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Abstracts

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OP 1
Forms of Therapy I: New Insulins

1

INSULIN REPLACEMENT BY SOMATIC CELL GENE THERAPY: PILOT STUDY

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Somatic cell gene therapy is a potential method to achieve insulin delivery in diabetes. To assess the feasibility of this approach cultured pituitary AtT20 cells were transfected by calcium phosphate coprecipitation with a plasmid construct (pMtNeohppI/1) containing the human preproinsulin gene and a metallothionein promoter. Transfected cells were isolated and cultured in modified Dulbecco's medium containing 10mM glucose. The cells constitutively released insulin at approximately 4ng/10^6 cells/24h. Exposure to several standard insulin secretagogues (raised concentrations of glucose, potassium and calcium) did not significantly alter insulin release, but IBMX stimulated insulin release two fold. 2 x 10^6 transfected cells were implanted intraperitoneally into athymic nude mice. Release of insulin in vivo was evaluated using a specific human C-peptide assay. Human C-peptide was detected in the plasma after implantation, rising to a concentration of 0.167±0.015 pmol/ml (n=10) after 14 days. Following injection of streptozotocin (200mg/kg, ip) human C-peptide concentrations were maintained at about 0.15pmol/ml. Development of hyperglycaemia was delayed in these mice, but severe hyperglycaemia eventually occurred (26.7±4.0 mmol/l, n=7 after 21 days despite C-peptide concentrations of 0.10±0.01 pmol/ml) associated with severe insulin resistance. The results suggest that somatic cell gene therapy could be a feasible approach to insulin delivery.

2

SUBCUTANEOUS ABSORPTION OF FAST-ACTING INSULIN ANALOGS: KINETICS AND BIOEFFECTIVENESS

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We used two series of insulin analogs in pigs with surgically pre-implanted intravenous catheters to examine rapidity of subcutaneous absorption and effects on glucose removal. These analogs were based (i) on the substitutions X(B28)Pro(B29) and Asp(B10)X(B28)Pro(B29) with X=Lys, Val and Glu, and (ii) on the substitution Asp(B10) and deletions des(B27-30), des(B28-30), and des(B29-30). Parameters of analog kinetics were determined from a 2hr step intravenous analog infusion (12.5μg/kg-hr). Analog sensitivity (S_I) was determined from the ratio: (mean glucose infusion rate to maintain fasting glycemia)/(mean analog concentration). Absorption rates, determined following subcutaneous injection are reported as time for 90% absorption. In series (i) absorption time of all analogs (except Val(B28)Pro(B29)-insulin: 139±13 min) was near 100 min and faster than Humulin (151±17 min, $p< 0.05$). Plasma concentrations were lowest and S_I maximal for Lys(B28)Pro(B29)-insulin (702±78pmol/l and 0.24±0.04, respectively) and comparable to Humulin (696±66pmol/l and 0.21±0.03). Asp(B10)Val(B28)Pro(B29)- and Asp(B10)Lys(B28)Pro(B29)-insulin demonstrated similar parameters but higher concentrations. Of the series (ii) analogs those with the tripeptide deletion (des(B28-30) exhibited the lowest plasma levels and highest S_I des(B28-30)-insulin: (420±120pmol/l and 0.48±0.15); Asp(B10) des(B28-30)-insulin: (450±144pmol/l and 0.51±0.17). However, only Asp(B10)des(B28-30)-insulin was absorbed more rapidly than Humulin (84±9 min, $p< 0.05$). In conclusion, Lys(B28)Pro(B29)-insulin and Asp(B10)des(B28-30)-insulin are optimal demonstrating the lowest plasma concentration, highest sensitivity and rapid absorption.

3

ACTION PROFILE OF THE RAPID ACTING INSULIN ANALOGUE, HUMAN INSULIN B28ASP.

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The insulin analogue (IA) B28Asp has higher absorption rates from subcutaneous tissue compared to regular human insulin but identical receptor binding properties. We measured the time-action profile of B28Asp and regular human insulin (euglycemic glucose clamp, blood glucose 5.0 mmol/l, 14 healthy volunteers). SC injection of 0.15 U/kg body weight (9.5-14.3 U) of either insulin preparation resulted in comparable values of maximal insulin action (maximal glucose infusion rates (GIR)), total amount of glucose infused and area under the GIR curve. However, half-maximal GIR was reached significantly earlier after injection of B28Asp (44±9 min) as compared to regular insulin (56±23 min, $p<0.01$). 45 and 60 min after injection of regular insulin GIR had increased by 3.4±1.8 and 4.8±2.3 mg/kg/min, reflecting 30±15 and 42±17 % of maximal action of 10.6±2.4 mg/kg/min. Following the injection of B28Asp, GIR had increased by 6.3±2.7 after 45 min and by 7.9±2.8 mg/kg/min after 60 min, reflecting 64± 28 % and 81±26 % of maximal action of human regular insulin. After 240 min GIR was significantly lower after injection of B28Asp than after regular insulin. Serum insulin concentrations increased after injection of B28Asp to peak values of 451±115 pmol/l within 40 min and declined within 240 min to 127±57 pmol/l. In contrast, peak insulin concentrations of 256±48 pmol/l were reached 120 min after injection of regular human insulin and were still 232±49 pmol/l after 240 min. The IA B28Asp showed a significantly faster onset of action and higher peak insulin levels as compared to regular insulin.

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CARCINOGENIC EFFECT OF THE HUMAN INSULIN ANALOGUE B10 Asp IN FEMALE RATS

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In the human insulin analogue B10 Asp, His is substituted by Asp at the B10 position by genetic engineering technology. B10 Asp is absorbed twice as fast as human insulin after s.c. injection. B10 Asp has a 3 times higher affinity for the insulin receptor and the IGF1 receptor than human insulin, whereas the mitogenic activity in various cell types has been reported 10-20 times higher than human insulin. The aim of this study was to evaluate potential long-term effects of high doses of B10 Asp in rats. Groups of 20 rats were injected for one year with saline (A); human insulin 200 U/kg/day (B); B10 Asp in the following doses: 12.5 U/kg/day (C); 50 U/kg/day (D), 200 U/kg/day (E). Complete necropsy including macroscopic and microscopic examination was carried out. The following incidences of mammary tumours were found in the female rats:

	A	B	C	D	E
Benign tumours	0	0	11%	0	44%
Malignant tumours (adenocarcinomas)	0	0	0	11%	23%

5% of male rats, group D, had benign tumours. No other neoplastic lesions were found. Thus a dose-dependent carcinogenic effect was found. Whether this is directly related to the increased mitogenic activity remains to be elucidated.

5

FAST ACTING HUMAN INSULIN ANALOGUES WITH A SINGLE AMINO ACID DELETION IN THE B-CHAIN.

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A new class of human insulin analogues has been developed and produced by biosynthetic and semisynthetic methods. These analogues are characterized by the deletion of a single amino acid residue close to the C-terminal of the B-chain. The analogues have been evaluated by measuring the *in vitro* biological potency in mouse fat cells, by osmometric determination of the association state in solution at neutral pH and by the blood glucose lowering effect found after subcutaneous injection in pigs. The *in vitro* biological potencies relative to human insulin were found to be 218% for des[Phe^{B25}]-human insulin, 157% for des[Tyr^{B26}]-human insulin and 168% for des[Thr^{B27}]-human insulin. The average molecular weight at neutral pH in Zn-free 1 mM solutions of des[Phe^{B25}]-HI and of des[Tyr^{B26}]-HI was determined to 9.0 kD for both analogues. After subcutaneous injection in pigs of a preparation containing des[Thr^{B27}]-HI a substantially faster decrease of the blood glucose level was found in comparison to the human insulin preparation Actrapid® and the maximum effect was achieved after 80 min. vs. 150 min. for Actrapid®. In conclusion, human insulin analogues with an amino acid residue deleted close to the C-terminal of the B-chain are new candidates for improved fast acting insulin injection preparations.

6

New Insulin Preparations With Prolonged Action Profiles:
A21-Modified Arginine Insulins

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New insulin analogues have been designed to obtain soluble preparations with protracted action profiles better than the standard crystal suspensions. Arginine insulins are intermediates of the proinsulin/insulin conversion with a protracted action due to a shift of the isoelectric point towards physiological pH. Replacement of Asn(A21) with other uncharged residues through site-directed mutagenesis resulted in a dramatic improvement of this effect. Analogues were characterized by free fat cell assay, decrease of blood glucose after i.v. injection in rabbits, 3 month immunization of pigs and thymidine incorporation in cultured aortic endothelial cells. Evaluation of profiles was performed by measuring serum insulin and glucose after s.c. injection in rabbits and dogs. Compared to human insulin, the potency of these analogues is not substantially reduced. The slight structural modification does not effect the immunogenicity and growth promoting activity. Subcutaneous injection of acidic solutions of Gly(A21),Arg(B31),Arg(B32) insulin in dogs and rabbits shows a protracted action profile which is twice as potent as the human NPH insulin used as a standard. Our results clearly state the importance of position A21 not only for stability but also for pharmacokinetic properties of insulin analogues.

7

EFFECTS OF ORALLY ADMINISTERED INSULIN NANOCAPSULES IN DOGS.

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Polyalkylcyanoacrylate nanocapsules (< 300 nm), used as a drug carrier for insulin, have been previously shown to preserve the therapeutic effect of insulin when administered orally to rats. This work was designed to investigate the effects of oral insulin nanocapsules (NC) in normal and diabetic dogs. In normal dogs (n = 6) a single oral administration of NC (100 U/kg) decreased both hyperglycemia and insulinemia induced by an i.v. glucose injection (0.3 g/kg) ; this effect was maximal after 9 days (- 72 and - 70 % respectively, $p < 0.01$) and had disappeared on the 15th day. However, fasted glycemia remained unchanged. In dogs made diabetic by alloxan (50 mg/kg i.v.) (n = 6) a single oral administration of NC at the same dose resulted, from the 2nd to the 7th day, in a reduction of postprandial hyperglycemia (- 26 %, $p < 0.01$), a drop of glucosuria (- 77 %, $p < 0.01$), a decrease in elevated plasma glucagon and somatostatin levels (- 37 and - 27 % respectively, $p < 0.01$). In conclusion, our results suggest that oral administration of insulin nanocapsules induces beneficial persistent effects on glycemia and hormonal profiles in dogs, suggesting that this new route of administration could be of interest in the treatment of diabetes.

OP 2
Gastrointestinal Tract in Autonomic Neuropathy

8

GUT MOTILITY IN DIABETES MELLITUS
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Autonomic neuropathy (AN) is a frequent complication of diabetes mellitus (DM). Cardiovascular tests only detect high grade AN. Gastrointestinal symptoms (S) in DM pts have been related to gut motor disorders due to AN. We evaluated whether gastrointestinal motility tests are more helpful than the cardiovascular tests. METHODS: 39 DM patients (6 type I/33 type II) were evaluated by means of gastric (GE) liquid/solid, esophageal (ET) radionuclide transit; orocaecal transit time (OCT) by H2 breath test and esophageal function by manometry. abnormal (Mp): rabbit ear phenomenon, alterated propagation velocity, amplitude of contraction. AN was assessed by Ewing-Clarke and E/I-test. RESULTS:(mean ± SD)

group	S %	Mp %	ET s	GE:li/so t 1/2 min	OCT min	E/I bt/min	EQ
I	60	60	9,2	35,2/59,6	175	8,5	1,07
		±	3,4	16,7/11,3	55	1,7	0,03
II	21	66	8,4	23,8/70,5	155	5,3	1,07
		±	5,2	16/26,5	61,9	0,9	0,03
Control:			7,1	23,9/46,2	95,7	13,6	1,08
		±	4,7	9,1/16,3	15,1	6,8	0,03

CONCLUSION: Concerning the symptoms there were significant differences between the two groups (60% vs. 21%). Only one pt had marked delay of ET. In DM I gastric emptying of liquids was significantly delayed but not in DM II suggesting that this test only reveals severe neuropathy. OCT-measurement showed a tendency for prolongation. Esophageal manometry is more helpful in detection of AN than the cardiovascular tests even in DM II patients.

9

SMALL INTESTINAL HYPERACTIVITY IS THE PREDOMINANT MOTOR ABNORMALITY IN THE UPPER GUT OF TYPE I DIABETICS WITH AUTONOMIC NEUROPATHY.
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The reports on motility abnormalities in type I diabetics are inconsistent. The aim of our study was to characterize the interdigestive and postprandial motor abnormalities in type I diabetics with cardiac autonomic neuropathy (CAN). Antro-duodeno-jejunal manometry was performed in 20 type I diabetics (age 47±2,7 yrs; duration of diabetes 25±9 yrs) with CAN (7 severe, 7 definite and 6 early involvement) and in 11 controls. During the study euglycemia was maintained and symptoms were monitored. During fasting the phase of motor quiescence (I) was shorter in diabetics than in controls (6±1 and 21±4 min respectively; $p<0.002$). In phase II the number of antral contractions and motility index (MI) were not different, but the number of small intestinal contractions was higher in diabetics than in controls ($p<0.05$). The fed state was shorter in diabetics with postprandial symptoms during the study than in controls and diabetics without symptoms (73±27, 156±12 and 140±13 min respectively; $p<0.02$). The diabetics had a lower number of antral contractions only in the first 30 min after the meal. The number of small intestinal contractions was higher in diabetics than in controls ($p<0.03$). This hyperactivity had a pattern of non-propagated high-amplitude contractile activity at maximal frequency ("bursts") in 18 diabetics (90%) and in only 3 controls (27%), during 7.9±1.6 % and 0.8±0.5 % of the total time respectively ($p<0.002$). No correlation between motility variables and severity of CAN was found. We conclude that in particular small intestinal motor abnormalities, predominantly in the interdigestive state, are found in type I diabetics with CAN. These abnormalities, mainly hyperactivity, are associated with symptoms, but are not related to the severity of CAN.

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EFFECT OF HYPERGLYCAEMIA ON ANTRAL, DUODENAL AND JEJUNAL MOTILITY IN HEALTHY INDIVIDUALS
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Impaired gastrointestinal motor activity is commonly seen in diabetic patients. However, factors controlling gastric and small intestine motility are poorly understood. The aim of this study was to investigate the influence of acute hyperglycaemia on antral, duodenal and jejunal motility.
Seven healthy subjects (age 22-33) years were investigated twice; before and during acute hyperglycaemia clamped at 15 mmol/l with 20% glucose infused i.v. for 90 min. Motility was measured with a manometric technigue using tube with eight channel recordings; 3 recording points in antrum and 5 recording points along the duodenum and proximal jejunum. The area under the curve (AUC) for the control period of 30 minutes of phase II motility was compared with a similar period during acute hyperglycaemia.
The integrated AUC for the pressure, calculated for all intestinal segments, before and during hyperglycaemia was 7424+627 and 3933±390 mmHg/30 min, respectively ($p<0.0001$), i.e. 47% reduction during hyperglycaemia. Compared with the control period hyperglycaemia decreased the pressure in antrum 49% ($p<0.001$), in the proximal duodenum 34% ($p<0.05$), in the distal duodenum 51% ($p<0.01$) and in the proximal jejunum 52% ($p<0.05$). Thus acute hyperglycaemia reduces antral, duodenal and jejunal motor activity in healthy subjects.

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Gastric emptying in type 2 diabetes: role of hyperglycemia.
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The effect of hyperglycemia on gastric emptying and the function of the emptying rate on subsequent blood glucose profile was examined in 26 type 2 diabetics without neuropathy and 22 healthy controls. Gastric emptying of glucose (75 g/400 ml) was measured by marker technique. The diabetics exhibited a significantly delayed gastric emptying (glucose delivered at 75 min.: 64.8 ± 4.2 controls vs. 44.6 ± 5.9 g diabetics; $p < 0.01$). To evaluate the role of hyperglycemia the glucose load was repeated in controls during I.V. glucose infusion which mimicked the blood glucose response of the diabetics. At comparable blood glucose levels the gastric emptying was markedly delayed and even significantly slower than in diabetics. To examine the effect of slowed gastric emptying on blood glucose profile in diabetics the anticholinergic drug butylscopolamine (20 mg i.v.) was injected 5 min. before the glucose load, thereby delaying only the initial emptying rate by 20 per cent. This marginal effect reduced the peak glucose in diabetics from 15.7 ± 1.3 to 9.8 ± 0.9 mmol/l ($p < 0.02$) and curtailed the plasma levels of insulin, C-peptide and gastric inhibitory polypeptide by more than 30 per cent. Conclusion: Based on identical blood glucose levels the gastric emptying in diabetics is enhanced. Moreover, the initial gastric emptying is a crucial determinant for the subsequent blood glucose response.

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PATTERN OF GASTROINTESTINAL TRANSIT DISORDERS IN LONG-STANDING TYPE 1 DIABETES MELLITUS

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Gastroparesis is a common feature in patients with diabetes mellitus (DM). In contrast, there is only limited information concerning small intestinal transit time (SIT) and colonic transit time (CT) in diabetics. As most previous studies focused on only one segment of the gut, the extent of gastrointestinal (GI) dysfunction in DM cannot be discerned. The aim of the study was to evaluate the frequency and the extent of GI transit disorders in DM by a non-invasive technique which allows the selective determination of gastric emptying (GE), SIT and CT.

Methods: In 11 controls and 20 patients with long-standing (>15 y) DM, GI transit of a copper pellet was measured by a metal detector. The pellet can be accurately located in the stomach, duodenum, cecum and rectum.

Results: GE in diabetics was significantly prolonged (217±26 min) compared with controls (73±7 min; p>0,0001). In 17 diabetics (85%), GE was above the normal range, being delayed by 164±22 min compared with controls. SIT was also significantly delayed in diabetics (202±19min; controls: 115±17 min; p=0,005), but SIT was above the normal range in only 8 patients (40%) The delay in SIT compared with controls was 173±18 min. CT was considerably, but not significantly (p=0,2) prolonged in diabetics (70±12 h vs 40±5 h in controls). 42% of diabetics had a CT above the normal range, being delayed by 71±7 h compared to controls. Involvement of one GI segment (usually the stomach), was established in 45% of diabetics, 2 segments in 30% and all 3 segments in 15%.

