centres in the common aims of i) improving the quality and accessibility of diabetes care ii) providing peer support for Diabetes Centres and services and a forum for pooling ideas and information and iii) lobbying funding sources to increase ambulatory care services for people with diabetes. The NADC comprises a National body which oversees policy development, membership and accreditation of Diabetes Centres, and lobbying at a national level; and State Sections which deal with local implementation issues, local lobbying, partnerships with other state level organisations, and developing and supporting local service networks. Since its inception in 1994, the NADC has documented goals and objectives, developed structure and process criteria for accrediting Diabetes Centres, policy for optimising diabetes control, and criteria for auditing the clinical outcomes of diabetes care. Several of the NADC State Sections are working with their respective State Health Departments to develop and/or advise on diabetes programs; or networking with rural and remote health professionals to provide specialist training and support. Although the NADC is a new organisation it has more than 30 member Centres and its potential to support improvements to the quality and accessibility of diabetes care are already apparent. New horizons include the use of accreditation criteria to lobby for resources to increase the number of accredited Diabetes Centres, extending the scope of the NADC to include smaller diabetes services, improving network links with non-specialist service providers and implementing national initiatives to improve the quality of diabetes care and information.

2617

RESEARCH SOCIETY FOR THE STUDY OF DIABETES IN INDIA (RSSDI) : TWENTY-FIVE YEARS OF ACADEMIC ACTIVITIES

P.V. Rao on behalf of the RSSDI Secretariat.
Nizam's Institute of Medical Sciences, Hyderabad, India.

RSSDI founded in 1972 by Prof. M.M.S. Ahuja, Prof. B.B. Tripathy, Prof. Sam G.P. Moses, Late Prof. M. Viswanathan and Late Prof. B.R. Sengupta, is the largest diabetes education, training and research organization supported by 795 clinicians as life members representing all Indian States and Union Territories. Diabetes Bulletin : International Journal of Diabetes in Developing Countries, the official bi-monthly Journal of RSSDI is in 16th year of publication. Continuing medical education programs of RSSDI organized by senior teachers of Medicine, Endocrinology and Diabetology throughout the country over past 25 years in particular RSSDI Post Graduate Courses in Diabetology were popular with many senior and young diabetologists. To maintain high standards of CME programs and to train more clinicians in diabetes care, RSSDI has commissioned National Accreditation Committee for Diabetology in 1995. RSSDI Diabetes Consensus Development Program was initiated for providing physicians with a current consensus statement in 1997 on diagnosis, follow-up, targets, screening for complications, management and education. Further proposed RSSDI activities include School of Diabetology, Indian Text Book of Diabetes Mellitus and, workshops for patient empowerment and training in diabetic education technology.

2618

RESULTS OF ACTIVITY OF KARAGANDA DIABETES CENTER ON DIABETES CARE IN KASAKSTAN IN 1996

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1996 characterized by aggravation of finacial supporting of diabetic patients by Government, intensive desorganization of system of providing of patients by drugs accompanied by collapse of finacial state of biggest part families of diabetic patients. As result we have uncontrollable increasing of hard complications, mortality and uncontrollable situation with diabetes care in result of paralysis of economic of Kazakstan. In this difficult conditions in result including our intensive efforts the national Diabetes program (1997-2001) was confirmed by goverment on the base of program prepared by us in 1994. 29 schools for diabetic patients were organized by us in main clinics and policlinics in capital towns of 15 Lands from 19 which has been provided free of payment by a good materials. Control of blood glucose and selfcontrol by using of test stripes and glucometers was introduced in 14 Lands. Regional diabetes programs were prepared for 14 Lands. 29 regional meetings were organised in 14 capital towns and cities of 12 Lands including main conference in Karaganda. Our practical activity were widely supported by Boehringer Mannheim.

2619

NATIONAL DIABETES PROGRAM IN BELARUS

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The St. Vincent Task Force for Diabetes was set up in Belarus by the Ministry of Public Health and Endocrinology Association in 1993. Regional and local health authorities have been visited to understand the problems encoutered in local services and to discuss ways in which the St.Vincent Declaration (SVD) aims might be met. A number of recurrent themes have been identified: education, the role of patient, epidemiological studies and registar. Following the recommendation of the SVD, an Action Programme for preventing lower limb amputation has been designed, Diabetic Foot Center was organized in Minsk. Basic characteristics of the National Diabetes Program in Belarus are the following: National Diabetes Plan endorsed by government and the most important objectives of the National Program are the following: early diagnosis and therapy in order to decrease morbidity and mortality rates; prevention, early diagnosis and treatment of acute complications; primary preventive system (screening for those at reask for the development of diabetes mellitus), secondary (good metabolic control of the disease and prevention of further development), tertiary (up-to-date treatment, rehabilitation) prevention; providing specialized care for children with diabetes, reinforcing existing diabetic center, organizing training and teaching in diabetes management, prevention of congenital malformation in infants of diabetic mothers, initiation of the central republican registar for diabetes; secure increased funding for diabetic care, monitoring and control systems for diabetes.

2620

DIABETES EDUCATION IN TURKEY

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In Turkey, as a developing country, diabetes education is not readily available to patients and health professionals, despite the fact that Turkey, with a population of 65 million, has two million citizens with diabetes. Nonspecific education programs for patients with diabetes existed in our country until the last 1993. National Diabetes Program (UDP) of Turkey, a governmental organization, was established in March 1994 to improve diabetes care. One of its goals is to educate not only diabetics but also health professionals. From March 1994 until today a lot of patients have been educated at inpatient and outpatient clinics. Multidisciplinary course program for physicians, nurses and dietitians was held on December 1994 in Ankara. Two postgraduate courses were organized for general practitioners on April, and June 1996 in Istanbul and Samsun respectively. In January a special course on diabetic foot and September and December 1996 the 4 days training courses that are named "Education Educators" for nurses were held respectively in Istanbul. In addition to these, except for UDP, a TV program was arranged in a private TV for two months. During this program telephone contact was made with diabetics, families and public. A lot of published materials were prepared with financial help of medical companies. Briefly, we have continued diabetes education in Turkey. But we have a lot of difficulties and deficiencies on this subject. These are: Low educational status of patients, cultural and language barriers between professionals and people with diabetes, lack of continuously formal training for patients, over workload of professionals and lack of time, lack of cooperation among professionals, lack of motivation, lack of resources, or wasting of resources on effective and inefficient programs, and the conventional strict separation of secondary and tertiary care from primary care is never really appropriate.