Conclusions: Gastroparesis is the predominant GI transit disorder in DM, occuring in 85% of patients, although in about 40% of diabetics SIT or CT are delayed as well. The results exhibit a heterogeneous pattern of transit disorders in the different GI segments studied.

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EFFECTS OF ERYTHROMYCIN DERIVATIVE (EM523) ON GASTRIC EMPTYING AND POSTPRANDIAL INSULIN REQUIREMENT IN GASTROPARETIC INSULIN-DEPENDENT DIABETIC PATIENTS

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We studied the effects of EM523, an erythromycin derivative on gastric emptying and postprandial insulin requirement in 6 insulin-dependent diabetic patients with gastroparesis. Postprandial insulin requirement was evaluated by measuring the insulin infusion rate during the feedback control with a Biostator. At the same time, gastric solid and liquid emptyings were evaluated by measuring the disppearance rate of ^{99m}Tc in stomach and the plasma acetaminophen concentration after a test meal, containing both substances, respectively. Studies with and without 30-min infusion of EM523 (4 mg, given 15 - 45 min after the test meal) were performed in the same patients on separate days. An intravenous EM523 significantly increased the postprandial insulin requirement during the first 90 minutes (from 2652 [862] to 5595 [818] mU, mean and [SE]) and plasma acetaminophen concentrations during 30 - 120 min, and decreased the remaining isotope in stomach during 30 - 120 min after the test meal, respectively. Patients showed no apparent peak for postprandial insulin requirement during the 3-hour control study without EM523, whereas the peak rate was observed within 90 min after the test meal with EM523 administration. The results suggest that EM523 may improve the gastric emptyings and insulin requirement in insulin dependent diabetic patients with gastroparesis.

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LONG-TERM EFFECT OF CISAPRIDE ON GASTROPARESIS IN TYPE 1 (INSULIN-DEPENDENT) DIABETES MELLITUS.

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Delayed gastric emptying is a well-recognized complication of long-standing diabetes mellitus. Various pharmacologic agents have been used for treatment, but none has been demonstrated to have long-term efficacy.

To assess the long-term effect of cisapride on gastric emptying, gastrointestinal symptoms and glycemic control 8 long-standing Type 1 (insulin-dependent) diabetics (mean age 40±12 yr, diabetic duration 18±5 yr, HbA1c 10±2.1%) were treated with cisapride (10 mg t.i.d.) for one year. Initially, after 4 weeks and 1 year isotope (99Tc) scintiscanning technique (T_{50}) was performed to measure gastric emptying. Gastrointestinal symptoms were evaluated by a standard questionnaire (score). Glycemic control was assessed by HbA1c and post-prandial plasma glucose. In the beginning all diabetics had a delayed gastric emptying (54.8±2.9 min). The administraion of cisapride increased gastric emptying siginficantly to 42.8±14 min after 4 weeks (p=0.011) and 36.2±16.9 min after 1 year (p=0.021). In 2 diabetics gastric emptying was continiously prolonged (> 60 min) under treatment. The total score for gastrointestinal symptoms improved from 12±1.2 to 2±1 (p=0.001) and 3±4 (p=0.009) respectively. HbA1c decreased from 10±2.2% to 8.9±1.8% and 8.9±1.5% but did not reach statistical significance. There was no siginificant change in mean insulin dose and postprandial plasma glucose.

These results indicate that cisapride is effective in long-term treatment of diabetic gastroparesis considering the significant increase of gastric emptying and reduction of gastrointestinal symptoms. However the therapeutic effect should be controlled by isotope scinti-scanning technique.

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BASAL GASTRIN AND POSTHEPARIN DIAMINE OXIDASE PLASMA ACTIVITY IN DIABETICS WITH AUTONOMIC NEUROPATHY

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In diabetics with autonomic neuropathy (AN) there is significant higher basal gastrin secretion. There are also reports that hypergastrynemia can be found in patients with chronic small bowel diseases. Postheparin diamine oxidase (DAO) plasma activity reflects small bowel morphologic and functional integrity. The aim of this study was to find out if, in the course of diabetic AN, high basal gastrin values were accompanied by low postheparin DAO plasma activity. The study was carried out in 3 groups. Group A)-12 Type 2 (non-insulin-dependent) diabetics with AN diagnosed by the use 5 common tests, B)-20 Type 2(non-insulin-dependent) diabetics without any complications, C)- control group-20 patients. Blood samples were taken for the measurement of basal gastrin and DAO activity and the next at 60'after i.v. injection of 150 mg heparin only for DAO.The basal values of gastrin in group A were significantly higher (147pg/ml) compared to group B (78pg/ml) (p< 0,05) and to group C (62pg/ml) (p<0,05).There were no significant differences according to basal gastrin between group B and C.Basal values of DAO activity were nearly the same (50pmol/ml).Postheparin DAO plasma activity was significantly lower in group A (247 pmol/ml) compared to group B (441 pmol/ml) (p<0,01) as well as to group C (541 pmol /ml)(p<0,01).No differences were found in postheparin DAO plasma values between group B and C. In the course of diabetes mellitus with AN relatively high basal gastrin concentrations are accompanied by low postheparin plasma DAO activity. It could be supposed that in these cases there is a certain degree of small bowel mucose damage.

OP 3
Metabolism In Vivo

16

PRECURSOR AND REGULATORY EFFECTS OF GLUTAMINE ON GLUCONEOGENESIS IN MAN
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Although glutamine is the most abundant amino acid in plasma and muscle, little is known of its metabolism in man. In vitro studies suggest that it may be a major precursor of alanine and an important gluconeogenic substrate. Therefore, to assess the effect of glutamine availability on production of glucose and alanine from glutamine, we infused normal postabsorptive volunteers with glutamine at 3 times its basal turnover rate for 4h along with [3-3H]glucose and [U-14C]glutamine. Glutamine infusion increased plasma glutamine 4-fold from 0.63±0.05 to 2.20±0.24 mmol/liter ($p<0.01$); glutamine turnover (µmol/kg/min) also increased approximately 3-fold (4.2±0.5 to 11.8±0.3). Production of glucose from glutamine increased more than 6-fold from 0.36±0.03 to 2.33±0.32 µmol/kg/min ($p<0.01$) so that the proportion of hepatic glucose output due to glutamine increased from 3.4±0.1 to 20.5±4.0% ($p<0.02$) and the proportion of glutamine disposal used for gluconeogenesis increased 2-fold (10.5±1.3 vs 24.3±3.5%; $p<0.05$). Plasma alanine increased from 0.33±0.03 to 0.42±0.04 mmol/liter ($p<0.05$) and the proportion of alanine derived from glutamine increased from 2.0±0.4 to 9.7±2.2% ($p<0.05$). **Conclusion:** The disproportionate increase in glucose formation from glutamine compared to the increase in glutamine availability suggest that glutamine acts no only as a gluconeogenic precursor but also as a regulator of gluconeogenesis.

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PERSISTENT ENDOGENOUS GLUCOSE PRODUCTION DESPITE SUPRA-PHYSIOLOGICAL INSULIN LEVELS DURING EUGLYCAEMIC CLAMPS.
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In euglycaemic clamp studies performed in normal subjects at high insulin levels (>100 mU l^{-1}), endogenous glucose production (EGP) is usually assumed to be completely suppressed. However this assumption is based on non-steady state tracer measurements of EGP which are prone to negative errors. We have used purified [6-^{3}H] glucose in an optimal tracer infusion protocol to assess the suppression of EGP in 4 hr euglycaemic clamps performed in 6 normal men at supraphysiological plasma insulin concentrations (561±52mU l^{-1})(mean ±SEM) while plasma glucose (5.3±0.1mmol l^{-1}) and glucose specific activities (mean 100±3% of basal) were maintained constant from 80-240 min. In all subjects isotopically determined glucose appearance was greater than the glucose infusion rate both at 80-120 min (62.9±6.6 vs 52.9±3.6 umol $kg^{-1}min^{-1}$) and 200-240 min (73.8±4.9 vs 63.8±3.4 umol $kg^{-1}min^{-1}$) (both $p<0.001$) and calculated EGP was always greater than zero (+10.0±3.2 at 80-120 min and +10.0±2.4 umol $kg^{-1}min^{-1}$ at 200-240 min). In 5 subjects EGP was partly suppressed but showed a wide variation (EGP 20 to 91% of basal at 80-120 min and 31 to 87% of basal at 200-240 min) while in one subject EGP increased (by >50%) to greater than basal levels. We conclude that supraphysiological insulin levels do not completely suppress EGP during euglycaemic clamps.

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VAGOTOMY INCREASES HEPATIC GLUCOSE PRODUCTION

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A physiological role of the parasympathetic nerves to the liver is not proven, but it appears that these nerves interact with and complement the effects of insulin. In order to evaluate the effects of parasympathetic denervation on insulin-stimulated glucose metabolism, we studied two groups of awake, unrestrained male Sprague-Dawley rats, after a 24-h fast: vagotomized rats (VAG; n=5) and control animals (CAV; n=5). All rats underwent a euglycemic hyperinsulinemic (3 mu/kg·min) clamp study, with a prime, continuous infusion of [3-^{3}H]-glucose. Basal hepatic glucose production (HGP) was increased in VAG when compared to CAV (46.7±1.1 vs 42.2±1.7 µmol/kg·min; $p<0.04$). Following insulin infusion, HGP was significantly higher in VAG than in CAV rats (27.8±5.0 vs 12.2±3.3 µmol/kg·min; $p<0.03$). However, no significant difference was observed in either glucose uptake (CAV: 121.1±7.2 vs VAG: 117.2±8.3 µmol/kg·min) or whole body glycolysis (CAV: 82.2±3.9 vs VAG: 77.2±8.9 µmol/kg·min). Therefore, these data indicate that vagal innervation plays a role in the regulation of glucose metabolism in the liver. In conclusion, it could be suggested that parasympathetic disruption in diabetic autonomic neuropathy may contribute to the worsening of metabolic control.

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EFFECTS OF LIPID MANIPULATIONS ON GLUCOSE UTILIZATION IN OBESE PATIENTS

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Seven obese non-diabetic patients participated in three protocols in order to see the effects of changing lipid oxidation on glucose utilization (storage and oxidation). 1) Intralipid 2) ß-pyridylcarbinol (ß-PC), a FFA lowering agent, or 3) isotonic saline (NaCl) were infused during 2 hours, and followed by a 2-hour euglycemic hyperinsulinemic clamp. When lipid oxidation was increased prior the clamp with a 2-hour Intralipid Infusion, glucose uptake (2.6 ± 0.6 mg/kg·min) and glucose storage (0.4±0.5 mg/kg·min) during the clamp were diminished compared to NaCl infusion (glucose uptake: 3.5 ± 1.1 mg/kg·min and glucose storage:1.0± 0.8 mg/kg·min glucose). In contrast ß-PC lowered lipid oxidation and induced an increase of glucose uptake (4.1 ± 1.0 mg/kg·min. vs 3.5 ± 1.1 mg/kg·min. after NaCl) and glucose storage (1.6±0.6 mg/kg·min. vs 1.0± 0.8 mg/kg·min. after NaCl). Changes in glucose metabolism were significantly different between the ß-PC and Intralipid tests ($p<0.01$). In addition the changes of glucose uptake were negatively correlated with those in lipid oxidation prior to the clamp ($p<0.005$). The changes of glucose storage due to the lipid oxidation modifications were smaller than those observed in the group of control subjects. In conclusion, the negative relationship between lipid oxidation and glucose storage exists in obese patients.

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RELATIONSHIPS BETWEEN GLUCOSE- AND LIPID METABOLISM IN TYPE 2 DIABETES MELLITUS

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The role of the Randle cycle in the pathogenesis of insulin sensitivity in type 2 diabetes is still a matter of debate. In 46 patients with type 2 diabetes (26F/20M, age:59.1±6.1 yrs, BMI:29.3±4.8 kg/m2, HbA1c:7.4±1.2%) we determined various measures of insulin action by means of indirect calorimetry and euglycemic hyperinsulinemic clamp (65mU/kgLBM/h) with assessment of glucose turnover rates ([3-^{3}H]glucose). The rate of basal lipid oxidation (mean±sd, 6.2±1.1 µmol/kgLBM/min) correlated inversely with basal glucose oxidation rate (mean±sd, 2.8±2.4 µmol/kgLBM/min): r=-0.46;P<0.001, and positively with %bodyfat: r=0.45;P<0.001, whereas no correlation was found with basal levels of plasma NEFA. During the clamp the increase of total glucose disposal rate (22.2±3.6 to 30.3±13.3 µmol/kgLBM/min), was significantly correlated with enhancement of the glucose oxidation rate (2.8±2.4 to 9.5±4.1µmol/kgLBM/min): r=0.74;P<0.001. The incomplete suppression of lipid oxidation rate during the clamp (6.2±1.1 to 4.2±1.3 µmol/kgLBM/min) correlated inversely with glucose oxidation rate (r=-0.62;P<0.001) and with both total (r=-0.52; P<0.001) and nonoxidative (r=-0.42;P=0.01) glucose disposal. Conclusion: our results suggest that in the basal state increased Randle cycle activity is associated with low glucose oxidation rate, while hyperinsulinemia stimulates glucose oxidation either directly or via suppression of lipid oxidation.

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BASAL AND GROWTH HORMONE PULSE STIMULATED METABOLISM DURING FASTING IN HUMANS; EVIDENCE FOR INCREASED LIPOLYTIC GH RESPONSIVENESS.

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To assess the effects of short-term fasting on basal and growth hormone (GH) stimulated substrate metabolism 7 normal healthy subjects were studied for 2h in the basal state and thereafter for 4½h after an i.v. bolus injection of 140 µg GH in randomized order following: 1) 12h and 2) 36h ("fasting") fast.
Fasting induced: 1) Decrements in circulating concentrations of insulin and C-peptide ($p<0.05$) and increments in glucagon concentrations ($p<0.05$). 2) Reduced plasma glucose values, isotopically determined glucose turnover and forearm glucose uptake ($p<0.05$). 3) Increments in circulating levels of all measured lipid intermediates (free fatty acids (FFA), 3-hydroxybutyrate (3-OHB) and glycerol) and forearm uptake of 3-OHB ($p<0.05$).
Following injections serum GH rose to a peak of 17.0 ± 2.7 ng/ml. This was associated with: 1) Unchanged concentrations of hormones and parameters of glucose metabolism. 2) Increased levels of lipid intermediates, the increase being most pronounced ($p < 0.05$) in the fasting state (FFA: 1775 ± 150 (fasting) vs 980 ± 100µmol/l, 3-OHB: 1780 ± 360 (fasting) vs 230 ± 90 µmol/l, $p < 0.05$); with high concentrations of lipid intermediates in the fasting state a net forearm release of 3-OHB was observed.
These data show that short-term fasting is characterized by increased mobilization of fat. This may partly be due to enhanced lipolytic responsiveness to GH, which in absolute terms appears to be increased with fasting.

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KETONE BODY METABOLISM BY HUMAN ADIPOSE TISSUE.

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Ketone bodies (KB) are an important metabolic fuel for heart, skeletal muscle, kidney and brain. Little is known, however, regarding their role as oxidative fuel for adult human adipose tissue (AT). We measured the in vivo uptake of total KB (acetoacetate + ß-hydroxybutyrate) and oxygen (O_2) uptake by AT in 7 healthy male volunteers (26.8 ± 1.3 y, 74.6 ± 2.1 kg, BMI 23.4 ± 0.5 $kg.m^{-2}$, mean ± SEM) after an overnight fast. Arterial and abdominal subcutaneous vein cannulae were sited for measurements of arteriovenous differences across this tissue bed. AT blood flow was measured with ^{133}Xe and total body fat (TBF) content by dual photon X-ray absorptiometry. Abdominal venous KB concentration was lower than arterial (249 ± 62 vs 265 ± 65 $\mu mol.l^{-1}$, $p<0.02$). Net uptakes of KB and O_2 in adipose tissue were 0.15 ± 0.06 and 3.4 ± 0.5 $\mu mol.min^{-1}.100\ g^{-1}$ respectively. KB oxidation would account for 21.2 ± 10.5 % of adipose tissue O_2 uptake. TBF was 12.8 ± 1.4 kg; systemic adipose tissue KB uptake was estimated at 0.24 ± 0.13 $\mu mol.kg^{-1}.min^{-1}$. Assuming systemic KB turnover of ≈3 $\mu mol.kg^{-1}.min^{-1}$, AT accounts for ≈8% of systemic KB disposal. Thus AT is a minor, but significant site of KB disposal in the postabsorptive state. KB are an important fuel for adipose tissue, accounting for ≈20% of postabsorptive O_2 consumption by this tissue; KB might be even more important in this regard in ketotic conditions such as fasting or poorly controlled insulin-dependent diabetes.

OP 4
Rheology

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VASOREACTIVITY OF AORTIC RINGS FROM DIABETIC RATS IS DEPENDENT ON DIABETES DURATION AND GLUCOSE CONCENTRATION.

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In this study we compared the in vitro contractile response to phenylephrine (Phe, 100 μM) and relaxation response to acetylcholine (Ach, 100 μM) of isolated aorta rings from Sprague-Dawley rats with short term (DS, 9 days, blood glucose 27.0 ± 5.2 SD mM), long term (DL, 6 wks, blood glucose 26.0 ± 5.0 mM) streptozotocin-diabetes, and non-diabetic controls (C) in normal (5mM) and high (30mM) medium glucose concentration. Aortic rings were obtained at the age of 12 wks and mounted between an isometric force transducer. No difference in maximum Ach-induced relaxation (63%) was observed between C and DL in 5 mM glucose; exposure to 30 mM impaired relaxation in C (to 27% $p<0.001$ vs C 5mM), but improved relaxation in DL (95% $p<0.01$ vs C 5mM). Relaxation was impaired in DS in 5 mM and 30 mM glucose (22 % $p< 0.01$ vs C 5mM). Phe-induced contraction was reduced by 19% in C in 30 mM, and in DS in 5 and 30mM compared to 5 mM glucose; contraction in DL in 5 and 30mM was impaired by 47% ($p<0.01$) compared to C (5mM). Thus, endothelium-dependent relaxation is dependent on diabetes duration and *in vitro* glucose concentration. Relaxation, but not contraction, of DL arteries is improved by 30mM compared to 5mM glucose.

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IMPAIRED VASCULAR REACTIVITY IN TYPE 1 (INSULIN-DEPENDENT) DIABETES MELLITUS

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Altered reactivity of resistance arteries may play a role in the pathogenesis of diabetic microangiopathy by modulating systemic blood pressure and/or tissue blood flow. This study documents the reactivity of isolated resistance arteries taken from gluteal subcutaneous fat (<300 μm internal diameter), from 8 Type 1 (insulin-dependent) diabetics (mean age 28 years; mean duration 19 years (range 13-25) and 9 normotensive age-sex matched controls, in a Mulvany myograph preparation [Ethical Committee Approved]. All were normotensive (supine random zero blood pressure; control vs diabetic [mean ±SEM], 112.2±4.3/72.3±3.9 vs 118.0±5.4/73.7±3.0 mmHg, NS), had normal urinary microalbumin excretion (3.7±0.9 vs 3.8±0.8 μg min^{-1}) and no family history of hypertension. Contractile responses to potassium (127mM), noradrenaline (NA, 10^{-8}-10^{-5} M) and angiotensin II (AII, 10^{-11}-10^{-7} M) were recorded. relaxation studies were performed in maximally contracted vessels using acetylcholine [endothelium-dependent] (ACH, 10^{-8}-10^{-5} M), and sodium nitro-prusside [endothelium-independent](SNP,10^{-9}-10^{-5} M). Vascular sensitivity (ED_{50}) to NA and AII was similar in controls and diabetics. However, maximal contractile responses were depressed in diabetics to potassium (2.86±0.38 vs 1.77±0.16 mN/mm, p=0.012), NA (3.67±0.59 vs 2.33±0.38 mN/mm, p=0.08) and AII (2.11±0.51 vs 0.49±0.27 mN/mm, p=0.019. Relaxation to ACH was impaired (70±9 vs 38±13 %, $p<0.05$), but normal with SNP (90±2 vs 83±5 %, NS). These findings reveal impaired vascular reactivity and endothelial function in Type 1 (insulin-dependent) diabetics, factors that may contribute to the development of diabetic microangiopathy.

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DOES PLASMINOGEN ACTIVATOR INHIBITOR PLAY A ROLE IN DETERMINING THE OUTCOME FROM THROMBOLYTIC THERAPY?

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The role of plasminogen activator inhibitor (PAI-1) in reperfusion and reocclusion after myocardial infarction is still unclear. We measured PAI-1 activity in 110 non diabetic and 45 diabetic subjects admitted with acute myocardial infarction. Levels of PAI-1 activity on admission were significantly higher in diabetic compared to non diabetic subjects (24.6±6.9 vs 18.6±7.9 AU/ml;p=0.0001). We calculated time to peak creatine kinase - MB (CK-MB) isoenzyme in 123 (80%) subjects as a short time to peak has been shown to be a valid index of reperfusion. In the 98 subjects receiving thrombolytic therapy, median time to peak CK-MB was 15.5 h (7.5 to 24) in diabetic subjects (n=26) compared to 12 h (5 to 26) in non diabetic subjects (n=72) (p=0.005). In subjects with time-to-peak ≤ 12 h, indicating likely successful reperfusion, PAI-1 activity was 17.5±7.8 compared to 22.8±7.7 in those with time-to-peak > 12 h (p=0.001). In multiple regression analysis including both diabetes and admission PAI-1 activity in the equation, both diabetes (p=0.0001) and PAI-1 activity (p=0.029) were significantly related to reperfusion. These results suggest that (1) diabetic subjects have elevated PAI-1 activity on admission with acute myocardial infarction and (2) both diabetes and elevated PAI-1 activity were independently associated with a reduced likelihood of reperfusion following myocardial infarction.

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PLASMINOGEN ACTIVATOR INHIBITOR 1 LEVELS IN PLASMA ARE ASSOCIATED WITH INSULIN RESISTANCE.

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The fibrinolysis inhibitor plasminogen activator inhibitor type 1 (PAI-1) has been identified as a cardiovascular risk marker. It is associated with hypertriglyceridaemia, hyperinsulinaemia and type II diabetes, conditions in which insulin action is impaired. To determine whether PAI-1 is related to insulin action, we studied 9 obese nondiabetics and 10 obese type II diabetics by means of a sequential hyperinsulinaemic euglycaemic clamp study. Insulin sensitivity was determined by the insulin level at which glucose uptake by the peripheral tissues is half-maximal (ED_{50}pgu). PAI-1 antigen at 8:30 hrs correlated significantly with ED_{50}pgu ($r=0.865$, $p<0.001$) in both nondiabetics ($r=0.977$, $p<0.001$) and in diabetics ($r=0.868$, $p<0.01$). Multiple regression analysis disclosed ED_{50}pgu, fasting plasma proinsulin level, and body mass index as the only significant determinants of PAI-1, accounting for 94, 3.5 and 0.2 % of the total variance of PAI-1 respectively, but insulin level was not independently related to PAI-1. The findings suggest a causal relationship between insulin action and PAI-1 levels, and suggest that PAI-1 may have a role in the pathogenesis of athero-thrombosis, induced by insulin resistance.

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POLYMORPHONUCLEAR SUPEROXIDE PRODUCTION IS ACTIVATED IN POOR-CONTROLLED TYPE 2 DIABETES MELLITUS.

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Activated polymorphonuclear leukocytes (PMN) have been associated with cardiovascular risk and atherogenesis through leukocyte-endothelial interaction and lipid peroxidation. Spontaneous and phorbol ester stimulated superoxide production was investigated in hyperglycemic state and after glycemic normalization (insulin or antidiabetic drugs administration) in 10 poor-controlled type 2 diabetics (HbA1C = 9.38±1.34%) without evidence of infection or macrovascular complications. Superoxide anion generation by PMN was assessed by determination of superoxide dismutase inhibitable reduction of ferricytochrome C (nmol/10^6 cells/5min) and oxygen metabolism of PMN from diabetic subjects was compared to 5 non-diabetic controls. Glycemic values reached 1.84±0.56 mg/ml initially and decreased to 1.18±0.19 mg/ml after antidiabetic treatment. Diabetic PMN exhibited a greater superoxide production in hyperglycemic state compared to non-diabetics, in stimulated conditions (17.72±2.74 vs 14.04±1.36,$p<0.05$) but not spontaneously (2.94±1.31 vs 3.1±0.95,NS). After treatment, a decrease of superoxide generation from diabetic PMN was noted (17.88±2.52 and 16.23±4.13,NS) and compared to controls, PMN activation was restored to normal while no different values could be found between the PMN from diabetics and non-diabetic subjects (18.34±4.86 vs 16.04±9.08 and 3.52±1.18 vs 4.08±1.6,in stimulated and non stimulated condition respectively,NS). These findings provide evidence of PMN activation in poor-controlled Type 2 diabetes and show that metabolic control is associated with a reduction of superoxide anion production to normal values. Thus, PMN activation must be related to the metabolic abnormalities associated with uncontrolled non-insulin-dependent-diabetes mellitus and the hypothesis of its participation in diabetic vascular complications can be suggested.

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GLUTATHIONE AND SUPEROXIDE ANION PRODUCTIVE ACTIVITY IN DIABETIC POLYMORTHONUCLEAR LEUKOCYTES

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It is accepted that one of the cause of the susceptibility to certain infections in diabetic patients is the impairment of superoxide anion(O_2^-) productive activity in polymorphonuclear leukocytes(PMNs). But its mechanism has not been clearly elucidated. Glutathione is important as the oxidation-reduction agent in vivo. We investigated the correlation between glutathione concentration and O_2^- production in PMNs. Formyl-methionyl-leucyl-phenylalanine stimulated O_2^- production was assayed spectrophotometrically by the reduction of ferricytochrome C. Glutathione was extracted by hyperchloric acid and measured by glutathione reductase-DTNB recycling method. O_2^- productive activity in diabetics(n=17) was significantly lower than that in controls(n=21) (9.5 ±2.4 vs 14.4 ±3.4nmol/10^6cells/ 5min, $p<0.001$). Significant decrease in Glutathione concentration of diabetic PMNs(n=13) was found compared with that of control PMNs(n=14) (1.09 ±0.22 vs 1.42±0.24nmol/10^6cells, $p<0.001$). After masking of glutathione by N-ethylmaleimide, O_2^- production was significantly decreased in controls. There was a correlation between concentration of glutathione and O_2^- productive activity in control PMNs(correlation coefficient= 0.780, $p<0.01$). These results suggest that decreased glutathione concentration might be one of the factors accounting for the impairment of O_2^- productive activity in diabetic PMNs.

OP 5
Experimental Nephropathy

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EFFECT OF ATRIAL NATRIURETIC FACTOR ON CYTOSOLIC FREE CALCIUM CONCENTRATION IN RAT MESANGIAL CELLS

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We previously reported that plasma atrial natriuretic factor (ANF) levels increase in early stages of diabetic nephropathy. In this study, we examined the effects of ANF on glomerular mesangial cells (MCs) through the changes of responsiveness to angiotensin II (AII) and ATP. Rat MCs were cultured for 5 days in RPMI 1640 medium. Cytosolic free calcium concentration ($[Ca^{2+}]i$) was measured in fura-2 loaded MCs by dual wave length spectro-fluorometry. Both AII and ATP caused a rapid increase in $[Ca^{2+}]i$ in dose dependent manner. Preincubation of MCs with 10^{-7}M ANF for 10 min significantly reduced the peak response of $[Ca^{2+}]i$ to 10^{-7}M AII (from 244.8±32.7% to 178.3±23.9, expressed as percent increase above the basal, $p<0.01$). Moreover, ANF treatment caused the same response of MCs to 10^{-4}M of ATP (238.2±23.0 % vs 164.7±30.4, $p<0.01$). Recently, it has been reported that extracellular ATP exerts significant effects on MCs function. Therefore, these results suggest that elevated levels of ANF modulate the physiological regulation of Ca^{2+}-mediated second messenger systems in MCs and have some implications in the pathogenesis of diabetic nephropathy.

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EFFECT OF GLUCOSE CONCENTRATIONS ON THE RESPONSE OF CYTOSOLIC FREE CALCIUM LEVELS TO ANGIOTENSIN II IN RAT MESANGIAL CELLS

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The contractility of glomerular mesangial cells (MCs) is regulated by the change of cytosolic free calcium levels ($[Ca^{2+}]i$). To assess MCs function in diabetes, we studied the effect of glucose concentrations on the responsiveness of $[Ca^{2+}]i$ to angiotensin II (AII) in MCs. The MCs were cultured for 5 days in RPMI 1640 media containing glucose at 5.5mM (N), 25mM (H) or 5.5mM plus mannitol 19.5mM (M). $[Ca^{2+}]i$ were measured in Ca^{2+} free buffer with EGTA, using fura-2 and dual-wavelength spectrofluorometry. $[Ca^{2+}]i$ showed a rapid peak increase in response to 10^{-7}M AII (the first response), then returned to basals within 3 min in the three groups. After the AII-induced transient increase in $[Ca^{2+}]i$, addition of $CaCl_2$ caused a second increase in $[Ca^{2+}]i$ in N and M groups. In H group, however, the AII-induced first peak response (209±24%; percent increase above the basal) was significantly lower than those in N (295±15%) or M (308±28%) groups, and $CaCl_2$-induced second increase in $[Ca^{2+}]i$ was abolished. These results suggest that high glucose level itself, and not high osmolality, affects the response of MCs to AII through the change of Ca^{2+}-mediated second messenger systems.

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MECHANISMS OF GLUCOSE-ENHANCED SYNTHESIS OF EXTRACELLULAR MATRIX BY RAT MESANGIAL CELLS

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To investigate mechanisms of glucose-enhanced extracellular matrix synthesis by rat mesangial cells, we assessed effects of D-glucose (5.5 [G5.5] and 30 [G30] mM): 1) vs iso-osmolar concentrations of sugars which do not enter the cell (mannitol [M]) or gain limited (D-galactose [Gal]) or no (L-glucose [LG]) access to metabolic pathways but are capable of inducing nonenzymatic glycosylation; 2) ± the aldose reductase inhibitor (ARI) Alcon 1576 (14 uM, gift of Alcon) or 1 mM sodium pyruvate ([P], which is able to correct glucose-induced polyol pathway-dependent increased NADH/NAD+, via the lactate deydrogenase reaction). Cells were cultured for 4 weeks through 6-8 passages in DMEM + 17% FBS under the conditions indicated above. Sub-confluent cells were exposed for 24 h to β-aminoproprionitrile (100 ug/ml) and ascorbic acid (50 ug/ml) and levels of type I (C-I) and IV (C-IV) collagen, fibronectin (FN), and laminin (LAM) released in media were quantified by an enzyme immunoassay. FN, LAM, C-IV, but not C-I synthesis was increased by G30 compared to G5.5, starting at week 2 (FN: 876.8±89.9 ng/ug DNA vs 591.5±46.4; LAM: 13.6±1.4 vs 9.2±1.4 C-IV; 365.0±62.8 vs 202.1±54.4; $p<0.0001$) and persisting throughout the remaining 2 weeks. These changes were partly mimicked by Gal (FN: 726.6±90.5, $p<0.0001$ vs G5.5; LAM: 11.6±2.2, $p<0.003$; C-IV: 301.7±65.7, $p<0.0001$) and LG (FN: 723.5±100.2, $p<0.0001$; LAM: 11.9±2.2, $p<0.002$; C-IV: 303.7±60.1, $p<0.0001$), but not by M. Matrix changes were delayed but not prevented by P and ARI. These results suggest that multiple mechanisms may be operating in glucose-induced matrix overproduction, including nonenzymatic glycosylation and glucose- (and polyol pathway-) dependent cytosolic redox changes; increased osmolarity seems to play a minor role, if any.

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PARACRINE RELATIONSHIPS BETWEEN PGE_2, HYALURONAN AND MESANGIAL CELL PROLIFERATION AND SULPHATION ACTIVITY.

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Exposure *in vivo* or *in vitro* to elevated glucose leads to increased production of vasodilatory prostaglandins (PGs) by glomeruli and mesangial cells. This study aimed to determine whether this increased PG production, could, in addition to its early functional effects, provide a link with the later structural changes of diabetic nephropathy. From analogy with other tissues, the effects of PGs on hyaluronan (HA) production by glomerular cores was examined. HA production by glomerular cores from streptozotocin diabetic rats was increased compared with control rats (13.6 ± 0.6 vs 11.1 ± 0.3 μg/mg protein, $p < 0.025$). Exposure *in vitro* to PGE_2 1.5 x 10^{-9}M caused a further increase (to 21.6 ± 1.8 $p < 0.005$ diabetic and 14.4 ± 0.4 control $p < 0.05$). HA (250 ng/ml) led to a decrease in the relative degree of sulphate incorporation into glycosaminoglycans and proteoglycans in the pericellular matrix (sulphation ratio 0.078 ± 0.003 vs 0.088 ± 0.003, $p < 0.05$). Additionally, in cultured mesangial cells, HA 250 ng/ml and PGE_2 10^{-8}M led to an increase in ^{3}H-thymidine incorporation at 24h (to 179 ± 14% and 161 ± 12% of control respectively, $p < 0.005$). It is concluded that increased PG synthesis secondary to a high glucose environment can lead to changes in glomerular HA synthesis which can affect sulphate incorporation and mesangial cell proliferation. These paracrine effects provide possible links between initial biochemical consequences of hyperglycaemia and later structural changes in the glomerulus.

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GLOMERULAR FILTRATION AND TUBULAR REABSORPTION OF ALBUMIN IN EARLY AND ESTABLISHED STZ-DIABETIC RATS

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Microalbuminuria is considered to be an indicator of the onset of diabetic nephropathy in Type 1 (insulin dependent) diabetes. However, early glomerular abnormalities in Type 1 diabetics may result in increased filtration of albumin prior to any observed increase in albumin excretion (U_{alb}). Glomerular and tubular albumin kinetics (with ^{125}I-albumin) were examined in streptozotocin diabetic Munich-Wistar rats (STZ-D, 65 mg/kg BW i.v.) at 7-10 days (7d, untreated, blood glucose (BG) = 20±1 mmol) and 50-70 days (50d, poorly controlled, BG = 17±1 mmol) after onset of STZ-D. These animals were compared to non-diabetic rats (CON, BG = 7±1 mmol). Sub-groups of each condition received acute lysine treatment (+L, 200 mg/kg BW/hr) to block tubular protein reabsorption. In addition, non-vascular albumin distribution volumes (Vd_{alb}) were measured in the kidney and compared to morphometric measurements of interstitial space (INT) to assess tubular uptake of albumin. U_{alb} in CON was 20±3 μg/min and was not altered at 7d STZ-D (19±3 μg/min, NS) but increased in 50d STZ-D to 118±13 μg/min ($P<0.05$). With +L, U_{alb} was 30±6 μg/min in CON+L (NS to CON) and increased in 7d STZ-D+L (67±10 μg/min, $P<0.05$) and was 126±11 μg/min in 50d STZ-D+L (NS to 50d STZ-D). Vd_{alb} increased from CON (23±1% of cortical volume) in both 7d STZ-D (37±4%) and 50d STZ-D (28±1%). +L decreased Vd_{alb} in 7d and 50d STZ-D to values not different from either CON or INT (INT=21±2% in CON, 19±1% in 7d, and 20±2% in 50d STZ-D) indicating that increases in Vd_{alb} were due to tubular reabsorption of albumin.

Conclusions: Increased glomerular filtration of albumin occurs early in STZ-D, but albumin does not appear in the urine due to the inherent tubular capacity for albumin uptake. In 50d STZ-D, despite increased glomerular filtration of albumin, tubular reabsorption of protein was decreased by >50% compared to 7d STZ-D resulting in both factors increasing U_{alb}.

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RENAL AND NEURAL ASCORBIC ACID METABOLISM IN THE SPONTANEOUSLY DIABETIC INSULIN-DEPENDENT BB/EDINBURGH RAT.