2621

A COMPREHENSIVE SYSTEM FOR QUALITY IMPROVEMENT IN MEDICAL CARE OF BLIND DIABETIC PATIENTS - 7 YRS EXPERIENCE

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The prevalence of blindness in persons with diabetes in different countries ranges from 0.2 to 0.6%. According to St. Vincent Declaration goals, Polish Assoc. of the Blind in cooperation with Dept. of Gastroenterology and Metab. Dis. of School of Medicine in Warsaw started in 1989 a comprehensive programme for improving quality of life of blind diabetic patients (BD) in Poland. A pilot study (62 BD aged 20-50 yrs with type 1 diabetes) showed that the main problems of BD were low level of knowledge about diabetes, poor metabolic control, difficulties with self-preparation of meals, self-control and self-administration of insulin. The first step a comprehensive programme for education and rehabilitation of BD was started and was effective with respect to improvement of metabolic control of diabetes and of self-dependence of patients. As a result of this trial 11 courses for BD and for theirs families were organized in different parts of Poland with over 300 of participants. In the next step a Registration System of Blind Diabetics has been developed and a comprehensive study of diabetes care was performed.A total of 824 BD (219 type I, and 605 with type II diabetes) were studied. In 1992 a Centre for BD was founded in Warsaw in 1992 and also 4 others and 11 Clubs for BD in different parts of Poland. Conclusions: This study showed that visually impaired patients with diabetes needs special attention and care. The quality of life of BD may be improved based on education and rehabilitation programmes provided by interdisciplinary teams.

2622

GLYCOSYLATED HEMOGLOBIN AS A DIAGNOSTIC METHOD FOR DIABETES.

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Our aim was to compare glycosylated hemoglobin (HbA1c) level with OGTT as a screening and diagnostic method for diabetes mellitus. 405 patients (158 male, 247 female, median age 61 and 56 yr respectively) sent by their physicians for a 2-hour OGTT (75 g load) had a fasting HbA1c level measured with the immunoassay DCA 2000 (Ames). There was good correlation between HbA1c and fasting ($r = .78$) or 2-hr glucose level ($r = .80$). Patients were classified by the NDDG criteria into three categories: Normal ($n = 309$), intolerant ($n = 29$) and diabetic ($n = 67$). Mean ± SD of HbA1c levels were $5.38 \pm .42\%$, $5.92 \pm .53\%$ and $7.02 \pm 1.32\%$ respectively; all differences being significant ($p < 0.01$). With a cut-off level of $< 5.5\%$ for normality, the test was able to discriminate diabetics from nondiabetics (normal + intolerant) with a high sensitivity (100%) and low specificity (53%); the 5.8% cut-off gave 87 and 80% and the 6.1% limit, 78 and 93% respectively. The Receiver Operating Characteristic curve shows the greatest deflection at the 5.5; 5.7 and 5.9 levels. With the WHO classification criteria results were similar. We conclude that HbA1c measurement may be a valid alternative to the OGTT for screening (cut-off level 5.5%) or for diagnosis of diabetes (cut-off value 6.1%). The greater cost is counterbalanced by its simplicity, rapidity and less discomfort to the patient. Time should tell which method will be more adequate to predict chronic complications.

2623

MONITORING DIABETES WITH CAPTURE-RECAPTURE IN DOMINICA: A MODEL FOR THE WEST INDIES.

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It is argued that use of capture-recapture (CAP-RECAP) may provide more accurate estimates of diabetes frequency and complications than the practice of pooling data from two or more sources. Accurate information about diabetes frequency is required in developing countries for proper allocation of limited resources for diabetes care at the local and national level. **Aim:** The aim of this study was to determine the utility of CAP-RECAP for developing a surveillance system to monitor diabetes frequency and consequences on the Caribbean island of Dominica. **Methods**: Three sources of information (the Dominica Diabetic Association, Regional Health Clinics, and the Queen Elizabeth Hospital) were used to identify individuals who were listed as having diabetes between January 1, 1995 and December 31, 1995. CAP-RECAP and log-linear modeling were used to calculate national and regional estimates of the number of diabetes cases. **Results**: As of December 31, 1995 there were 1945 observed cases of diabetes representing a crude prevalence of 2.71 %. The ascertainment- corrected estimate was 2688 [95%CI(2548-2828)] with a prevalence of 3.75%. At the national level, the pooled data from the sources of ascertainment accounted for only 72.3% of the estimated number of cases. Regional ascertainment % (observed/estimated) varied, ranging from >90% in rural regions to <50% in the most urban area. **Conclusion**: Surveillance with CAP-RECAP can yield more accurate estimates of diabetes frequency than current methods used in Dominica, particularly in the urban regions. This surveillance model may help other Caribbean countries improve their efforts to monitor diabetes .