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Reduced plasma ascorbic acid (AA; vitamin C) concentration in human diabetic subjects and streptozotocin-induced diabetic rats has suggested a link between AA metabolism and diabetic microangiopathy. This study investigated plasma, kidney and sciatic nerve concentrations of AA in the spontaneously diabetic BB/Edinburgh (BB/E) rat. Samples of blood, kidney and sciatic nerve were removed from age-matched (mean±SEM age = 174±5 d) non-diabetic and diabetic (duration of diabetes = 79±4 d) BB/E rats. Plasma was deproteinised using 5% metaphosphoric acid (MPA). Kidney and sciatic nerve (lyophilised) samples were homogenised in 5% MPA. Total AA was determined by fluorimetry. Mean glycated haemoglobin (HbA1), measured by electroendosmosis, was significantly higher ($p<0.05$) in the diabetic rats (5.9 ±0.3%) than the non-diabetic animals (4.9±0.2%). There was no significant ($p>0.1$) difference in mean values for either plasma (19.9±4.5 μmol/l vs 30.7±4.5 μmol/l) or nerve (239±40 pmol/mg dry weight vs 244±45 pmol/mg) AA levels in the diabetic (n=8) and non-diabetic (n=10) groups, in fact two diabetic animals had significantly elevated nerve AA concentrations (1940 pmol/mg and 3890 pmol/mg). Mean kidney AA level was significantly lower ($p<0.02$) in diabetic rats (340±40 pmol/mg) than non-diabetic animals (500±40 pmol/mg). We conclude that increased oxidative stress in the kidney may be important in the development of diabetic nephropathy but seems unlikely to be involved in diabetic neuropathy.

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DECREASED ENDOTHELIN-1 mRNA IN THE KIDNEY OF STREPTOZOTOCIN (STZ) INDUCED DIABETIC RATS

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Endothelin-1 (ET-1) caused reduction in renal blood flow, glomerular filtration rate, urinary sodium excretion, and was capable to stimulate mitogenesis in mesangial and glomerular cells. Moreover mRNA for preproendothelin is present in renal glomerular cells and in the inner medulla. Some reports indicated that the development of diabetic nephropathy is associated the local production of growth factors. In this study, we try to investigate whether renal ET-1 local production is affected in the STZ-induced diabetic rats. Twenty Wistar rats were induced to diabetes by STZ, and sacrificed on the 2, 4, 7, 14, 21, 28, 35 and 42 days later to obtain the kidney. The random plasma glucose levels of diabetic rats were higher than 350mg/dl on the 3rd day after STZ injection and on the sacrificed day. Renal total RNA was extracted by guanidine isothiocyanate. Ten-microgram total RNA per lane was fractioned on 1% formaldehyde-agarose gel and then transfered to nylon membrane, and hybridized with a random-primed ^{32}P-labelled *Eco*RI fragment of rat ET-1 cDNA. The result revealed that renal ET-1 mRNA expression decreased progessively from day 7 to day 42. Our finding suggested that local production of ET-1 on the kidney may be decreased in early stage of STZ-induced DM.

OP 6
Signal Transduction

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GTP-BINDING PROTEINS IN ISLET SUBCELLULAR FRACTIONS: REGULATION BY LIPID PRODUCTS OF PHOSPHOLIPASES A_2 AND D

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Low molecular-weight GTP-binding proteins (LMW-GBPs) have been implicated in late stages of stimulus-secretion coupling, but have not been studied in normal islets. Therefore, we studied [α-^{32}P]GTP binding and GTPase activity in islet subcellular fractions. Four LMW-GBPs (23-28 kDa) and one heterotrimeric GBP (39-40 kDa) were labeled in secretory granules (SG) > heavy membranes > cytosol. Kinetic analysis of GTP-specific hydrolysis revealed 3 GTPases in homogenates (K_m 370 nM, 2.2 μM, and 720 μM GTP). The highest affinity GTPase was ribosylated and thereby inhibited by pertussis-toxin pretreatment, and thus presumably corresponds to heterotrimeric G_i and/or G_o. We investigated regulation of these GTPases by lipid products of insulinotropic phospholipases (A_2 and D) endogenous to islets. In individual and/or reconstituted fractions, arachidonic acid, phosphatidic acid,and lysophosphatidylcholine (but not control lipids phosphatidylinositol, phosphatidylcholine, and lysophosphatidylethanolamine) inhibited GTPase in a concentration-dependent manner (by up to 60-80% at 100 μg/ml lipid). These effects on GTPase were not due to inhibition of GTP binding and/or exchange, as assessed by [^{35}S]GTPγS binding. Therefore, inhibition of GTPase would maintain GBPs in their activated, GTP-bound state. These data identify regulatable GBPs in islet fractions which may be a locus (i.e., "G_E") at which phospholipase activation culminates in insulin secretion.

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AN EFFECTOR DOMAIN PEPTIDE OF GTP-BINDING PROTEIN RAB3 STIMULATES INSULIN RELEASE FROM PERMEABILIZED HIT CELLS

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rab3 is a low molecular weight GTP-binding protein of the ras superfamily. This protein is present on cellular vesicles including insulin granules, and thus may control vesicular transport and membrane fusion. A synthetic peptide of rab3 (rab3AL), corresponding to the effector domain of ras which interacts with GTPase-activating protein (GAP), was used to examine whether rab3 plays a role in exocytosis of insulin. Insulin-secreting HIT-T15 cells were permeabilized with 1.5 U/ml streptolysin-O for 5 min. Optimal GTPγS (100 µM) stimulated insulin secretion 2-fold at basal Ca^{2+} level (0.1 µM), but did not increase further high Ca^{2+}-induced release (3-5 fold) during 5 min incubation. rab3AL (50 µM) enhanced (2-fold) the insulin secretion at either low (0.01-0.1 µM) or high (1-100 µM) Ca^{2+}. The effect of GTPγS was also doubled by 50 µM rab3AL. The effect of rab3AL was dose-dependent with EC_{50} at about 15 µM. In contrast to rab3AL, the synthetic peptide of the effector domain of ras or rab1, at 50 µM, had no effect on basal or stimulated insulin release. rab3AL did not promote insulin secretion from intact cells. These results suggest that i) rab3 and its effector may be involved in the regulation of insulin secretion and ii) the mechanism underlying rab3AL action is distinct from that of Ca^{2+} and GTPγS.

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INDUCTION OF HEPARIN-SENSITIVE OSCILLATIONS OF CYTOPLASMIC Ca^{2+} IN HYPERPOLARIZED ß-CELLS.

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Glucose induction of sinusoidal Ca^{2+} oscillations in pancreatic ß-cells requires periodic depolarisation with a resulting entry of the ion. Combining whole cell configuration of the patch clamp technique and dual wavelength fluorometry with 100 µmol/l Indo-1 it was analysed whether it is possible to obtain Ca^{2+} oscillations also when maintaining ß-cells in a hyperpolarized state. Activation of the G-proteins either by internal perfusion with 100 µmol/l GTP-γ-S or external application of 100 µmol/l carbachol in ß-cells held at -70 mV resulted in oscillations of cytoplasmic Ca^{2+} from a basal level of 60-100 nmol/l to tenfold higher values. These Ca^{2+} transients were sufficiently pronounced to activate 70-150 pA K^+ currents in a K^+-rich medium. The oscillations obtained after external application of carbachol had a similar frequency (1.52 ± 0.13 /min) as those recorded during internal perfusion with GTP-γ-S. When omitting Indo-1 the frequencies recorded from current measurements after injection of GTP-γ-S increased from 1.40 ± 0.07 to 2.32 ± 0.22 /min. Irrespective of whether the oscillations reflected a response to GTP-γ-S or carbachol they were completely abolished by injection of 100 µg/ml heparin. It is concluded that both external and internal activation of G-proteins result in ß-cell oscillatory activity, probably mediated by intracellular release of Ca^{2+} in response to activation of a heparin-sensitive inositol-1,4,5-trisphosphate receptor.

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EFFECT OF GLUCOSE CONCENTRATION ON INSULIN AND "GLUCOSE-SENSOR" GENES EXPRESSION IN INS-1 CELLS

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It is suggested that glucokinase and glucose transporter 2 (GLUT2) are the so-called "glucose-sensing" apparatus of ß cells. Increase in glucose from 2.8 to 20 mmol/l resulted in a 4 fold increase of insulin secretion in INS-1 cells. We investigated the expression of these genes and that of insulin at different glucose concentrations. Cells were cultured at 2.8, 10, and 20 mmol/l glucose and 0.1% FCS for 72h. At 10 mmol/l the mRNA levels of all these genes were equal to control levels (10% FCS and 10 mmol/l of glucose) and reduced to 50% at 2.8 mmol/l glucose. At 20 mmol/l glucose, glucokinase mRNA slightly increased, GLUT2 mRNA moderately decreased and proinsulin mRNA reduced to about 75%. GLUT1 mRNA was not detected under the culture conditions we tested. In similar culture conditions secreted immunoreative insulin were 0.485 ± 0.02, 1.39 ± 0.01 and 1.07 ± 0.2 and the contents were 3.28 ± 0.02, 5.05 ± 0.03, 1.79 ± 0.09 mg/ 10^6 cells at 2.8, 10, and 20 mmol/l of glucose respectively. Therefore, glucokinase GLUT2 and insulin mRNAs levels do not vary similarly at different glucose concentrations. Furthermore glucose has opposing effects on insulin secretion and synthesis at 20 mmol/l.

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MALONYL-COA IN ß-CELL SIGNALLING : CONTROL OF FATTY ACID OXIDATION OR SUBSTRATE FOR LIPID BIOSYNTHESIS ?

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We have proposed that malonyl-CoA is a metabolic coupling factor in nutrient-induced insulin release. Malonyl-CoA is the first intermediate in lipid biosynthesis pathway and it also controls fatty acid oxidation. Its concentration results from the balance between its synthesis by acetyl-CoA carboxylase (ACC), degradation by malonyl-CoA decarboxylase and usage by fatty acid synthase (FAS). We therefore studied the expression of ACC and FAS in rat pancreatic islets and clonal ß-cells (HIT). Two ACC isozymes were detected in both islets and HIT cells : a 265 kDa isoform present in lipogenic tissues and a 280 kDa isoform thought to be implicated in fatty acid oxidation regulation. Islets and HIT cells contained high amounts of ACC mRNA, protein and enzymatic activity. In contrast, FAS mRNA and enzymatic activity were undetectable in islets whereas they were abundant in lipogenic tissues. These observations demonstrate that *de novo* lipid biosynthesis is not involved in ß-cell signalling. The data rather suggest that malonyl-CoA formation in ß-cell is strickly involved in regulating fatty acid oxidation. In addition, the results explain why malonyl-CoA rises markedly and rapidly upon ß-cell nutrient stimulation i.e. it is not diverted to lipid biosynthesis.

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HYPOGLYCEMIA INDUCES AN HYPERSENSITIZATION OF THE INSULIN RECEPTOR TYROSINE KINASE ACTIVITY.

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To investigate the effect of hypoglycemia "per se" on the regulation of muscle-derived insulin receptor tyrosine kinase activity (TKA), 2 groups of Sprague-Dawley rats were infused with either Insulin ($4mU\cdot Kg^{-1}\cdot min^{-1}$) (INS) or Phloridzin ($3\ mg\cdot Kg^{-1}\cdot\ min^{-1}$) (PHL); plasma glucose was maintained at mean values of 3.3 and 3.5 mM in INS and PHL respectively, by a variable glucose infusion (clamp technique); 6 hour-fasted rats represented the control group (C). Receptors were isolated under conditions designed to preserve their in vivo phosphorylation state, and their TKA towards poly(Glu-Tyr) was measured in the absence and presence of in vitro exposure to insulin. Insulin infusion resulted in an enhanced "in vivo" TKA ($p<0.05$ vs C and PHL). Surprising was the finding of a slight increase ($p<0.05$ vs C) of the in vivo TKA in PHL. The in vitro insulin dose response curves of TKA showed no significant differences between INS and C. In contrast, there was a marked increase of the insulin-stimulated TKA in PHL ($p<0.001$ vs C and INS); at 100nM insulin TKA was 1.8 fold more responsive when compared to both C and INS. Moreover, in PHL the half-maximal stimulation of TKA was <0.5 nM, being over 10 fold more sensitive to insulin than both INS (3.8 ± 0.03 nM; mean±SE) and C (4.2 ± 0.05 nM; mean±SE). In conclusion: hypoglycemia associated with low plasma insulin concentrations determines an hypersensitization of the intrinsic tyrosine kinase of the insulin receptor.

OP 7
Forms of Therapy II

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Nasal Insulin Outpatient Study in Type I Diabetes

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19 patients (15 male) with Type I diabetes on basal and prandial insulin therapy completed an open, randomised, crossover study comparing U500 Novolin® nasal insulin with meals and U100 soluble subcutaneous insulin 30 minutes before meals with four week outpatient treatment periods culminating in 24 hour inpatient metabolic profiles. Mean age was 39.0 y (SD 9.9), diabetes duration 11.2 y (SD 9.7), body mass index 24.5 kg m^{-2} (SD 3.6) and total daily insulin dose 0.7 IU kg^{-1} (SD 0.1). Four subjects noted slight and one moderate nasal irritation. Frequency of transient hypoglycaemia was similar for both therapies apart from a more severe episode in one patient who took nasal insulin without a meal. When on nasal insulin, mean fasting plasma glucose levels were not significantly different but incremental mean postprandial glucose values were elevated (4.4 *v* 3.0 mmol/l, p=0.011), mean HbA1c values higher (9.1 *v* 8.5, p<0.001), mean weight lower (71.7 *v* 73.3 kg, p<0.001) and admitted dietary intake tended to be less. U500 Novolin® nasal insulin, given as a single administration immediately before meals, was effective in treating Type I diabetic outpatients but achieved less satisfactory glycaemic control with the limited dose range available.

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A DOUBLE BLIND, PLACEBO CONTROLLED DOSE RESPONSE STUDY OF NASAL INSULIN IN RELATION TO A STANDARD MEAL IN TYPE 2 DIABETIC SUBJECTS

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The aim of this study was to compare the efficacy of nasal insulin (500U/ml), placebo nasal spray and subcutaneous insulin injection in relation to standard meal in nine insulin treated type II diabetic subjects (fasting C-peptide >0.30nmol/l) Each received either active nasal insulin (50, 100 or 150 U), placebo nasal spray or subcutaneous insulin (8-9 U) on separate days in random order immediately (nasal insulin) or 20 min before (subcutaneous insulin) a standard meal. Following nasal insulin, free insulin concentrations rapidly increased to reach peak values after 20-40 min of 75, 108 and 155 mU/l for 50, 100 and 150 U of nasal insulin respectively before returning to baseline by 120 min. In contrast, after subcutaneous injection, free insulin concentrations reached a peak around 120 min thereafter remaining above control day values for the remainder of the 300 min study. The area under the blood glucose curve (0-120min) was significantly lower following 100 (16.8±3.0 (±SD)) and 150 U (15.4±4.9) of nasal insulin compared to placebo (20.8±3.8; p<0.05). Following 150 U nasal insulin, blood glucose concentration did not rise significantly above baseline during the 300 min study. Nasal insulin was well tolerated and did not cause hypoglycaemia. In these well controlled, insulin sensitive type II subjects, nasal insulin improved meal related blood glucose control in a dose dependent manner.

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Long term, Large-scale Clinical Evaluation of Performance of the M1000 Programmable Implantable Insulin Pump.

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We report the performance of 123 M1000 pumps (INFUSAID ®, Norwood) which have been implanted for the **delivery of U100 Hoecsht 21 PH Insulin in 177 Type I diabetic patients at 15 centers. The total duration of pump implant is 203 years (mean 19,8 months/pump)** . One pump failure, in the stop mode (circuit break) has been reported, and one pump has been explanted due to suspicion of battery depletion. Eleven irreversible catheter occlusions have been reported. Glycemic control **was assessed by monthly HbA1c values and daily blood glucose readings. A decrease/ stabilization of mean HbA1c** levels was realized, as was an increase in the frequency of glucose readings in the normal range during the first 18 months of pump therapy. Analysis of 24 hour glucose profiles performed at baseline and month 6 indicates that mean glycemia decreased in 63 %, standart deviation decreased in 76 %, and M-value decreased in 76 % (n=49 patients). **The incidence of severe hypoglycemia was monitored during a three-month intensive therapy phase pre-implant (10.8 %/pt yr) and during daily pump therapy (2.0 %/pt yr). Hence the increase in glycemic control is realized without increase in glycemic control is realized without a concurrent increase of severe hypoglycemia.**

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THE FRENCH EXPERIENCE OF INTRAPERITONEAL INSULIN THERAPY WITH IMPLANTABLE PROGRAMMABLE INFUSION PUMPS.

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Implantable insulin pumps are evaluated pre-routinely on a large-scale in France, under the scrutiny of the EVADIAC Group. As of January 1992, 7 centers have reported 171 pump implantations in 162 type I diabetic patients, aged 39± 1 yrs, with diabetes duration of 18 ± 1 yrs, representing 145 patients-years of experience (range : 3-25 months). There has been no death, pump overflow or severe infection, and only 2 patients elected to return to conventional therapy. Rates of reintervention for catheter malfunction and on pump site were 15.3 and 8.3 % patient-years respectively, including 3 pump failures and 6 pump-pocket erosions/infections. Severe hypoglycemias and ketoacidosis rates were 6.2 and 5.5 % patient-years. Overall diabetes control and glycemic fluctuations slightly improved when compared to preimplantation : HbA1c 7.1 ± 0.1 vs 6.6 ± 0.2 (NS), SD of home blood glucose testings 69 ± 2 vs 60 ± 3 mg/dl (p < 0.05) at 0 and 15 months respectively. We conclude that implantable programmable insulin pumps have proven reasonable safety and efficacy on a large-scale although still relatively limited duration basis.