2624

REINFORCED HEALTHY LIVING ADVICE FAILS TO PREVENT DIABETES IN SUBJECTS WITH INCREASED GLUCOSE LEVELS

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Few randomised clinical trials have investigated whether dietary and exercise advice can delay or prevent progression to NIDDM in subjects with increased but not diabetic fasting glucose levels. 189 subjects with fasting plasma glucose (FPG) 5.5 – 7.7 mmol l^{-1} on two occasions were randomised to either reinforced at 3 monthly clinic visits (50%) or basic (50%) healthy living advice in a factorial design with sulphonylurea (50%) or placebo (50%) therapy. 152 (80%) subjects (45% male), mean (SD) age 51 (9) years, body weight 81 (14) kg, median (iqr) FPG 6.1 (5.6,6.5) mmol l^{-1}, HbA1c 5.8 (5.5,6.1)% and 26% diabetic on OGTT completed 3 year follow-up. Subjects were advised to adopt a low fat, high unrefined carbohydrate diet, lose weight if BMI > 22 kg m^{-2} and increase physical activity until exercising 3–4 times per week. Admitted dietary intake was assessed by 3 day food diaries and fitness by maximal oxygen uptake (VO_2 max) during a submaximal bicycle ergometer test. At 3 years the reinforced group significantly increased VO_2 max (2.3 vs 2.1 l min^{-1}, p = 0.0009), % energy from carbohydrate (47.5 vs 44.5, p = 0.016) and reduced % energy from fat (32.4 vs 35.5, p = 0.0095). There were no significant differences between the groups for mean changes in body weight (–0.2 vs 1.1 kg), blood pressure (–1/0 vs 1/1 mm Hg), FPG (0.1 vs 0.0 mmol l^{-1}), HbA1c (0.0 vs 0.0 %) or lipid levels (LDL cholesterol –0.1 vs 0.0 mmol l^{-1}) and no difference in proportion progressing to diabetes in the reinforced group 29% to 43% diabetic compared with the basic group 24% to 33%, p = ns. In conclusion, conventional reinforced healthy living advice resulted in increased physical fitness and some dietary change but did not delay transition to diabetes over 3 years.

2625

NEED BASED SERVICES FOR IDDM PATIENTS IN THE COMMUNITY - THE CHENNAI (MADRAS) EXPERIENCE

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Typical Type I or IDDM occurs mainly in children or young adolescents. In the western countries its prevalence is 0.1 to 0.3% while in India it has been reported at about 0.01%. Inspite of this low Incidence, in India, its large sub-population of children and young adults (1/3 of total population at any given time), coupled with the fact that nearly 50% are below the poverty line, makes IDDM a major health issue wherein serious economic, social, cultural, personal and psychological problems arise in addition to medical complications of IDDM. A detailed study of 30 Juvenile Diabetics (16 males and 14 females) in the age group 10-25 years and 30 parents (16 fathers and 14 mothers) was undertaken. 70% of parents reported severe financial strain and were greatly relieved by the comprehensive "free medicare" offered by our Juvenile Diabetes Comprehensive Care Centre. Negative academic performance and ignorance regarding the role of the social worker was also noted. Based on their needs, our centre aims at providing such comprehensive care by integrating medical and para medical workers' efforts (Diabetologists, Diabetes Educators, Social Workers, Psychologists, Active Rehabilitation Services, Trained Diabetic Nursing Services, Counsellors, Awareness Programmes etc) through voluntary effort supplimented by Governmental and Philanthropic support, in the Non-Governmental sector. This is perhaps the only way a developing country can cope with the vast Medical and Socio Economic challenges encountered in caring for the young IDDM patients as illustrated in individual case studies.

2626

SOCIAL REHABILITATION OF YOUNG PEOPLE WITH DIABETES IN KHARKOV.

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The aim of work is to help young people with diabetes in their personality realization. The Diabetes Youth Club "Juventas" and Kharkov boarding school for children with diabetes are the main subject of work. The development of main psycho-social problems and the determination of those in wich the help is possible, the results evaluation is making by means of questionnaires, interviewing and intercourse. The practical correction based on the longitude method (5 years) is included in all club events. As a result at the time there is the increasing of social adaptation and social activity, increasing of emotional satisfaction in members of the club. 60% state not less 2 times increasing of life quality. It is supported by the medical data of compensation stabilization in the club members.

2627

REGIONAL SURVEY OF WORKING-AGE PHARMACOLOGICALLY TREATED DIABETICS IN CENTRAL FINLAND

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A total of 2375 pharmacologically treated diabetics aged 16 - 64 were surveyed in Central Finland, which has a total population of 258 000. The sample contained 87 per cent of the diabetics in that age group who received insurance reimbursement for insulin or peroral hypoglycaemic agents in the year 1994. Most of the diabetics in the region are treated by their own general practitioners and local diabetes nurses. The central hospital outpatient department is responsible for the care of 20 per cent of the diabetics studied, but it provides consultation and education for the whole region. The pharmacological treatment consisted of insulin in 58 percent, tablets in 30 per cent, a combination of insulin and tablets in 11 per cent, and insulin pump in 1 per cent of the patients. The mean HbA1c levels (with normal values 4 - 6 per cent) in these four groups were 8.6, 8,2, 9,0 and 7,0 per cent, respectively. In the IDDM subgroup (817 patients on sole insulin therapy whose diabetes was diagnosed before the age of 30 year) 22 per cent had a HbA1c level below 7.5 per cent (normal values 4 - 6 per cent), and 14 per cent had a level above 10 per cent. There was a highly significant improvement in the distribution of HbA1c levels in the same IDDM subgroup between 1988 and 1994 (chi square, $p = 0.00001$), the proportions of patients with HbA1c levels of 10.5 per cent or more being 20 per cent, and 10 per cent, respectively. A diabetes care system based on primary care, diabetes nurses, and a strong consultant support is able to achieve acceptable goals and to improve its performance.