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ACARBOSE SIGNIFICANTLY IMPROVES GLUCOSE CONTROL IN NON-INSULIN-DEPENDENT DIABETES MELLITUS SUBJECTS (NIDDM): RESULTS OF THE MULTICENTER CANADIAN TRIAL. S. Ross*, Calgary, J.-L. Chiasson*, J. Hunt*, Vancouver, R. Josse*, Toronto, J. Mukherjee*, Toronto, C. Palmason*, Toronto, W. Rodger*, London, E. Ryan*, Edmonton, and M. Tan*, Halifax and T. Wolever*, Toronto, Canada.

Persistent elevation of post-prandial glucose will contribute to long-term poor control of diabetes in NIDDM subjects. In a randomized double-blind placebo-controlled one year trial, the efficacy and safety of Acarbose, an alpha glucosidase inhibitor, was studied in 354 NIDDM subjects treated with either diet alone (D) or diet plus sulfonylurea (S), metformin (M), or insulin (I). Glucose control was assessed by fasting glucose (FG), HBA1C, and area under the curve following a standard test meal (AUC). The mean FG for all subjects was 12.1 ± 0.6 mmol/L and was not affected by Acarbose in all four groups. The AUC, however, was significantly decreased ($p = < 0.0001$) in all four treatment groups. There was a significant decrease in HbA1C in D ($p = < 0.0335$); S($p = < 0.0020$); M($p = < 0.0058$). There was a clinically relevant but not significant fall in HbA1C in the I group. A multivariate logistic regression model was fitted to the data using treatment, demographic, baseline biochemical and nutritional parameters as predictors. The treatment parameter (Acarbose vs Placebo) proved to be one of the strongest predictors of response except in the I group. Response is defined as an HbA1C of < .07 and/or a drop of 1% or more from baseline. Other strong predictors were body mass index and baseline HbA1C. The odds of being a responder to Acarbose, was 4:1(D); 3:1(S); 12:1(M). While GI adverse events were increased in the Acarbose group, none of these events were serious or life threatening. These data indicate that Acarbose is an effective and safe drug to use as adjunct therapy in NIDDM patients.

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Multicentre Trial of Miglitol in Patients with Non-Insulin Dependent Diabetes

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A six month, double-blind trial of an alphaglucosidase inhibitor, recruited 315 diet or sulphonylurea treated diabetics in 22 UK centres. Equal numbers were randomised to placebo, Miglitol 50mg or 100mg tds. Body weight, glycaemic indices and lipid levels were monitored regularly, with initial and final 3 day diet diaries. 201 (64%) with median age 61 y, diabetes duration 4 y, mean HbA1c 8.0 % (SD 1.2), fasting plasma glucose 10.7 mmol l^{-1} (SD 3.0) and body mass index 27.8 kg m^{-2} (SD 4.8) completed the study. 67% of the 114 who withdrew cited flatulence or diarrhoea, particularly if on active therapy. No significant changes occurred in mean body mass index, admitted dietary intake, triglyceride or HDL cholesterol levels. Final mean total cholesterol levels were significantly lower in the placebo group than the 50 or 100 mg tds active therapy groups (6.3 *v* 6.7 *v* 6.8 mmol l^{-1}, $p<0.01$) whilst higher levels were seen in the placebo group for HbA1c (8.7 *v* 8.2 *v* 7.9 %, $p<0.01$) and fasting plasma glucose (12.1 *v* 11.4 *v* 10.0 mmol l^{-1}, $p<0.005$). Improved glycaemic, but not cholesterol, levels were obtained over a six month period in patients allocated to Miglitol.

OP 8
Clinical Neuropathy

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VIBRATORY PERCEPTION AND THERMAL DISCRIMINATION IN RELATION TO GLYCEMIC LEVEL IN A CAUCASIAN POPULATION

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To establish the relationship between glucose metabolism and the occurrence of neuropathy, we studied subjects with various degrees of glucose intolerance, as assessed in an epidemiologic survey in a general Caucasian population, aged 50-76 years (the Hoorn Study). Included were 65 subjects with previously diagnosed diabetes mellitus, 87 with newly-detected diabetes, 236 with impaired glucose tolerance and 238 with normal glucose tolerance. In this age/gender/glucose tolerance stratified random sample, we assessed vibratory perception (VP) and thermal discrimination (TD) thresholds of the foot. Both thresholds were significantly related to age and gender, women having lower thresholds than men. In a multiple regression analysis, controlling for age and gender, neither VP nor TD was related to post-load plasma glucose. TD showed a weak positive relation with serum concentration of fructosamine only ($p=0.06$). However, VP was positively related to fasting plasma glucose ($p<0.01$), HbA1c ($p=0.01$) and fructosamine ($p<0.0001$). The relation of VP with fructosamine persisted, even when excluding diabetic subjects from analysis ($p=0.02$). Thus, our cross-sectional data suggest that, in a Caucasian population, the function of thick, myelinated nerve fibres, as measured by vibratory perception threshold, is inversely related to glycemic level, even in the non-diabetic range.

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BLOOD GLUCOSE CONTROL AND PROGRESSION OF DIABETIC NEUROPATHY: EIGHT YEARS RESULTS FROM THE OSLO STUDY.

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Forty-five type 1 (insulin-dependent) diabetic patients (age 18-42 years, diabetes duration 7-23 years) were treated either with continuous insulin infusion, multiple injections (x4-6 daily) or conventional (x2 daily) treatment and followed prospectively for eight years. Mean HbA1 at start 11.2±2.2(SD)%. Mean HbA1 during eight years 9.5 ± 1.5(SD)%, range 7.3-13.2% (normal <7.6%) ($p< 0.01$). Motor and sensory nerve conduction velocities (NCV) were measured at start and after eight years. A significant reduction of NCV was observed during eight years in patients with mean HbA1>10% (n=12) compared to patients with mean HbA1<10% (n=33). ΔMNCV n.peroneus: -4.8±4.9 vs -2.2±5.2 m/sec ($p<0.01$). ΔMNCV n.tibialis post: -6.8±5.7 vs -3.9±5.5 m/sec ($p<0.05$). ΔSNCV n.surealis: -8.9±7.8 vs -4.6±5.3 m/sec ($p<0.05$). Multiple regression analysis showed that a change in HbA1 of 1% resulted a 1.3 m/sec change in NCV during eight years. In conclusion: Less progression of diabetic neuropathy was observed in the group of patients with improved blood glucose control during eight years.

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ACTH$^{4-9}$-analogue in a randomized, double-blind, placebo controlled trial in diabetic patients with neuropathy.
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The ACTH$^{4-9}$-analogue ORG 2766 is effective in the prevention and treatment of experimental diabetic neuropathy. The aim of this study was to see whether ORG 2766 is effective for neuropathy in type 1 diabetic patients. Sixty-two patients were selected; mean age 46.3 ± SD 11.4 years, duration of diabetes 24.8 ± 11.3 and HbA1 9.4 ± 2.4%. Inclusion criteria: vibratory threshold (VT) > p95 and/or warm thermal threshold (TTw) in the hand > p95. Patients were randomly divided into 2 groups: group 1 (n=32) received placebo and group 2 (n=30) was treated with ORG 2766 3mg sc. every 24 h. At 0, 4, 8 and 12 months the following neurophysiological parameters were measured: VT, TTw, cold thermal threshold, sensory nerve conduction velocity (NCV) in ulnar and sural nerve, motor NCV in ulnar and tibial nerve, Hoffmann reflex latency, cardiovascular autonomic score, darkness adapted pupil size and pupil constriction latency. Results: mean changes ± SD versus baseline at 4, 8 and 12 months for VT are: ORG 2766; -0.17 ± 0.40, -0.27 ± 0.45 and -0.34 ± 0.24 μm, and placebo; -0.14 ± 0.55, -0.17 ± 0.49 and -0.20 ± 0.46 (difference at 12 months p=0.05). There were no differences for the other variables. Conclusion: ORG 2766 improves vibration threshold in patients with diabetic neuropathy, necessitating further and larger studies to ascertain effects on other neurophysiologic parameters.

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UNMYELINATED FIBRE PATHOLOGY IN DIABETIC PATIENTS WITH MILD NEUROPATHY
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We studied the unmyelinated fibre pathology in sural nerve biopsies of 15 diabetic patients (mean age 47 years, 8 type 1, mean duration of diabetes 16.3 years) and compared them with 8 age-matched controls. The results were also related to neuropathy symptom score (NSS) and quantitative sensory testing including current perception threshold (CPT) assessed with a neurometer. There was no difference in the mean unmyelinated fibre density between diabetic patients and controls (50511 fibres/mm^{-2} ± 6084 SE vs 56059 ± 5054) but the fibre diameter in diabetic patients was smaller (0.521 μm ± 0.034 vs 0.876 ± 0.018, p< 0.001). An increase was found in the unassociated Schwann cell profile density (36205 ± 4039 vs 8905 ± 1105, p< 0.0001), the total Schwann cell profile density (61393 ± 4472 vs 44805 ± 3616, p< 0.02) and the % of unassociated Schwann cell profile density (58.11 ± 4.25 vs 20.83 ± 3.09 p< 0.0001). A positive correlation was found between NSS and the unassociated Schwann cell profile density (r = 0.55, p< 0.05), unmyelinated fibre density and CPT at 250 Hz (r = 0.58, p< 0.05) and fibre diameter and CPT at 2 kHz (r = 0.59, p< 0.05). No correlation existed between any unmyelinated fibre pathology and myelinated fibre density, warm thermal threshold, vibration perception threshold and electrophysiology. We conclude that unmyelinated fibre pathology with increased degeneration rates and axonal atrophy are present in patients with early neuropathy and that CPT may be of use in assessing this abnormality.

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AN ASSOCIATION BETWEEN TISSUE OXYGENATION AND NERVE FUNCTION WITH EVIDENCE OF REVERSIBILITY
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Diabetic peripheral neuropathy is probably caused by a combination of metabolic factors and neuroischaemia. We assessed the relationship between nerve function and transcutaneous tissue oxygenation (TcPO2) in diabetic neuropathy and non-diabetic peripheral vascular disease. Thirty-four diabetic patients, mean age 41 (range 30-70) years, mean duration 21 (9-34) years, were studied. On the dorsum of each foot, TcPO2 was measured at 44°C with a TCM3 monitor (Radiometer, Copenhagen), skin temperature with an infra-red thermometer (Mikron, USA) and peroneal motor conduction velocity (PMCV). PMCV correlated with TcPO2 r= 0.59 p<0.001 but not with skin temperature r= 0.16 p>0.2. In each patient comparing the higher TcPO2 leg (mean ± SD) (70.2±9.3 mmHg) with the lower TcPO2 leg (61.7±10.0 mmHg), PMCV was significantly higher 45.3±7.1 m/s vs 41.5±6.3 m/s p<0.01, but not temperature, 31.4±0.4 vs 31.1±0.5°C p=NS. We also studied ten non-diabetic patients before and after unilateral femoro-popliteal bypass, mean age 59.5 (49-77) years. TcPO2 (59.3±10.7 to 70.7±7.2 mmHg p<0.01) and PMCV (42.6±6.1 to 46.7±3.2 m/s p<0.01) increased significantly in the operated leg, but not in the unoperated leg (63.2±8.8 vs 63.0±4.6 mmHg and 45.1±7.8 vs 43.4±7.2 m/s p=NS). Skin temperature did not change in either leg, 30.3±0.4 vs 30.4±1.3°C and 30.8±1.3 vs 30.2±1.2°C respectively (p=NS). These studies provide further evidence for the role of ischaemia in the aetiology of neuropathy and suggest that increasing tissue oxygenation can improve nerve function.

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USE OF MORPHOMETRY IN THE ASSESSMENT OF BIOCHEMISTRY IN SURAL NERVE BIOPSIES FROM DIABETIC NEUROPATHIC PATIENTS
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In diabetic animal models correlations of nerve biochemistry and function are easily made since they precede morphologic changes. In human neuropathic patients nerve fiber damage complicates these comparisons. We have used nerve morphometry to interpret the relationship between nerve biochemistry and function. Following nerve conduction studies sural nerve biopsies were obtained from 160 diabetic neuropathic patients. Nerve levels of glucose, sorbitol, and fructose were quantitated by GC/MS. Nerve morphometry was assessed by light and electron microscopy. As expected, the nerve levels of sorbitol and glucose were positively correlated (p<0.001), but nerve sorbitol content was not positively correlated with the degree of nerve fiber loss. Instead the sorbitol/glucose ratio correlated positively with fiber density (p<0.01) and fiber occupancy (p<0.01) (a measure of intracellular volume) presumably reflecting the intracellular localization of sorbitol versus the total tissue distribution of glucose. The sorbitol/glucose ratio also correlated with sural nerve amplitude, (p<0.001) an electrophysiological measure of nerve fiber number. In summary, nerve sorbitol correlates with measures of nerve fiber number and volume confirming its intracellular localization. Therefore, human nerve biochemistry can be interpreted only on the basis of a simultaneous assessment of nerve structure in diabetic neuropathy.

OP 9
Metabolism In Vitro

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STIMULATION BY GLUCOSE OF LIPOGENIC ENZYME GENE EXPRESSION IN RAT ADIPOSE TISSUE.

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The expression of fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) is low in the adipose tissue of suckling rats and increases markedly at weaning to a high carbohydrate diet. We have studied in vitro the factors regulating this phenomenon. Inguinal adipose tissue pieces from 19-day old suckling rats were incubated for 6-hr in Minimal Essential Medium. Total RNA was extracted, blotted and analysed using cDNA probes specific for FAS and ACC. Insulin (10^{-7}M) added in the presence of lactate and pyruvate did not stimulate the expression of FAS and ACC. Glucose (20mM) alone resulted in a 5-7-fold increase of FAS and ACC mRNA. Insulin potentiated the glucose effect. 3-O-Methyl-Glucose, a non-metabolizable glucose analog had no effect. However, 2-deoxy-glucose (1mM) of which metabolism stops after its phosphorylation into glucose-6-phosphate, leading to the accumulation of this latter compound, stimulated the expression of FAS and ACC to the same extent than 20mM glucose. Assay of glucose-6-phosphate in adipose tissue pieces cultured in various conditions showed a striking parallelism between its concentration and the induction of FAS and ACC mRNA. We conclude that glucose-6-phosphate could be the messenger metabolite involved in the stimulation by glucose of lipogenic enzyme gene expression.

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Insulin and non esterified fatty acid regulation of triglyceride and apo-B secretion - a proposed mechanism.

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Increased plasma triglyceride and apo-B concentrations may contribute to the premature development of ischaemic heart disease in diabetes. Insulin and non esterified fatty acids (NEFA) are potent regulators of triacylglycerol and apo-B secretion and yet their mechanism of action is poorly understood. The present study was undertaken to examine regulation of triglyceride and apo-B secretion by insulin and NEFAs over periods of time up to 72h in the human hepatoblastoma cell line Hep G2. Insulin decreased intracellular apo-B by 20% ($p<0.001$) and secreted apo-B by 30% ($p<0.001$), whereas insulin increased intracellular triglyceride by 16% ($p<0.01$) and decreased triglyceride secretion by 40% ($p<0.001$). Oleate increased intracellular apo-B by 21% ($p<0.01$), secreted apo-B by 50% ($p<0.001$), intracellular triglyceride by 100% ($p<0.001$) and secreted triglyceride by 30% ($p<0.001$). Regression analysis showed there was no correlation between intracellular triglyceride content and triglyceride and apo-B secretion but there was a significant correlation between intracellular apo-B content and triglyceride and apo-B secretion ($r=0.89$, $p<0.001$ for both analyses). These results show that insulin inhibited triglyceride and apo-B secretion throughout the 72h. We conclude that the availability of intracellular apo-B to form lipoproteins regulates lipoprotein secretion and both insulin and NEFAs regulate triglyceride and apo-B secretion by this mechanism.

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EFFECT OF DIET ON INSULIN AND CONTRACTION MEDIATED GLUCOSE TRANSPORT AND GLUCOSE TRANSPORTERS IN RAT MUSCLE.

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A diet rich in fat is known to diminish insulin mediated glucose uptake in muscle. This study elucidated the mechanism and explored whether also contraction mediated glucose uptake is affected by diet. Rats (3 wks old) were fed a diet rich in either fat (F, 73% of energy) or carbohydrate (CHO, 66%) for 5 wks. Hindquarters were perfused. Contractions were elicited by stimulation of the sciatic nerve. Glucose transport was measured by uptake of 3-O-(^{14}C)-methyl-D-glucose (40mM) in fast-twitch white superficial gastrocnemius (FW), fast-twitch red deep gastrocnemius (FR) and slow-twitch red soleus (SR) muscles. Amount of GLUT 1 and GLUT 4 glucose transporting proteins was determined by Western blot. Glucose uptake was lower ($p < 0.05$) in hindlegs from F than from CHO rats at 150 uU/ml insulin (0.7 $\pm$ 0.07 (SE) vs 0.9 $\pm$ 0.05 mg/min/leg) as well as during contractions (0.8 $\pm$ 0.08 vs 1.0 $\pm$ 0.1). Glucose transport elicited by maximum insulin (20.000 uU/ml)(SR: 1.7 $\pm$ 0.2 vs 2.6 $\pm$ 0.2 umol/g/5 min, $p < 0.05$) and contractions (SR: 1.8 $\pm$ 0.2 vs 2.6 $\pm$ 0.3, $p < 0.05$) were in red muscle decreased in parallel in F compared to CHO rats. GLUT 4 was decreased by 29% (FW, $p < 0.05$), 21% (FR, $p < 0.05$) and 13% (SR, $p < 0.1$), whereas GLUT 1 was identical in F compared to CHO rats. Conclusions: Both insulin and contraction mediated glucose uptake in muscle are reduced by fat feeding. This reflects a decrease in glucose transport due to decreased GLUT 4 content.

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INSULIN-LIKE GROWTH FACTOR II STIMULATES GLUCOSE TRANSPORT IN HUMAN SKELETAL MUSCLE.