2628

DETECTION AND TREATMENT OF DIABETIC RETINOPATHY USING STAGED DIABETES MANAGEMENT

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Although retinopathy (DR) is a serious complication of diabetes no clear guidelines for its detection and treatment have been implemented in the primary care setting. The aim of this project was to develop guidelines for management of DR in primary care practice. In our region, 95% of the individuals with diabetes are treated by primary care physicians. A recent assessment of quality of diabetes care in 22 rural primary care settings indicated that <25% of individuals with diabetes have annual dilated eye examinations. A one year follow-up chart audit after adoption of Staged Diabetes Management (SDM) (a comprehensive set of guidelines and clinical pathways for the management of diabetes in the primary care setting) revealed a significant ($p<.01$) improvement (47%) in the number of patients receiving an annual dilated eye exam. While this was a notable improvement, many individuals with diabetes were still not receiving adequate eye examinations. More importantly, the quality of the eye examination and follow-up care was variable. In order to assure consistency in screening and diagnosis as well as to provide treatment options, a separate section on diabetic retinopathy was developed as part of SDM. At the core of the section are a practice guideline and DecisionPaths for diabetic retinopathy. The practice guideline covers criteria for diagnosing mild to moderate nonproliferative diabetic retinopathy (NPDR), severe NPDR, and proliferative diabetic retinopathy (PDR). It also describes the current treatment options for diabetic retinopathy (metabolic control, panretinal and focal photocoagulation, and vitrectomy) along with establishing clear screening and follow-up schedules. A DecisionPath (algorithm) for screening and diagnosing DR was generated to guide the primary care provider through the assessment of risk factors for diabetic retinopathy (level of control, duration and type of diabetes), dilation of pupils, description of retinal lesions, and appropriate time frames for referral to eye care specialists. In addition, a quick reference table listing the stage of retinopathy and a detailed description of the clinical presentation of the retinal lesions associated with DR is included. A series of color retinal photographs provides a visual reference for identifying microaneurysms, dot and blot hemorrhages, lipid exudates, cotton wool spots, neovascularization of the disc and elsewhere, as well as vitreous hemorrhage.

2629

DIABETES RESEARCH IN CANADA.

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Research is an important and integral component of a nation's diabetes program. Defining its magnitude and type for a nation can be difficult. We determined the status of diabetes research in Canada in 1992-1993 from information obtained on funding of diabetes research in Canada, diabetes research in Canadian medical (13/16), nursing (14/31) and nutrition (9/19) schools, diabetes researchers in Canada, and publications on diabetes-related topics from Canadian institutes. The total funding on diabetes research was $21.2 mm, with $19.5 mm for operating grants and $1.6 mm for personnel support. Governmental agencies funded $5.7mm, non-governmental diabetes-related agencies $6.3 mm and non-diabetes-related $0.8 mm, industry $6.3 mm, and foundations $2.1mm. Canadian Federal agencies funded $0.19/capita for diabetes research. Whereas all medical schools (13) conducted diabetes research, very little research was done in nursing (2/14) and nutrition (1/9) schools. Areas of research included etiology, pathogenesis, complications, care, clinical trials, genetics and epidemiology. Three non-medical departments were also involved in diabetes research. There were 144 individuals involved in diabetes research, varying from 3 to 35 per medical school. Seven others (6 in nursing and 1 in nutrition) were also involved. Publications on diabetes-related research from databases for 1989-1993 revealed 199-290 publications/ year from Medline and 0-10/year from CINAHL. There were 1026 publications from Medline and only 20 from CINAHL databases, reflecting the little diabetes research in nursing and nutrition schools. Government, diabetes organizations and industry are the 3 major funding agencies for diabetes research in Canada. On a per capita basis, Canadian federal agencies provided relatively low funding for diabetes research. Most of the diabetes research are done in medical schools with very little diabetes research in nursing and nutrition schools.

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ANALYSIS OF DIABETOLOGICAL HUMAN AND STRUCTURAL RESOURCES IN PIEDMONT, AN ITALIAN REGION.

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Piedmont, a region of North-Western Italy, has a population of 4.5 milion and a prevalence of 3% diabetes mellitus. According to a national law and its regional implementation, a network of diabetological public services takes care of most cases of diabetes, both IDDM and NIDDM.

Aim: to give a detailed analysis of the present situation of the diabetological network.

Methods: direct interview of the chief physician of each service to collect data about the medical and paramedical staff, the structure and organization of the clinic, technical resources and time allocated to patients. The interviewers were 8 diabetologists specially trained for this task. The data, stored in a data base, were statistically analysed.

Results: 71 services (over 95% of the region) were interviewed; of these 40% are diabetological outpatients clinics (2 with dedicated wards and/or 6 with day hospital), 49% internal medicine clinics, 5% endocrinological and 6% pediatric clinics. In more than 50% of cases the clinics are open 5 days/week, for an average of 8 hours/day. 20% of clinics have less than 500 patients, 62% between 500 and 2000 and 8% over 2000 patients. 30% of clinics have a specialized and dedicated diabetes team, 50% have an internist or an endocrinologist. 60% of clinics have general nursing staff, while 50% have at least 1 diabetes nurse specialist; of these 71% have followed special diabetological courses. 53% of clinics do not have a dietitian and the diet is prescribed by a doctor. No service has a psycologist or chiropodist internal to the staff. In 39% of service, medical time is 60'-120' per patient per year, while it is 180' in 8%. Nursing time risulted 60'-120' per year in 42% of cases, 180' in 8%. 48% of services carry out an average of 2 or 3 visits for patient/year, only 5% carry out more than 4 visits. 80% of services can also offer ophthalmological, nephrological, cardiological and neurological counselling.

Conclusion: in Piedmont there is a very effective public diabetologic network which provides care for 85% of the diabetic population and a good provision of counselling services; on the other hand there is a discrepancy between nursing and medical time, there is a lack of dietitians and a total absence of chiropodists and psycologists.

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PATIENTS' EDUCATION AND COMPLICATION SCREENING IN ROUTINE OF DIABETIC CLINICS. RESULTS OF A SURVEY IN AN ITALIAN REGION.

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INTRODUCTION: Piedmont, a region of N.W. Italy, has a population of 4.5 million.The estimated prevalence of diabetes is around 3%. A national and regional law has been passed to follow diabetic patients through a network of public diabetes clinics. AIM: To assess how patients' education and complication screening are carried out in the network of diabetic outpatient clinics in Piedmont. METHOD:Direct interview of the head of the clinic to collect data about the organization. Particular attention was paid to patients'education and to complication screening. RESULTS: 98% of clinics in Piedmont were interviewed. Patients' education is implemented in 85% of clinics. Individual education is the most frequent type (87%). Combined education , i.e. both individual and group, is less frequent (35%). As for the personnel in charge of education, doctor plus nurse is the most frequent finding (35%), whereas doctor alone is the second (28%).Dieticians are involved rarely (23%). Material such as checklists, booklets, videotapes are seldom used (30%). Financial support from public or private institutions is hardly reported (10%). Eye complication screening is carried out in a large proportion of clinics (92%), mainly through direct ophtalmoscopy performed by an ophthalmologist. Nefropathy is screened by means of microalbuminuria and renal function tests in most clinics (88%). Periodical physical examination, EKG, blood lipids and blood pressure checks are the most used tools to screen for cardiovascular complications (75%). Routine neuropathy (37%) and routine diabetic foot screenings (35%) are carried out remarkably less frequently. These complications are usually addressed when any symptom or sign develops. Yet most clinics take charge of the treatment of foot lesions (83%). CONCLUSIONS: The effort to improve patients' knowledge by making individual training and education a widespread practice is being made. However, apart from individual approach, other effective methods of education and dietician's involvement rate unsatisfactory. A fairly good result was found as regards eye, cardiovascular and renal complication screenings which seem to be fairly addressed. Something more should be done to screen for diabetic foot in a routine way.

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INFLUENCE OF THE GREAT HANSHIN-AWAJI EARTHQUAKE ON GLYCEMIC CONTROL IN DIABETIC PATIENTS

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We investigated the influence of the Great Hanshin-Awaji Earthquake which occurred on 17th January, 1995, on glycemic control in diabetic patients. We investigated the dietary and living conditions and the changes in HbA_{1C} level in 177 diabetic patients at our hospital; one year before and one year after the disaster. The mean value of HbA_{1C} increased significantly after the earthquake($8.34 \pm 2.07\%$ in March 1995 vs $7.74 \pm 1.82\%$ in December 1994, $p<0.01$). Ninety nine of 177 patients showed more than a 0.5% increase in HbA_{1C} after the earthquake. The causes of this exacerbation were examined by multiple regression analysis of the following factors: inappropriate diet, discontinuing drug therapy, reduction of exercises, total destruction of the patients' houses, a long-term stay in shelters, sex, age, difference between therapies. Inappropriate diet had the highest partial regression coefficient. Investigation of dietary conditions revealed that excessive carbohydrates and insufficient vegetables were prominent. Within the two months after the earthquake, we observed ketoacidosis in 2 patients, gangrene of the foot in 3 patients, and pneumonia in one patient. The level of HbA_{1C} gradually declined with time after the earthquake and returned to its usual level in September 1995. This study showed an increase of HbA_{1C} in diabetic patients after the Great Hanshin-Awaji Earthquake. The increase in HbA_{1C} was mostly caused by inappropriate diet. Therefore we should always educate diabetic patients on how to manage diet and drugs in emergencies.

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FACILITIES FOR DIABETES CARE IN SOUTHERN GHANA

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Ghana is a Tropical country with a population of 16 million. The country is divided into 10 political regions. The regions in Southern Ghana comprise Central, Eastern, Volta, Western and Greater Accra, with a total population of 8.3 million inhabitants.The study was initiated to determine the state of diabetes care in the 4 Regional referral hospitals [Central(C), Eastern(E), Volta(V), Western(W) and the Teaching Hospital in the Greater Accra(GA) regions] in Southern Ghana. Each of the five Health facilities was requested to complete a Health Care Facility and Standards of Care Questionnaire. Of the five health referral health facilities in the Southern Ghana only one(20%, GA) ran a regular diabetes clinic and had diabetologists. Only two(40%) facilities had an eye specialsit(E,GA). Two hospitals(40%,GA,W) had a dietitian but none of the five facilities had a trained diabetes educator or foot care specialist. A stadiometer was available at 1(GA), and a weighing scale at 2(W,GA) facilities for diabetes care. All had equipment for measuring blood pressure, urinalyis for glucose, protein and ketones. Fasting blood glucose, oral glucose tolerance test , blood urea, serum creatinine were available at all facilities. Creatinine clearance, 24-hour urine protein, Glycated Haemoglobin and serum lipids including HDL cholesterol were available at one centre(GA). C-peptide, islet cell antibody and microalbumunuria were not possible in any of the facilities. All facilities used 100U insulin. Insulin was always availabe in 3(E,V,GA). In 2 facilities insulin supply was erratic. All utilized 100U syringes. One hospital had regular but informal diabetes education. 3(GA,E,W) of the 5 regions had active Diabetes Associations. Facilities for diabetes care is less than satisfactory in Southern Ghana. There is therefore an urgent need for provision of basic and essential facilities in the referral centres and to put in place a comprehensive diabetes care disease management programme in Southern Ghana to improve diabetes care.

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COCHRANE DIABETES GROUP: THE EPIDEMIOLOGY OF RANDOMISED CONTROLLED TRIALS IN DIABETES.

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A register of all randomised controlled trials (RCTs) in diabetes is being established by the Cochrane Diabetes Group, an international collaboration preparing, maintaining and disseminating systematic reviews in all areas of diabetes, with the specific aim of reducing the undesirable delay in research findings being translated into clinical practice. This developing register has allowed an examination of the 'epidemiology' of RCTs in diabetes. Using sophisticated MEDLINE search strategies 4957 possible RCTs have been identified in 865 journals between 1966 and 1995, 32% appearing in ten principal journals, and 14% in languages other than English. There has been an exponential rise from 26 in 1966 to 832 in 1994. Over 400 were carried out in each of the areas of retinopathy and hypertension with over 300 looking at treatments for neuropathy and nephropathy. 200 examined dietary interventions. Visual assessment of a sample of MEDLINE abstracts suggests that 43% of the references on the database are true RCTs with a further 15% being controlled clinical trials. The remainder are other study types or review articles often picked up by the search strategy because of the frequent use of expressions such as *random* and *control* in diabetes. Handsearch examination of original journals suggests that even this sensitive electronic search is not identifying a further 22% of RCTs in diabetes contained in journals indexed on MEDLINE. This survey highlights the importance of a centrally maintained reference resource both as a basis of systematic reviews in diabetes and for the identification of priority areas for primary research.