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Insulin-like growth factor II (IGF-II) has been reported to be elevated in type 1 diabetes mellitus and correlated with Hb_{A1C} levels. Furthermore, IGF-II has effects on glucose metabolism which are similar to insulin. In the circulation, IGF-II is associated with specific insulin-like growth factor binding proteins (IGFBP's) which may alter the effects of IGF-II at the target tissue. Although IGF production can occur within the musculature, the physiological role of IGF-II and the IGFBP's in human skeletal muscle is undefined. We investigated the effect of IGF-II and IGFBP-1 on 3-0-methylglucose transport in incubated human skeletal muscle strips. Increasing concentrations of IGF-II (0, 10, 30, and 100 ng/ml) stimulated glucose transport in a dose-dependent manner (0.79 ± 0.07, 0.86 ± 0.17, 1.11 ± 0.15, $p<0.05$, and 1.47 ± 0.19, $p<0.01$, μmol x ml^{-1} x hr^{-1}, respectively). Glucose transport was maximally stimulated in the presence of 100 ng/ml (13.4 nM) of IGF-II ($P<0.01$), which corresponded to the effect of 100 μU/ml (0.6 nM) insulin (1.51 ± 0.18 μmol x ml^{-1} x hr^{-1}, $P<0.01$). Exposure of muscle strips to IGFBP-1 (500 ng/ml) inhibited the IGF-II (100 ng/ml) stimulated glucose transport by 40% ($P<0.05$). These data demonstrate that IGF-II stimulates the glucose transport process in human skeletal muscle. Furthermore, IGFBP-1 appears to play a counter-regulatory role in the IGF-II stimulated glucose transport.

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SKELETAL MUSCLE MAY BE A SITE FOR DEGRADATION OF CGRP AND POSSIBLY AMYLIN

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Amylin and calcitonin gene-related peptide (CGRP) have potent inhibitory effects on insulin-mediated glucose metabolism in skeletal muscle. CGRP is located and released from nerves to bind to receptors on muscle fibres. The mechanisms that operate to degrade, and hence inactivate, these peptides are unknown. We investigated the degradation of ^{125}I-hCGRP, measured by increased solubility of CGRP peptide fragments in trichloroacetic acid, in incubated rat skeletal muscle preparations. The degradation of 1% of ^{125}I-hCGRP.mg wet wt^{-1}.2h^{-1} of muscle is defined as one unit of activity. The rate of degradation of ^{125}I-hCGRP was significantly lower in extensor digitorum longus muscles (type II fibres), 0.30 ± 0.02 Units versus soleus (type I fibres) 0.39 ±0.03 Units (n=7; P <0.05). Neither insulin nor substance P affected the rate of ^{125}I-hCGRP degradation. The rate of degradation was significantly diminished by some protease inhibitors, including phenathroline and phenylmethanesulphonyl fluoride. Phenanthroline (1 mM) decreased the degradation rate in soleus preparations from 0.47 ± 0.02 Units to 0.14 ± 0.02 Units. The rate of degradation of ^{125}I-hCGRP was also decreased in soleus muscle isolated from insulin-resistant dexamethasone-treated rats (0.36 ± 0.02 Units) versus controls (0.44 ± 0.05 Units; n=8). These results suggest that a mechanism operates in skeletal muscle to degrade CGRP and possibly amylin. The effectiveness of this degradative process might play a role in determining the magnitude and/or duration of the response of skeletal muscle to both peptides.

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VANADATE-INDUCED GLUCOSE PRODUCTION IS BLOCKED BY INSULIN AND INDOMETHACIN

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Vanadium compounds have been shown to exert insulin-like effects on isolated rat skeletal muscle and adipocytes. Similarly, vanadate treatment decreased blood glucose levels of diabetic rats and mice. On the other hand, both stimulation and inhibition of hepatic glycogen synthesis by vanadate have been reported. To test the net effect of vanadate on hepatic glucose metabolism we perfused isolated livers obtained from fed Sprague-Dawley rats in a flow-through system with oxygenated Krebs-Ringer buffer (pH 7.4) and dialyzed human erythrocytes (hematocrit 2o%) at a flow rate of 7.0 ml.min^{-1}. Continuous infusion of 50-500 μmol.ml^{-1} sodium-orthovanadate resulted in a dose-dependent long-lasting elevation of net hepatic glucose release (maximum: 6.3$\pm$0.9 μmol.min^{-1}.100gbw^{-1}). Vanadate did not increase the cAMP levels (0.11$\pm$0.02 nmol/l at 10 min) in the effluent perfusate, while the subsequent infusion of glucagon stimulated both glucose (maximum: 12.6$\pm$1.0 μmol.min^{-1}.100gbw^{-1}) and cAMP output (0.99$\pm$0.39 nmol/l at 10 min). The vanadate effect was completely blocked by indomethacin (10^{-4} mol/l), but not by omission of Ca^{2+} from the perfusion medium. Insulin inhibited vanadate-induced glucose production dose-dependently ($p<0.01$).In conclusion, orthovanadate stimulates glycogenolysis in the perfused rat liver, which might be mediated by prostaglandins.

OP 10
Endothelium

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IDENTIFICATION AND REGULATION OF THE ENDOTHELIAL GLUCOSE TRANSPORTER BY GLUCOSE.

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The regulation and mechanisms of glucose uptake by the endothelium have not yet been identified. Therefore, we studied the uptake of glucose by bovine aortic endothelial cells, identified the respective glucose transporter, and proved the influence of insulin and glucose on the expression of the glucose transporter. The uptake of glucose followed Michaelis-Menten kinetics (Vmax=12 nmol/min/mg protein, K=2 mM). Insulin did not influence the uptake of glucose, although high affinity insulin receptors (Kd=0.4 nM) are present. By immunoblotting glut 1, but no Glut 4 could be identified on endothelial cells. Preincubation of BAEC with increasing concentrations of glucose (10-44 mM) reduced the uptake of glucose for 40-50% within 4 hours. In parallel the amount of glut 1 protein was diminshed. The changes in glut 1 were specifically induced by glucose and reversible. Preliminary data indicate furthermore that the reduction in glut 1 protein is associated with a diminuition of the appropriate m-RNA (Northern blot). Similar results were obtained with microvascular endothelial cells from rat heart. In summary, macro- and microvascular endothelial cells containe Glut 1, but no Glut 4. Glucose distinctly influences the translation and transcription of glut 1. Both kinds of endothelial cells, in which the acute uptake of glucose is not influenced by insulin, dispose of effective mechanisms to prevent an overloading of the cells with glucose by reducing the amount of Glut 1 and thus to reduce the damaging effects of hyperglycemia on endothelium.

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INVESTIGATION OF INSULIN INFLUENCE ON ENDOTHELIAL PERMEABILITY AND OF INSULIN EXCHANGE

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Bovine endothelial cells from aorta (macrovascular, BAEC) and retina (microvascular, BREC) were cultivated on polycarbonate filters to investigate the barrier function of endothelium and the influence of insulin on endothelial cell permeability. Endothelial cells bound insulin specifically with a dissociation constant of $K_d=4.1\pm2.1\cdot10^{-10}$M for 1150$\pm$280 high affinity receptors per cell. The uptake of insulin was <0.5% per hour. In spite of high affinity receptors, insulin did not influence BAEC and BREC permeability for several diffusion markers such as ^{14}C-sucrose and fluorescent dextranes (4-70kD). Insulin (radius 1.3nm) itself passed the endothelium mainly through the intercellular clefts (two different pore sizes: 0.5nm and 8.6nm). This paracellular exchange was not saturable and increased linearly with time, following the equation y=0.11t+0.09 (r=0.999, y=% exchanged insulin of total insulin, t=time in minutes). There was no difference in insulin exchange between micro- and macrovascular cells (BAEC: 2.24%, BREC: 2.15% exchange of total insulin in 20min). Monensin, an inhibitor of pinocytosis, did not influence the exchange of insulin (up to 50μM Monensin). Taken together, insulin has no influence on the permeability of bovine aortic and retinal endothelial cells. Furthermore, the exchange of insulin across BAEC and BREC is not receptor mediated and follows diffusion kinetics. These data do not support the hypothesis of the endothelium as an active storage pool for insulin.

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INSULIN INCREASES AVP-INDUCED ENDOTHELIN-1 RELEASE FROM HUMAN VASCULAR SMOOTH MUSCLE CELLS.

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Endothelin-1 (ET-1) is a vasoactive peptide produced also by vascular smooth muscle cells (VSMC) under suitable stimuli, including arginine vasopressin (AVP). Endothelin-1 is involved in the control of vascular tone through vasoconstrictor effects and is a mitogen for VSMC. Insulin plays a role in the pathogenesis of hypertension and atherosclerosis. Therefore, we investigated whether insulin influences basal and AVP-stimulated ET-1 release from VSMC obtained from human microvessels and cultured in MEM supplemented with 10% FCS, antibiotics, glutamine and buffers. VSMC were investigated at the passages 5-8 following quiescence by serum starvation. Insulin (80 and 320 μU/ml), AVP (10 nM) or their association were added to cell cultures; medium was collected after 6 hours and ET-1 was determined by RIA. ET-1 (pg/200 μl medium, m±SEM) in unstimulated cultures was 15.2±0.8; insulin at 320 μU/ml increased ET-1 levels (20.6±0.8, $p<0.01$), while at 80 μU/ml it failed to stimulate ET-1 (15.3±1.4). ET-1 levels with AVP were 20.4±1.6 ($p<0.025$), with 80 μU/ml insulin+AVP 26.2±2.4 ($p< 0.002$), with 320 μU/ml insulin+AVP 30.6±1.2 ($p<0.0001$). In conclusion, insulin at physiological concentrations enhances AVP-induced ET-1 release from human VSMC, whereas at higher concentrations it increases by itself ET-1 release. Therefore, it can be hypothesized that the insulin effects on vascular tone and atherogenesis are partially mediated by its influence on ET-1 release.

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PLASMA ENDOTHELIN LEVELS AND VASCULAR EFFECTS OF L-ARGININE IN TYPE 1 (INSULIN-DEPENDENT) DIABETES.

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Uncomplicated Type 1 diabetes is associated with vasodilation, the mechanism of which is unknown. To investigate the role of endothelial mediators, we measured plasma endothelin levels and studied the effects of iv L-arginine (precursor of nitric oxide (NO); 0.5 g/kg in 30 min) in 8 male Type 1 diabetic patients and matched controls. After L-arginine (compared to an equivolumic 0.9% NaCl infusion), blood pressure, heart rate and plasma atrial natriuretic peptide and endothelin levels did not change in either group. Baseline endothelin level was lower in the patients (4.3 ± 1.1 vs 5.7 ± 1.1 pg/ml; $P<0.05$ vs controls). Plasma cGMP (NO's second messenger) increased in the controls (from 5.0 ± 1.5 to 7.4 ± 2.8 nM; $P=0.05$ vs NaCl) but the respons in patients was blunted (from 4.2 ± 1.1 to 5.7 ± 1.9 nM; $P=0.19$ vs NaCl), consistent with increased basal NO release. Lower leg extracellular volume (electrical conductivity method) increased in the controls (1.6 ± 1.1 vs 0.1 ± 1.4%; $P=0.03$ vs NaCl), but not in the patients. These findings suggest a role for the endothelium in explaining the vasodilation observed in uncomplicated Type 1 diabetes.

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DOES MODERATE EXERCISE STIMULATE THE RELEASE OF ENDOTHELIN?

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In order to assess the possible role of moderate exercise in stimulating the release of endothelin by endothelial cells, 9 patients (4 diabetics and 5 controls) were asked to pedal for 30 min on a ergometer bicycle at a workload of 40% of the previously estimated maximum value (VO2 max). Platelet aggregation responses to ADP (1.25; 2.5 and 5 μM), and the following plasma parameters were measured before (T0) and immediately after (T30) exercise: norepinephrine (NE, nmol/l), 11 dehydro-TXB2 (pg/ml), GH (ng/ml) and endothelin (ET-1, ET-2 and big ET-1, fmol/ml). Plasma endothelin concentrations were not significantly different at T0 in both controls (median=8) and diabetics (median=13). In the 9 patients taken as a whole, the following parameters (mean ±SD) were increased at T30: aggregation response to ADP 2.5 μM ($p<0.05$); NE (1.5±0.5 vs 2.8±0.9, $p<0.01$); GH (1±3 vs 14±1, $p<0.02$); 11 dehydro-TXB2 (87±19 vs 123 ±72, NS). ET levels remained unchanged (12±8 vs 14±6) but changes in plasma levels of ET and NE were strongly correlated ($r=0.84$, $p<0.02$). In conclusion, these preliminary results demonstrate that changes in plasma endothelin levels during moderate exercise depend strongly on changes in NE levels, involving probably the α adrenergic receptors of the endothelial cells.

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VASCULAR RESPONSES IN THE FOREARM OF MICROALBUMINURIC TYPE 1 (INSULIN-DEPENDENT) DIABETIC PATIENTS: EVIDENCE OF ALTERED NITRIC OXIDE BIOSYNTHESIS

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Objective: To determine whether microalbuminuric diabetics (MICRO-AER) have impaired endothelium-dependent vascular reactivity. **Patients**: We studied fourteen (8M,6F) normal controls (CONTROLS), fourteen (8M,6F) normoalbuminuric diabetics (NORM-AER) (median, range albumin excretion rate 8, 4-18 μg/min), fourteen (11M,3F) MICRO-AER (54, 21-199 μg/min) age (mean±SEM: 36±2, 40±3, 39±2 years), duration (23±2, 23±2 years), with similar cholesterol and fructosamine. Mean BP was 90±3, 95±2, 100±3 mm Hg. **Methods**: All subjects were euglycaemic when studied. We measured forearm bloodflow using plethysmography during brachial artery infusions of saline and an inhibitor of endothelial nitric oxide biosynthesis N^G-monomethyl-L-arginine (LNMMA). Endothelium-dependent (carbachol) and independent (sodium nitroprusside, SNP) vasodilation was also measured before and during LNMMA infusion. Data were analyzed by analysis of variance. **Results**: Basal bloodflow was similar in all groups as was dose response of SNP and carbachol. LNMMA caused vasoconstriction ($p<0.001$) but less so in MICRO-AER (25±5%) than in NORM-AER (46±11%) or CONTROLS (53±7%). LNMMA did not alter the response to SNP but significantly inhibited ($p<0.05$) the peak response to carbachol in CONTROLS (% increase over control with LNMMA 463±59% vs 356±48% without LNMMA) and NORM-AER (428±52% vs 295±22%) but not MICRO-AER (457±76% vs 444±89%). **Conclusion**: Euglycaemic MICRO-AER have normal basal forearm bloodflow despite evidence of altered basal and stimulated endothelial nitric oxide synthesis.

OP 11

Protein Metabolism In Vivo

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In vivo effect of insulin on protein synthesis in skeletal muscle and liver.

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Insulin fails to stimulate whole body protein synthesis in adult animals, including lactating goats. Whole body protein synthesis measurements may obscure a potential anabolic effect of insulin in some tissues. The aim of the present experiment was to determine whether or not insulin alter protein synthetic rate in vivo either in skeletal muscles or in liver. Two groups of animals were used. The experimental group was infused with insulin for 2.5 hours under a glucose clamp, resulting in a 60 fold increase in plasma insulin. In addition all blood amino acids were maintained at their basal levels by a continuous infusion of a spécific amino acid mixture. The control group received only saline. Protein synthesis was assessed by a flooding dose of L(2.3.4-^{3}H) valine. Fractional synthesis rate (FSR) of six skeletal muscles (tensor fasciae latae, longissimus dorsi, masseter, diaphragmus, anconeus and semitendinosus) and liver was calculated assuming that either tissue homogenate or plasma free valine specific radioactivity was representative of valyl-tRNA. Under these conditions, insulin was unable to affect FSR both in liver and various skeletal muscles. These results explain the lack of effect of insulin on whole body protein synthesis and suggest that the anabolic effect of insulin in vivo may be attributed to an inhibition in proteolysis.

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INSULIN SELECTIVELY AFFECTS HEPATIC PROTEIN SYNTHESIS IN HUMANS.

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Short-term insulin deficiency does not affect estimates of whole body protein synthesis (WBPS) but decreases albumin and increases fibrinogen fractional synthetic rates (FSR) in type 1 diabetic subjects. To establish the effects of physiologic increments in plasma insulin concentration on WBPS and on hepatic protein synthesis (albumin, fibrinogen and APOB100), 6 normal volunteers (27±2 yr, MEAN±SEM) were studied in a random order on two different occasions at three day intervals, during either a 240 min saline infusion (control study) or an hyperinsulinemic (0.4 mU·kg^{-1}·min^{-1}) euglycemic (4.7±0.1 mM) glucose clamp. WBPS and FSR of hepatic proteins were calculated using plasma [^{14}C]KIC specific activity (SA) as precursor pool (240 min infusion of [1-^{14}C]leucine) and the increase over the time in [^{14}C]leucine SA derived from purified and hydrolyzed proteins. Insulin (174±14 pM) reduced ($p<0.01$) WBPS (1.70±0.1 vs control 2.06±0.09 μmol·kg^{-1}·min^{-1}), increased ($p<0.02$) albumin FSR (7.2±0.4 vs control 6.2±0.6%·day^{-1}), decreased ($p<0.05$) fibrinogen FSR (18±1 vs control 23±2%·day^{-1}), whereas did not affect APOB100 FSR (51±4 vs control 49±4%·day^{-1}). In conclusion: plasma insulin increments in the post-prandial range increase albumin synthesis and decrease or do not affect the synthesis of other hepatic proteins. The stimulatory effect of insulin on albumin synthesis may serve to capture part of ingested essential amino acids and prevent their oxidative losses.

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DO NON-ESTERIFIED FATTY ACIDS REGULATE SKELETAL MUSCLE PROTEIN TURNOVER IN MAN?

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Elevated plasma non-esterified fatty acid (NEFA) levels contribute to the glucose insulin insensitivity in obesity and Type 2 (non-insulin-dependent) diabetes. We examined whether they also influence skeletal muscle protein metabolism by measuring forearm $^2H_5$-phenylalanine exchange. $^2H_5$-phenylalanine was infused (0.5 mg.kg^{-1}.hr^{-1}) for 300 min in 7 healthy subjects on 2 occasions. 10% Intralipid (30 ml/hr) or 0.154 mol/l NaCl were infused in random order from 120 min. Measurements were taken during baseline (90-120 min) and infusion (270-300 min) periods; δ represents change from baseline. Intralipid infusion increased plasma NEFA levels (1.31 ± 0.13 [mean ± SEM] *vs* 0.49 ± 0.05 mmol/l; $p<0.05$) and forearm NEFA uptake (+45 ± 76 *vs* -51 ± 44 nmol.100mlforearm[FA]$^{-1}$.min^{-1}; $p<0.05$). Serum insulin and blood ketone body levels were comparable throughout the study. Plasma phenylalanine levels fell with Intralipid (65 ± 6 *vs* 56 ± 5 μmol/l; δ comparison, $p<0.02$), but there was no effect on *net* forearm phenylalanine exchange (-9 ± 1 and -9 ± 2 nmol.100mlFA^{-1}.min^{-1}). However, forearm phenylalanine disposal [Rd] (12 ± 2 *vs* 17 ± 2 nmol.100mlFA^{-1}.min^{-1}; δ comparison, $p<0.02$) and release [Ra] (21 ± 3 *vs* 26 ± 3 nmol.100mlFA^{-1}.min^{-1}; δ comparison, $p<0.05$) decreased to a comparable degree with Intralipid. Thus, elevated plasma NEFA levels decrease skeletal muscle phenylalanine and protein turnover, but not *net* phenylalanine exchange. Therefore, the fall in plasma phenylalanine levels appears to be secondary to an effect of NEFA at some other tissue.

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ACUTE GROWTH HORMONE (GH) EFFECT ON IN VIVO PROTEIN DYNAMICS AND LIPOLYSIS IN HUMANS.

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We investigated the mechanisms of GH induced protein anabolic effects in 12 normal males treated with GH (n=7) or Saline (n=5). We infused ^{13}C-leucine (LEU) and ^{2}H$_5$-phenylalanine (PHE) for 420 min during a somatostatin clamp with replacement of basal serum levels of GH (2-4 ng/ml), insulin (60-80 pM/L), and glucagon (130-150 pg/ml). Femoral arterial and venous catheters were inserted, and regional blood flow was determined by iodogreen dye dilution. Between 210 and 420 min, GH was infused (in GH subjects) at a rate sufficient to increase serum GH concentrations into the high-physiologic range (15-20 ng/ml) for 3 hours, while the basal GH infusion rate was continued in C subjects. IGF-I levels remained constant (400-600 ng/ml) in both groups, and C-peptide levels were suppressed (less than 20 pM/L) and not different between groups. The period of high-physiologic GH exposure resulted in an increase in serum glycerol ($p=0.038$) indicative of lipolysis, but without alterations in serum glucose or A-V glucose differences. LEU oxidation was reduced in GH compared to C subjects ($p=0.02$), but no significant differences in LEU or PHE flux were observed. The nonoxidative portion of LEU flux (indicative of whole body protein synthesisi) was higher in GH ccompared to C subjects ($p=0.05$). No differences in LEU or PHE appearance rates or balances across-the-leg were noted. Conclusion: a 3 hour infusion of GH in the high-physiologic range increasedwhole body protein synthesis and decreased leucine oxidation. We could demonstrate no evidence for a simultaneous GH-induced increase in protein synthesis across-the-leg, a region comprised largely of skeletal muscle. These results indicate that the early effects of GH on protein synthesis occur predominantly in non-muscle tissue.

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PROTEIN ECONOMY IN INSULIN-DEPENDENT DIABETES DURING PRE- AND POSTHEPATIC INSULIN THERAPY
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To assess the extent of whole body protein catabolism in well-compensated, insulin treated diabetes, urea production rate was estimated in body weight balanced, chronic insulin-dependent diabetic dogs, which were normally meat-nourished. In resting, overnight-fasted state, they were insulin infused over 16 h, in paired experiments either i.v. or portally (p.o.). Over the final 6 h, a primed continuous infusion of $15N_2$-urea was applied. Insulin doses of 0.70±0.13 (i.v.) and 1.03±0.13 mU/kg.min (p.o., n=4 ea., x±SEM) were infused to establish peripheral-venous plasma IRI of 0.198±0.035 and 0.188±0.033 nmol/l, repectively (controls 0.040±0.01). Glycaemia was 5.2±0.1, 5.8±0.3 and 5.1±0.2 mmol/l, respectively. Average glucagon concentrations (antiserum No. 185) were 16±5, 9±2 and 12±4 pmol/l and total amino acid concentration amounted to 2.00±0.17, 2.02±0.27 and 2.22±0.12 mmol/l (NS). Urea turnover was unexpectedly high with only minor differences in relation to the route of insulin administration: i.v. 16.8±1.8, p.o. 14.8±3.1 (NS), controls 7.5±1.7 µmol/kg.min (S). If, however, the animals were fed a protein-reduced, carbohydrate-enriched diet, urea production decreased by 20 % (contr.) and 50 % (diabetics) with i.v. - p.o. differences preserved. Conclusion: carbohydrate-enriched nutrition may reduce urea production as a basis for the evaluation of therapeutic effects on protein metabolism in diabetes.

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LEUCINE METABOLISM AFTER LIVER TRANSPLANTATION IN HUMANS.
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End-stage liver disease is characterized by reduced leucine (leu) and branched chain amino acids (bcaa) concentration, increased protein catabolism, and insulin resistance. In order to assess the effect of Orthotopic Liver Transplantation (OLT) on leucine/protein metabolism, 6 patients with stable liver function after OLT [age=42±5y; IBW=98±1%; transplant age=5±1 mo.; prednisone=10mg·day^{-1};cyclosporin=5mg·kg^{-1}·day^{-1}; albumin= 3.3 (before) → 4.4 g/l (after OLT); bilirubin=2.2→0.9 mg/dl;AST=74→48 U/l;PT=1.5→1.08], and 6 control subjects (con) were studied by means of the [1-^{14}C]leucine infusion and indirect calorimetry. Basal plasma IRI (13±2 vs 7±2 µU/ml), C-peptide (3.1±0.9 vs 1.7±0.5 ng/ml) and glucagon (110±28 vs 55±10 ng/ml) were higher in OLT vs con respectively (p<0.01). Basal plasma glucose (4.3±0.2 vs 5.0±0.2 mmol/l), FFA (0.82±0.21 vs 0.64±0.19 mmol/l), lactate (0.54±0.12 vs 0.58±0.41 mmol/l), were similar in OLT and con. Basal plasma leu (69±5 vs 127±6), bcaa (279±21 vs 387±34 µmol/l) and α-ketoisocaproate (23±2 vs 30±2 µmol/l), were reduced in OLT with respect to con (p<0.01). No difference was observed in basal phenylalanine, lysine, glutamine, alanine and glycine. Basal endogenous leucine flux (ELF:33±4 vs 39±3) and non-oxidative leucine disposal (NOLD:30±2 vs 33±1 µmol·m^{-2}·min^{-1}) were similar in OLT and con (p=NS), while leucine oxidation (LO: 2.7 vs 5.5) was reduced in OLT vs con (p<0.01).After 180 min of basal equilibration period a 1 mU·kg^{-1}·min^{-1} euglycemic insulin (≈ 70 µU/ml) clamp was performed for additional 3 hrs. OLT demonstrated a defective decrease of leu (69→44 vs 127→52), ELF (33→28 vs 39→26) and LO (2.7→2.5 vs 5.5→4.6) respect to con (p<0.05). Tissue glucose disposal was reduced in OLT (4.6±1.2) vs con (7.8±1.0 mg·kg^{-1}·min^{-1})(p<0.001). In summary OLT show a)in the basal state:1. reduction of leu, bcaa and LO 2. two-fold increase of insulin 3. normal ELF (proteolysis) and NOLD (protein synthesis); b) during insulin infusion:1.defective inhibition of ELF and LO; 2. impaired stimulation of peripheral glucose uptake. In conclusion, the alteration of leucine/protein metabolism and the insulin resistance characteristic of liver cirrhosis do not appear to be normalized within five months after liver transplantation.

OP 12
Islets I

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EXPRESSION OF CERTAIN ANTIONCOGENES AND CELL CYCLE RELATED GENES IN PANCREATIC ISLETS
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To obtain a possible explanation for the low proliferative capacity of the adult pancreatic β-cell, expression of two antiproliferative genes (Rb and p53) as well as certain genes important for the cell cycle and mitosis (cyclin B 1, cyclin D, $p34^{cdc2}$ and $p33^{cdk2}$ kinases) was assessed by the polymerase chain reaction (PCR). cDNA was prepared from liver, spleen and islets and used as template for the amplification reactions. The optical density of the autoradiographs after Southern blotting was normalised by parallel PCR-amplification of cDNA coding for the enzyme glyceraldehyde-3-phosphate dehydrogenase. Islet expression of the p53 and Rb genes was lower or similar to that of spleen, making expression of these genes unlikely as an explanation for the low replicatory potential of the β-cell. Levels of cyclin D mRNA and mRNA encoding $p33^{cdk2}$ kinase did not differ from those of spleen. However, a decreased islet expression of the genes coding for cyclin B 1 and $p34^{cdc2}$ kinase compared with those of spleen was observed (0.7±0.2 vs 24.1±7.1, p<0.001 and 1.3±1.1 vs 25.7±2.3, p<0.01). These results suggest that low expression of cyclin B 1 and $p34^{cdc2}$ kinase may play a role in maintaining adult β-cell in a state of a low proliferative capacity.

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CLONING OF RHOMBOTIN, AN ONCOGENE OF THE LIM DOMAIN FAMILY NORMALLY EXPRESSED IN NEURONAL TISSUE, FROM A RAT ENDOCRINE CELL LINE.

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Rhombotin belongs to the family of LIM-domain proteins which contains a common metal binding, zinc finger-like motif. Normal rhombotin expression is detected predominantly in the developing nervous system. Rhombotin is considered a protooncogene since it is activated by translocations associated with lymphoid tumor formation. By PCR we have cloned rhombotin from rat endocrine cell line Rin-5AH cDNA. Direct sequencing of PCR products revealed a sequence homology to the human and mouse rhombotin genes of >93% and >96%, respectively. On pancreatic sections immunohistochemistry using antibodies raised against rhombotin-specific peptides reveals the presence of rhombotin-related immunoreactivity in glucagon-producing alpha cells. No staining is detected in other pancreatic cell types. Interestingly, a similar staining pattern is seen with an antibody raised against another LIM-domain protein, Cysteine Rich Intestinal Protein (CRIP) which consists of a single LIM domain. The high degree of evolutionary conservation of the rhombotin gene points at an important function for this oncogene. Rhombotin expression in alpha-cells and endocrine tumours could, in turn, suggest that alpha-cells possess an islet regenerating potential.

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MECHANISM OF ACTION OF GROWTH HORMONE IN THE PANCREATIC BETA CELL: MUTATIONAL ANALYSIS OF THE GROWTH HORMONE RECEPTOR

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Growth Hormone (GH) stimulates insulin expression and proliferation of rat pancreatic β-cell through specific receptors(GH-R). The aim of our study is to investigate the mechanism of action of GH in the insulin producing cell line RIN-5AH and the role of the internalisation of the complex GH/GH-R in its signalling mechanism. We previously showed that domains of the GH-R between amino acid residues 294 and 454 are required for efficient internalisation . By comparing the sequences for GH-R and PRL-R from several species a highly conserved region is found between amino acid residues 294 and 454 containing a proline-rich cluster that has been proposed to play a role in association with a signal transducer protein and/or it could be involved in the internalisation mechanism. In addition tyrosine phosphorylation has been suggested to play a role in ligand dependent endocytosis. To test these hypotheses we constructed one GH-R mutant lacking the consensus sequence GH-R$\Delta_{297-311}$, one carrying a stop codon after this sequence, GH-R_{1-319}, and another one in which the tyrosines in position 333 and 338 were mutated to phenylalanines GH-R_{FF}. We expressed them transiently in COS 7 cells, and we tested for internalisation. Analysis of GH-uptake mediated by single cohort of receptors was performed at 37° C after preincubation with ^{125}I-hGH at 4° C for 2 hours. As determined by acid wash stripping of surface bound ^{125}I-hGH the GH-R $\Delta_{297-311}$ and the GH-R_{FF} internalised 70 % of the specific cell associate radioactivity like the wild type GH-R_{1-620} whereas the clone expressing GH-R_{1-319} showed a markedly decreased internalisation. In conclusion, these results indicate that the domain responsible for internalisation is located between residue 319 and 454 and that phosphorylation of tyrosines in positions 333 and 338 is not involved.

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HORMONAL REGULATION OF GROWTH HORMONE AND PROLACTIN RECEPTOR EXPRESSION IN INSULIN PRODUCING CELLS.

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Growth hormone (GH), prolactin (PRL) and placental lactogen (PL) are known to stimulate insulin production and proliferation of pancreatic beta cells. We have recently shown that the receptors (R) for both GH and PRL are expressed in the embryonic mouse pancreas as early as day 9 and that the expression is elevated in the pancreas of pregnant rats. In isolated islets the GHR mRNA was increased by dexametasone (DEX) and progesterone (P), whereas the PRLR mRNA was increased by PRL, GH and estradiol (E). In order to study the time and dose dependence of these effects we measured the mRNA levels in RIN 5AH cells by a RNase protection assay. The effect of DEX and P on GHR mRNA was seen after 1 h and maximal after 16 h. In contrast the effect of GH and PRL on PRLR mRNA required exposure for 24 to 48 h. Significant effects were seen at 0.4 ng/ml DEX or 100 ng/ml P and 10 ng/ml GH or PRL. These results indicate that the expression of GHR and PRLR is stimulated by glucocorticoids and somatolactogenic hormones at physiological concentrations and it is hypothesized that these hormones are involved in the regulation of the growth of the endocrine pancreas both in the pregnant mother and in the fetus. Disturbances in this regulation may lead to impaired growth of the beta cell mass increasing the risk of developing diabetes.

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CHARACTERIZATION OF $GABA_A$ RECEPTOR STRUCTURE ON RAT PANCREATIC ISLETS

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Three major subunit families of the $GABA_A$ receptor have been identified named α, β and γ; different expression of each subunit leads to different biochemical and functional receptor characteristics. The presence of the $GABA_A$ receptor has been demonstrated on α cells of guinea pig pancreas by immunohistochemical sudies (Nature 341,233,1989). The aim of this study was to characterize the $GABA_A$ receptor structure on pancreatic islets studying the expression of the receptor subunits mRNAs on neonatal rat islets. Total RNA, extracted from cultured islets by the RNAzol method, was transcribed by M-MLV reverse transcriptase (200 U). The cDNA obtained was amplified by Hot Tub DNA polymerase (2.5 U) using specific primers for each subunit. Results demonstrated that rat neonatal islets express mRNAs for only 2 of the 5 subunits of the α family (α1 and α4), for all the subunits of the β family (β1, β2 and β3), and for only one of the 3 subunits of the γ family (γ3). Therefore $GABA_A$ receptor on pancreatic islets is ethero-hexamer. The characterization of $GABA_A$ receptor structure on pancreatic islets is important in order to understand the role of the GABAergic system in the modulation of the endocrine pancreatic secretion.

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MECHANISMS INVOLVED IN THE *IN VITRO* REGULATION BY GLUCOSE OF ISLET GLUT2 mRNA

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Abnormal glucose-induced insulin secretion has been ascribed to diminished expression of islet GLUT2 glucose transporter in several animal models of diabetes. Alternatively, decreased GLUT2 in these animals could be a consequence of a primary defect of β-cell glucose metabolism. To explore this issue we have investigated the mechanisms by which glucose normally regulates islet GLUT2 mRNA (GLUT2). GLUT2:β-Actin ratios were measured by Northern analysis from rat islets cultured for 24 hours under selected conditions. Glucose 16.7 mM vs 5.5 mM increased 2.8 kb-GLUT2 accumulation 353±69 %. Time-course studies with glucose 16 mM showed 251% GLUT2 induction after only 8 hours. Mannoheptulose 11 mM + Glucose 16.7 mM inhibited GLUT2 (25±2%), whereas glyceraldehyde 11.2 mM + glucose 5.5 mM partially mimicked the effect of high glucose (172±18%). Similarly, GLUT2 was enhanced by mannose, but not by 2-deoxyglucose. Tunicamycin, an N-linked glycosylation inhibitor known to modify the effect of glucose on another glucose transporter, did not affect GLUT2 levels. RNA synthesis inhibitor actinomycin D completely abolished stimulation by high glucose, but did not alter GLUT2 at glucose 5.5 mM. These results suggest that *in vitro* glucose acutely induces accumulation of islet GLUT2 mRNA, at least partly due to enhanced gene transcription, and that glucose metabolism is required for this effect.

OP 13
Immunology II

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APPEARANCE OF DIABETES-ASSOCIATED ANTIBODIES IN F1(NODxB10) MICE AFTER TRANSPLANTATION OF NOD BONE MARROW.

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Sera from 98% of Non Obese Diabetic (NOD) mice and from 35% human type 1 diabetics have antibodies recognizing a protein antigen (polar antigen) located at the secretory pole of cells on frozen sections of the RINm38 rat insulinoma. In NOD mice chimera studies have shown that diabetogenesis is dependent on expression of NOD derived susceptibility alleles at the level of hematopoietic stem cells, since autoimmune diabetes can be transferred from NOD into diabetes resistant F1 generation mice (NODxB10) by bone marrow transplantation. In the present study we aimed to estabilish whether the alleles responsible for the presence of anti-polar antibodies are expressed at the bone marrow level. Anti-polar antibodies were determined by indirect immunoperoxidase in sera from mice of the following strains: NOD (n=20); B10 (n=14); F1 (NODxB10) mice (n=17); F1mice (n=9) irradiated (1000R) at 4 weeks of age and reconstituted with bone marrow cells from NOD (n=9) or from F1(n=3) mice. Anti-polar antibodies were present in 100% NOD mice, were absent in B10 and F1mice, but were found in 77.8% (7/9) of F1 mice transplanted with NOD bone marrow and not in F1 receiving F1 bone marrow. These data provide the first evidence that expression of antibodies associated to ß cell autoimmunity can be induced, similarly to diabetes and insulitis, by transplantation of NOD bone marrow into F1 mice.