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IDDM IN CHILDHOOD AND ADOLESCENCE - PROSPECTIVE FOLLOW-UP AFTER ONSET

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Better care of diabetes in childhood and adolescence was declared a main objective of St. Vincent. There are only few population-based data about the course after diabetes onset. Aims of this study were the evaluation of health care utilization, diabetes management, and frequency of readmissions and severe hypoglycamias, especially in subjects that are not treated in spezialized diabetes centers. **Methods:** Using prospectively registered population-based incidence data on IDDM diagnosed before the age of 15 years in the Düsseldorf region (EURODIAB ACE study region), a cohort of cases from 1/1993 to 4/1995 (n=200) was followed up by telephone interviews and mailed questionnaires. Data on admission at diagnosis were obtained from clinic records. Age-, sex-, and region-matched controls were also followed up to compare morbidity outcomes in diabetic and nondiabetic subjects. **Results:** 112 subjects (65 boys, mean age at diagnosis [±SD] 8.2±3.9 ys) with a total of 194.6 person-years after diabetes onset (mean follow-up period 21 (range 8 to 38) months) were analyzed. Mean duration of hospitalization at onset was 17.6±7.7 days. 59% of the subjects stayed in the clinic of first contact for further treatment, 23% visited a general paediatrician or practitioner. HbA1c was measured in 97% of the subjects, in 70% at least once quarterly. In 33% blood glucose was measured 2 to 3 per day, in 65% at least 4 times. 50 readmissions for all reasons were observed in 35 subjects (33%). Incidence rate (IR) of readmissions was 0.26 [CI95% 0.19-0.26] per person-year. 26 events of severe hypoglycamia (with injection of glucose or glucagon) occured in 18 (17%) of the subjects. IR of severe hypoglycamias was 0.13 [CI95% 0.08-0.18] per person-year. **Conclusions:** Most of the followed patients with IDDM are treated in the clinic of first contact - most of them general hospitals without spezialization in diabetes care - or in a general praxis. Blood glucose self control and HbA1c measurements were performed according to consensus guidelines in the majority of the patients. Incidence rates of severe hypoglycamias and of readmissions were comparable to other studies. In ongoing analyses hospitalization in diabetic and nondiabetic subjects is compared by estimating relative risks.

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A POBLATIONAL STUDY ON DIABETES MELLITUS IN BUENOS AIRES DE PALMARES, COSTA RICA

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Objective: To realize an epidemiological and clinical description of diabeticc patients from a clinic in a rural area of Costa Rica.

Methods: Using registration documents from EBAIS (Basic Equipment for Integrated Health Care) 105 diabetics were visited to their homes: a clinical story and physical examination was done, feet prints were taken in order to classify their feet with the PATON-A scale. The laboratory test were taken from their clinical files.

Results: The prevalence of Diabetes among adults was 3.4%. There were 55 women and 22 men (71 NIDDM, 5 MODY, 1 IDDM). Adherence to treatment was 90%, most of them hand first level of education (six years); 67% had familiar story of DM. 7 were smokers, 5 drank alcohol in excess. 74 of them were sedentary and 7 were on a free diet. 49 had hypertension, 34 had hyperlipidemia, 11 had asthma, 8 with psyquiatric disorders, 6 had nephropathy and 5 tyrod dysfunction. 71% had a normal blood pressure (under 140/90), 35% with a BMI under 30. Glycemia was under 140 mg/dl in 38.5%, 140-200 mg/dl in 38.5, over 200 mg/dl in 26%. Tryglicerides media 207.9 mg/dl, 15% had more than 300 mg/dl. Cholesterol media 233 mg/dl, 38% had levels over 250 mg/dl. 29% had macroproteinuria. 8.5% had creatinine over 1.5 mg/dl. Treatment: diet 4%, glibenclamide 61%, Insulin 35%.

Conclusions: There was a good adherence to treatment. More educational intervention is needed because a considerable number of patients persist with harmful habits and without a proper diet. 1/3 were well controlled, 1/3 had regular control and 1/3 were badly controlled. 63% of them were found hypertensives andd 44% were found to have hyperlipidemia. It is necessary to keep a more frequent control on those with other risk factors. The feet abnormalities were correlated with the time of evolution of the diabetic disease.

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Diabetes Associations in Action

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TWINNING PROGRAMME: CROATIA HELPING DIABETIC CHILDREN FROM BOSNIA AND HERZEGOVINA

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The war in Bosnia and Herzegovina aggravated diabetes care, particularly in children. In spite of the war dangers and shortage of appropriate food, 80 diabetic children continued to live in Sarajevo throughout the war. Medical care was provided by young pediatricians with little experience in the treatment of diabetes mellitus, who remained in Sarajevo. At the initiative of SOS KINDERDORF INTERNATIONAL, which recognized the difficulties diabetic patients were facing in Sarajevo, and the Vuk Vrhovac Institute from Zagreb, a Zagreb-Sarajevo Twinning Programme was set for the field of diabetes mellitus. The programme of aid to diabetic children included regular insulin and its administration related equipment provisions; a monthžs aid in food and money for vegetable and fruit provisions; organizing educational meetings and parent and children education; education of the staff of the Košević Clinic at the Vuk Vrhovac Institute in Zagreb; efforts to organize educational-recreational camps for children. In 1995, a physician from Sarajevo underwent a one-month training in paediatry and diabetology in Croatia. In July 1996 we organized a summer camp for 20 Bosnian and 50 Croatian diabetic children, who were accompanied by the above mentioned physician and nurses, and participated in daily educational activities, exercise and sport games. Diabetes control (HbA1c) in children from Sarajevo had been much worse than in Croatian children ($p<0.001$), but was improved during their stay in the camp ($p<0.01$). Education and care for the children and medical personnel continues.

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THE ROLE OF LATVIAN DIABETES ASSOCIATON IN EDUCATION DIABETES PATIENTS OF LATVIA 1993 -- 1996.

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The main goal of Latvian Diabetes Association (LDA) is active and passive education of diabetes patients. LDA does it in a following ways: Creates library, fond of audio- and video recordings. It is the only library of this kind in Baltics, there are 22 magazines for diabetes patients from 12 countries in the fond of periodicals, in the fond of videorecordings there are both 10 plots on diabetes which LDA has created for the National TV channel of Latvia and video recordings on diabetes. In the fond of audiorecordings 18 tapes for medicine professionals and diabetes patients are found. LDA works at new informative and educational materials, copies and distributes them. LDA has received copy- and translating rights from editorial boards of 6 national diabetes associations. Over 60 materials have been translated in 3 years, 5 members of LDA has done it without reward. LDA regularly writes educational materials for health educational magazines "Veselība", "Sveiks un Vesels" (28 are published) and publishes them into the informative bulletin of LDA "Mēs & Diabēts", writes articles on diabetes for general press (55 are published). LDA participates in TV and radio programs on diabetes (8 radio and 5 TV programs), issues the informative bulletin of LDA (4 -- 16 A4 pages, circulation 1000 -- 2000). Bulletin is issued 2 -- 4 times a year with a supplement for medicine professionals. Specially trained LDA members 4 hours every weekday free of charge consul other diabetes patients, in case of emergency recommending them to visit a specialist. Every year LDA organizes WDD events in Latvia. LDA is the first organization in the history of the Republic of Latvia that started to celebrate WDD already 6 months after it was founded.

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THE SPECIALITY COMMISSION DIABETES - A NON-PROFIT ORGANIZATION FOR IMPROVEMENT OF DIABETES CARE IN BAVARIA, GERMANY

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In Bavaria the speciality commission Diabetes (Fachkommission Diabetes in Bayern - Landesverband der Deutschen Diabetes-Gesellschaft) has formed a structure for regional improvement of diabetes management with respect to the St. Vincent Action Programme. This non-profit organization was founded in 1993 and in April 1996 the commission was reshaped as the Bavarian section of the German Diabetes Association. Since then 152 members from primary care, diabetes specialists, city and community hospitals and university clinics are actively involved. The board of the commission has formed several sub-committees like quality management and epidemiology, diabetic foot, education, nephropathy, primary care, health care system and outpatient / inpatient cooperation. The implementation of DIABCARE Q-Net is widely supported by its members. So far over 9.000 patient data sets were aggregated by the Basic Information Sheet or by the software DiabData. The participating centers received data evaluations as benchmarking. At the moment the implementation of the DIABCARE fax system is put into action. Further aims in the future will include the Bavarianwide distribution of the carecard Diabetes and enforcing efforts in quality development by installation of quality circles.

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THE EDUCATION OF SOCIETY ABOUT DIABETES -- ACTIVITIES OF LDA 1993 -- 1996.

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The education of society about diabetes is one of the basic task of Latvian Diabetes Association (LDA). The importance of the task and peculiarities are determined by following factors: the diabetes morbidity rates are increasing sharply, society is insufficiently informed about diabetes and there is no uderstanding about the special needs of diabetes patients, the members of society and they have not enough resources for self -- education. LDA is the only organization that regularly informs society about the problems of diabetes and uses following ways to do that. 28 articles have been published in healt educational magazines "Veselība" and "Sveiks un Vesels" and 55 articles have been published in general press until the December 1996. LDA has prepared 8 radioprograms on risk factors of diabetes, early diagnosing and symptoms of diabetes, bases of healthy lifestyle. During World Diabetes Day a broad press campaign is always organized, specially turning attention to the first signs of ilness and early diagnosig them. Checking blood glucose level durin the WDD (for free), every year dozens of firstly diagnosed persons with too high glucose level are found (in 1996 -- 40 cases out of 908, that is -- 4,4%). In July -- September 1995 LDA carried out a sociological questionnaire "How much do you know about diabetes?". 1000 respondents were questioned. The results will be summarized and estimated during the first half of the year 1997. LDA will continue all the started works and hopes to publish a magazine devoted to diabetes theme for a broad audience of readers.

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IDF AND LITHUANIAN DIABETES ASSOCIATION.
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In order to implement the St.Vincent Declaration the Lithuanian Diabetes Association(LDA) was established on 9 December,1989.At present LDA has about 5000 members comprising both health care professionals,people with diabetes and their carers.The LDA plays the leading part in realization of the National Diabetes Programme.The LDA provides practical help and advice producing publications,publishes the newspaper "Diabetas",prepares the information on cassette for visualy handicapted members, organizes conferencies,seminars,summer camps, World Diabetes Days,gives information over the phone,helps to establish diabetes schools,to solve social problems,to remove discrimination (driving licence,list of specialities to study in higher schools,50 strips for self control, compensation for food).By making representations to the government,the LDA helps to ensure that standard of diabetes care in Lithuania is maintained.Since 1994 the LDA is a member of the IDF.The IDF helps us to understand new ideas and to learn from experience.It is essential for growth and development of the LDA.

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BLOOD GLUCOSE TESTING IN PUBLIC PLACES
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To promote public awareness of diabetes, blood glucose testing in public places has been performed. During the last two years three actions have been organized by the Zagreb Diabetic Society, two of them in the city park and one in a sports center. Blood glucose was measured using a small apparatus for selfcontrol of diabetic patients. The measuremnt was conducted by the members of the Society and supervised by the medical staff of diabetic centers in Zagreb. Persons with glucose levels higher than 6.7 mmol/l were advised to check the results of measurement in a doctor's office. A total of 1475 citizens was tested, including a certain number of registered diabetics. A total of 192 persons had suspected newly discovered diabetes, while 242 had had diabetes diagnosed earlier. The percentage of those with blood sugar higher than 6.7 mmol/l, previously not registered as diabetics, was different for the two locations: 13 and 20 % in the park, and 10 % at the sports center. This could be explained by the difference in average ages of the population visiting these two locations. On the occasion of the World Diabetes Day 1996 similar actions were organized in three locations in the city center, to alert the citizens of the dangers of diabetes and to promote association of patients.

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THE ALL-RUSSIAN DIABETES NEWSPAPER (ALL-RDN) AS A MEMBERSHIP CARD.
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All-RDA founded the All-RDN for free of charge distribution between individual & collective members of the All-RDA. The annual All-RDA membership fee is the 100 russian rubles -equivalent of 0,017 $ USA. It was very difficult to unificate more than 1.000.000 diabetics in the territory with the area of 17.075.400 squre kilometers. So our membership All-RDA politic for admittance to the All-RDA was acceptable only for 89 regional diabetic & diabetes organizations. But a lot of diabetics from rural areas could not receive the new diabetology information and could not receive social protection from the All-RDA. We changed our membership politic last month from collective to individual membership after publishing of the first All-RDN number. The circulation of the first All-RDN number was 5.000 samples. We will publish All-RDN monthly increasing the circilation & All-RDA membership to 1.000.000 in 1998. One of the principal of the points at issue in All-RDN is to ask IDF to distinguish one special "russian" region for membership in IDF.

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WHAT WE ARE DOING FOR PUBLIC AWARENESS OF DIABETES IN BEIJING — BDPTA IS WORKING.
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Beijing Diabetes Prevention & Treatment Association (BDPTA) is established in May 10, 1996. A lot of work have been done in last year: (1)Organization of BDPTA: 59 council directors including 13 patients, more than 2000 members;(2)Lectures on diabetes: 3 times in the park or theater, more than 1000 patients each time;(3) Diabetes Day(DD): Beijing DD is on May 10 every year of last 4 years, International DD is on Nov. 14 every year from 1992, with more than 10000 attendants in the last IDD only in Beijing;(4)Diabetes booklet: 2000 in Beijing, 50000 in whole country;(5)Diabetes Corner on newspaper: once a week on HEALTH TIMES ;(6) (Cross Talk on Diabetes) : on Beijing Broadcasting once a week for 1 year;(7)Diabetes help Card: more than 3000 delivered; (8)Hot line for diabetics: every Saturday morning; (9) Small sized consultation: twice monthly on the second and fourth Saturday; (10)Network of diabetes monitor and therapy, one in the countryside, another in the town; (11)To continue IDDM registry: once per 2 year for IDDM incidence;(12)To have training course for physicians, pediatrics, nurses and dietitians, writing teaching materials on the treatment both of IDDM and NIDDM.

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THE BERMUDA DIABETES EPIDEMIOLOGY PROJECT - A SUCCESSFUL PARTNERSHIP VENTURE.

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Diabetes is a major health problem in Bermuda. To define the magnitude of the diabetes problem, Bermuda Diabetes Association (BDA) determined the prevalence of diabetes and its risk factors in a random sample of the adult population. We describe here the cooperative efforts of many partners in making the Bermuda Diabetes Epidemiological Project (BDEP) possible in a nation where there is no official research funding agency. The need and importance of the survey were initially presented to the Premier, Minister of Health, health professionals and business community in Oct. 1994. In Dec. a similar presentation was made to the Cabinet which endorsed the BDEP. A steering committee, with members from the BDA, Ministry of Health, Heart Foundation, business community, health professionals and Statistics Department, was appointed. During 1995 it met regularly to plan for the survey tool, fund raising, pubic awareness and survey conduction. The Nova Scotia Heart Health Survey, modified to include an extensive diabetes component, was used as the Survey tool. Fund-raising events included "Bermuda Night" featuring Bermuda music & food, health screening & talks to companies for their donations, watermelon sales on Harbor Nights, fruit sales at Agricultural Show, "Tag Days" and Denim for Diabetes Day. Publicity for BDEP was organized by a local advertising executive who donated his services. The Canadian Diabetes Association shared its Diabetes Campaign materials. All three media in Bermuda publicized BDEP. One TV station televised "Bermuda Night " and opened it up like a telethon. When BDEP was launched in Sept. 1995 at Government House the Ministry of Health donated funds to BDEP. When the survey began in Jan. 1996 the Ministry of Health organized a press conference. The Survey was completed in Nov. 1996. Together BDA and its partners made BDEP a dream come true.

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ENHANCING PUBLIC AWARENESS OF DIABETES: A COMMUNITY PROJECT ON THE WHITE MOUNTAIN APACHE RESERVATION

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Background:The White Mountain Apache Tribe is located in rural eastern Arizona on the Fort Apache Reservation. With a current total population of 12,000, there are now 750 diabetics among the White Mountain Apache. New diabetics are being diagnosed at a rate of 1.1 per week. Methods:To raise local awareness about diabetes, interested community members were recruited by the physician-educator to begin a diabetes liaison program to spearhead community efforts. The first step was to educate liaison members. Five completed a comprehensive course in 1994; eight in 1995. Emphasis was placed not just on content but on teaching skills. Each session began with a case scenario involving information from the previous session. Community members then role played these cases as educators and patients in both English and Apache. By the end of the 1995 session; members were able to lead an entire review session without aid from the instructor. Results:The liaison committee members then were able to lead local efforts at community awareness. Members co-organized with the Indian Health Service two community wide diabetes education conferences in 1994 and 1995 attracting 150 and 100 diabetics and their relatives respectively. Two members were chosen to be part of the Supervisory Board for Diabetes Education at the Whiteriver Service Unit. The liaison committee helped focus the local Community Health Representatives on diabetes forming a diabetes team all of whose members, with renewed interest in health care, returned to school to get their Certified Nursing Assistant degrees. Conclusions:White Mountain Apache lay community members with medical instruction were able to spearhead efforts at raising tribal awareness about diabetes and its sequelae. Future measurements of the impact of this group will look at the percentage of community members educated and given foot care by the newly formed community health representative diabetes team.

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A GLOBAL INSULIN COLLECTION AND DONATION PROGRAM - UPDATE

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Often costing $ US 25 or more a vial, insulin affordability in many countries is one of the major, yet often unrecognised challenges facing the international diabetes community. Many children and adults die prematurely or suffer early complications as a direct result., and the problem is rapidly worsening, with many countries now obtaining less than 10% of the insulin they need. Insulin, syringes and test strips that would otherwise be wasted are collected through diabetes clinics, Associations and manufacturers and have been sent to 18 countries in urgent need. Between 1986 - 1996, 66,388 vials (adequate for 5,200 people with IDDM for one year), 1.19 million syringes and 2,138 boxes of test strips, valued at least at $US 1.7 million have been distributed and many lives have been saved. Much more insulin is available from such sources. This is part of an integrated global program at the Institute to help improve insulin availability and affordability in many countries and we invite others to join under the theme "No More Deaths Because Of Insulin Shortage".