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THE MECHANISM OF SULFONYLUREA-INDUCED DIABETES PREVENTION IN THE BB RAT.

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Glipizide prevents diabetes in the BB rat, although the mechanism is unclear. We thus examined islet histology after glipizide treatment (100mg/kg/day) from 30 to 200 days of age. The diabetes incidence at day 85 was 24% in controls and 5% in glipizide treated rats ($p<.025$). The inflammation prevalence in diabetic rats was 100% in both groups. In nondiabetic rats the prevalence was 93% in controls (n=27) and 88% in treated rats (n=34), but the severity of inflammation, scored quantitatively and blindly, was less in treated animals ($p<.002$). At 200 days diabetes incidence was higher in controls (47%) than in treated rats (24%)($p<.02$). At 240 days no rebound increase in diabetes incidence occurred. The inflammation at 240 days (nondiabetic) was 86% in controls and 44% in treated rats ($p<.01$), and the severity was also less ($p<.01$). Glimeperide also prevented diabetes similarly. These data indicate that the sulfonylureas in BB rats a)prevent diabetes, b)act by an autoimmune component since both prevalence and severity of islet inflammation are decreased, c)do not enhance the likelihood of diabetes-onset by increasing beta cell secretion and d)may be useful in human diabetic prevention studies since these drugs also improve remission rates in new onset patients and has direct in vitro actions to suppress human lymphocyte proliferation.

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PREVENTION OF HIGH- AND LOW-DOSE STREPTOZOTOCIN DIABETES WITH D- AND 5-THIO-D-GLUCOSE

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In mice the effect of glucose compounds on diabetes induced either by a single high dose of streptozotocin (HD-STZ) or by multiple low doses (LD-STZ) was studied. We found that 5-thio-D-glucose (5-T-G), a sulfur analogue of D-glucose (D-G), prevented dose-dependently both HD-STZ and LD-STZ-induced hyperglycemia and that D-G, which was only tested in the LD-STZ system, was also protective, albeit somewhat less than 5-T-G. This protective effect was achieved by injecting i.p. 5-T-G and D-G, respectively, right before STZ. In C57BL/6 mice pretreatment with 5-T-G significantly ($p<0.0002$) prevented diabetes in 100% animals treated with either HD- or LD-STZ compared to mice, having received STZ only. Comparable results were obtained in BALB/c mice. Interestingly, pretreatment with 5-T-G failed to prevent STZ-induced immune reactions: By using the popliteal lymph node assay the T cell-dependent immune reactions to STZ as antigen was not affected, and the STZ-induced perivasculitis of pancreatic islets were not changed by pretreatment with 5-T-G. In conclusion, our data indicate that 5-T-G and D-G protected against hyperglycemia by preventing the ß-cell toxic effects of STZ, not by preventing its immunologic effect in the LD-STZ model.

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PANCREATIC ß-CELLS EXPRESSING MHC CLASS II MOLECULES ARE INCAPABLE OF PRESENTING ANTIGEN.

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It has been proposed that pancreatic ß-cells aberrantly expressing MHC class II molecules may present self-antigens and initiate IDDM. In this study, we sought to establish: *(a)* the identity of class II^+ cells in rat islets (using the fact that only cells of haematopoietic origin have the Leucocyte Common Antigen, LC-A); *(b)* whether class II^+ ß-cells are capable of presenting antigen. Class II expression was measured by immunofluorescent double-labeling and flow cytometry analysis. Pre-incubation of pre-diabetic BB rat islets in IFN-γ and TNF-α resulted in an increase in the number of class II^+ cells (7.4 to 26.5%). Although the dispersed islets contained a small number of LC-A^+ cells, the increase of class II expression occurred predominantly (21.1%) in LC-A^- cells. Similar increases were observed using islets from Lewis rats and RIN-5mF cells, a source of pure ß-cells (2.9 to 25.9%). ß-cells did not present antigens to T-cell lines, even after pre-incubation with cytokines. Our results suggest that: *(a)* the class II molecules detected on dispersed islet preparations after treatment with cytokines are expressed on ß-cells, and are not found exclusively on haematopoetic cells; *(b)* this ability is widespread, and not restricted to ß-cells from BB rats; *(c)* expression of MHC class II molecules is not sufficient to allow ß-cells to function as antigen presenting cells.

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REPETITIVE INJECTIONS OF INTERLEUKIN 1ß INDUCE HYPERGLYCEMIA IN THE BB AND OTHER RAT STRAINS

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The effects of repetitive interleukin 1ß (IL-1) injections on blood glucose (BG), temperature (TP), and IL-1-binding plasma proteins in DP-BB, DR-BB and Wistar Furth (WF) rats were investigated. Thirty day-old DP-BB (n=40), DR-BB (n=55), and WF (n=55) rats were randomized to daily injections of human IL-1 (4.0 μg/kg) or vehicle + pair-feeding for 13 weeks or until diabetes onset. IL-1 induced hyperglycemic episodes (BG > 9 mmol/l) in a higher number of DP-BB rats (16/20) compared to DR-BB (4/27) ($p < 0.00005$) and WF rats (11/28) ($p = 0.01$). IL-1 induced fever during more weeks in DP-BB rats (11/13) than in DR-BB (4/13, $p = 0.005$) and WF rats (6/13, $p = 0.04$). In IL-1 treated rats an IL-1-binding IgG was demonstrated in 5/5 DR-BB and 5/5 WF rats by size exclusion chromatography, but only in 1/5 non-diabetic DP-BB and 0/5 diabetic DP-BB rats. Binding of ^{125}I-IL-1 to IgG could be displaced by cold IL-1, but not by IL-1α or TNFα. We conclude that IL-1 induces hyperglycemia in all the investigated rat strains and that the DP-BB rats are more sensitive to the hyperglycemic and fever-inducing effect of repetitive IL-1 injections compared to DR-BB and WF rats, explainable by a reduced capacity for IL-1-antibody production.

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ANTIBODIES TO COW'S MILK PROTEINS AS RISK DETERMINANTS FOR EARLY ONSET TYPE 1 (INSULIN-DEPENDENT) DIABETES

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To study whether antibodies to cow's milk proteins are risk determinants for childhood onset type 1 (insulin-dependent) diabetes we examined 116 recent onset diabetic children and 112 age and sex matched controls. Sera were blindly analysed for cow's milk IgA, IgG and IgM antibodies as well as antibodies to ß-lactoglobulin and islet cells (ICA). All antibody levels tended to be increased among cases but statistically significantly so for cow's milk IgA antibodies ($p<0.001$) and ß-lactoglobulin IgA ($p<0.01$) as well as for ICA that was found among 92% of cases versus 3% of controls. Breast-feeding duration was significantly inversely related to the log of ß-lactoglobulin IgG ($p=0.04$) and cow's milk protein IgA antibodies ($p<0.001$). Formula feeding was significantly correlated to ß-lactoglobulin IgG antibodies ($p=0.01$) and cow's milk protein IgA antibodies ($p=0.04$). In a logistic regression analysis it was found that IgA antibodies to ß-lactoglobulin was related to an increased risk for diabetes with an odds ratio of 8.83, ($p=0.046$). This increase in risk was independent of formula feeding as well as the presence of the other antibodies studied including ICA. It is concluded that IgA antibodies to ß-lactoglobulin are significantly associated with an increased risk for diabetes at young age independent of ICA-status and an early introduction of cow's milk formula.

OP 14
Epidemiology I

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CLINICAL CHARACTERISTICS AT ONSET OF CHILDHOOD INSULIN-DEPENDENT DIABETES MELLITUS IN EUROPE

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As part of the multinational EURODIAB ACE project on childhood onset insulin-dependent diabetes mellitus we studied clinical characteristics at disease onset. The analysis was based on 3062 cases, comprising all cases of insulin-dependent diabetes diagnosed 1989-1990 among children aged 0-14 yrs. in 24 study regions throughout Europe and Israel (total study population: 16.8 mill. children). In 98.2%, insulin was administered within a week after clinical diagnosis of diabetes; in 85.6%, the diagnosis was preceded by symptoms of less than 2 months of duration. At onset, 0.5% of the cases died. After grouping the study regions according to population risk of disease the distribution of age at onset varied statistically significantly (Chi-square: 104.1, df 70, $p=0.005$) between risk categories but without consistent patterns of variation. For all study regions, relatively fewer and relatively more cases were diagnosed during early summer and during autumn and/or winter, respectively. We conclude that (1) in epidemiological surveys commencement of insulin treatment may for practical purposes be equated with clinical onset of diabetes; (2) across Europe childhood diabetes shows distinct seasonality at onset; (3) further studies are needed to elucidate the geographical variability regarding disease precipitation at different age levels; and (4) death in conjunction with the onset may represent a hitherto unrecognized problem in Europe.

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EVIDENCE OF TIME AND SPACE CLUSTERING OF TYPE I DIABETES IN CHILDREN.

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Diabetic children are sometimes diagnosed in clusters. If this is not just random distribution it would strongly support the hypothesis that environmental factors sometimes elicit the disease. By using the method developed by Knox, time and space clustering was analysed in all 584 cases of type I diabetes diagnosed between 1977 - 1990 and below the age of 16 from 4 pediatric departments in South-East Sweden. The overall annual incidence for the study period was 25.2 per 100.000 children aged 0 - 15 years, with the highest peak 1983 (39.2) and lowest 1977 and 1989 (18.9 resp. 20.7). Statistically significant clusters were found both for time and space with the highest significance obtained for the cut off values of 15 km and 7 months, respectively ($p < 0.001$). There was no evidence of time clustering at distances more than 20-25 km. Analysis of subsets did not reveal any significant heterogeneity.
Our results indicate that locally appearing factors (infections?) sometimes elicit clusters of IDDM in children.

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INFECTION AND THE CAUSES OF CHILDHOOD DISEASES: EVIDENCE FROM COMMUNITY LIFESTYLE STUDIES

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A diabetes register for children aged 16 or under from 1978 to 1990 was compiled for Yorkshire in Northern England. There is evidence of substantial variation in diabetes incidence both between districts (X^2 = 81.35 on 19 df, p=0.0001), and wards (X^2 = 649.3 on 533 df, p=0.0004). District incidence rates ranged from 3.89 in Calderdale to 15.03 in Holderness. A strong linear relationship was found between high rates and low ward population density (1981 census). The relative risk for an increase in population density from one quintile to the next was 0.90 [95% CI = 0.85 to 0.95, LRS = 16.006 on 1 df, p = 0.000063]. The pattern of incidence by age was very different in rural areas than in urban areas. In rural wards the incidence rates were considerably higher for children under 8 [Rate ratio = 1.60; 95% CI = 1.21 to 2.00] than the rates in urban wards, whereas for older children the two rates were more similar [Rate ratio - 1.06; 95% CI = 0.84 to 1.34]. Children of pre-school age in rural areas are relatively shielded from the normal urban circulation of infectious agents. This pattern of age incidence suggests that age at first exposure to certain infectious agents may play a significant role in the aetiology of childhood diabetes.

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CHILDHOOD DIABETES AND INFANT FEEDING: A POPULATION BASED CASE-CONTROL STUDY

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The aetiology of Insulin-Dependent Diabetes Mellitus appears to involve both genetic and non-genetic factors. We aimed to study environmental factors with a population based case-control study. All incident cases in the 0-14 years age-group in New South Wales were ascertained for an 18 month period. Environmental exposures were determined by questionnaire. For each case, two age and sex matched controls were randomly selected from the population. Associations were evaluated with odds ratios (OR) and their 95% confidence intervals (95% CI), adjusted for the confounding effect of maternal education level by logistic regression. Longer duration of breast feeding was associated with a lower risk for subsequent diabetes. Exclusive breast feeding for 3 months or more was associated with a protective effect which remained significant after allowing for maternal education level. The introduction into the diet of cow's milk based infant formula before 3 months of age was associated with an increased risk.

		Cases	Controls	OR (95% CI)	Adjusted OR (95% CI)
Breast Feeding (months)	<2	85	69	1.0#	1.0#
	2-7	70	103	0.55 (0.36-0.86)	0.64 (0.40-1.00)
	>7	62	86	0.59 (0.37-0.92)	0.68 (0.42-1.11)
Exclusive breast feeding (months)	<3	114	105	1.0#	1.0#
	≥3	102	153	0.61 (0.43-0.88)	0.67 (0.45-0.98)
Introduction of cow-based formula (months)	>3	110	163	1.0#	1.0#
	≤3	107	94	1.69 (1.17-2.44)	1.51 (1.03-2.22)

reference category

In conclusion, early exposure to components of cow's milk in the diet and/or short duration of breast feeding may be associated with an increased risk for subsequent diabetes.

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FAMILIAL OCCURENCE OF INSULIN-DEPENDENT DIABETES MELLITUS (IDDM) IN DENMARK.

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Familial aggregation of IDDM is well known, though the prevalence of familial cases vary in published studies. In the present study the prevalence of familial IDDM was estimated in a population based manner. All Danish children aged 0-19 years with IDDM were identified by asking all pediatric departments and departments of internal medicine to report known cases of IDDM, and notified cases were asked to fill in a questionnaire. The questionnaire was filled in by 1418 of 1574 known patients giving a participation of 90%. Ascertainment is thought to be nearly complete (< 1-2% attending GPs). The male/female ratio was 753/665 ($p<0.001$). No sex difference was found in familial cases: 99/98. Mean age at diabetes onset was 8.1±4.2 yrs in familial cases vs. 8.6±4.2 in sporadic cases ($p=0.087$). The prevalence of probands with IDDM affected 1.degree relatives was 12.8%. Ninety probands (6.7%) had a father with IDDM and 28 (2.1%) had a mother with IDDM ($p<0.0001$). Sixty-six probands (4.9%) had a least 1 sibling with IDDM. A significant correlation of age at onset of affected siblings was observed ($p<0.036$). No significant trend in seasonal variation in diabetes onset was found neither in familial nor in sporadic cases.
The population based cohort of young IDDM patients and their relatives will be important for future genetic marker studies and for intervention studies in prediabetics.

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THE INCIDENCE OF TYPE 1 DIABETES IS RAPIDLY INCREASING IN SARDINIAN MALE ARMY CONSCRIPTS

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To provide further information on the recently reported high incidence of Type 1 (insulin-dependent) diabetes in Sardinia we performed a cohort study of a series of Sardinian male birth cohort. All male subjects borne live in Sardinia between 1 January 1936 and 31 December 1972 were investigated regarding the development of Type 1 (insulin-dependent) diabetes during the first 19 years of life using the files of the Sardinian Conscript Board. A total of 687 diabetic subjects were identified of whom all were of Sardinian ancestry. The cumulative rate of developing Type 1 (insulin-dependent) diabetes during the first 19 years of life ranged from values close to zero for the first eleven cohorts (1936-45) up to 3.96% for the 1966 cohort and lasted steadely thereafter. Poisson regression modelling was used to determinate the change in incidence over calendar time. Two significant non-linear increases in the cumulative incidence of the disease in the birth cohorts of 1945-65 (mean value 1.2%) and 1966-72 (3.96%) were found. The results indicate a rapidly increasing incidence of Type 1 (insulin-dependent) diabetes in males in Sardinia consistent with the concurrent high incidence observed in both sexes during the Eurodiab survey.

OP 15

Pregnancy and Diabetes

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PRECONCEPTIONAL CARE OF INSULIN DEPENDENT DIABETIC WOMEN

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Preconceptional care of diabetic women aims at the prevention of maternal and fetal complications. 698 insulin dependent pregnant diabetics (White B-C: 442 D-F: 256) were treated: in 25% of the mothers the intensive care has been started preconceptionally (group I. n=170 newborns 171) in the remaining cases during gestation (group II. n=528 newborns 535). Clinical features of infants in the two groups were as follows. Perinatal mortality (PM): I. 3,5% II. 4,5%; congenital malformations (CM): total: I. 5,3% II. 6,5% „diabetes attributed": I. 2,9% II. 4,3%; premature births: I. 13,5% II. 16,8% (N.S). In case of children (n=257) born to White D-F mothers PM: I. 1/70=1,5% II. 14/187=5,6% (P<0,05); CM total I. 3/70=4,3% II. 13/187=7,0%. Metabolic state of the mothers was followed by glycohaemoglobin (GHb) measurements in a group of women with (group I. n=30) and without (group II. n=30) preconceptional care. GHb: preconceptionally 6,3±1,4%; first trimenon I. 5,6±1,2% II. 7,4±1,3 (P<0,001); second trimenon I. 4,5±0,9% II. 5,7±1,0% (P<0,01); third trimenon I. 4,6±0,7% II. 5,4±1,2% (P<0,01). As a result of preconceptional care mothers were near normoglycaemic before conception and normoglycaemic from early pregnancy. Following prepregnancy care fetal outcome has been generally improved, mainly in infants of mothers with microvascular complications.

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GLUCOSE METABOLISM IN NORMAL PREGNANCY AND PREGNANCY COMPLICATED BY GESTATIONAL DIABETES

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The aim of this study was to measure insulin and glucose sensitivity in gestational diabetes (GD) using stable isotopes. We investigated glucose and insulin kinetics in normal pregnancy (n=4) and gestational diabetes (n=5) using an intravenous glucose tolerance test (IVGTT) labelled with 6,6$^2H_2$-glucose, analysed using a modification of Bergman's model. Subjects were studied in the third trimester of pregnancy (at diagnosis for those with GD) and 3 months post-partum. The rate of fall of glucose (Kd) following an IVGTT was significantly decreased ($p<0.05$) in GD (1.01 ± 0.16 %/min) compared with controls (1.65 ± 0.06 %/min) during pregnancy and increased after delivery in both groups to 1.62 ± 0.26 ($p<0.05$) and 2.44 ± 0.29 %/min (NS) respectively. Glucose sensitivity was 0.63 ± 0.08 x $10^{-2}min^{-1}$ in the pregnant GDs and 0.83 ± 0.12 x $10^{-2}min^{-1}$ in normal pregnancy (n=3) with values of 0.95 ± 0.18 x $10^{-2}min^{-1}$ and 1.06 ± 0.18 x $10^{-2}min^{-1}$ respectively after delivery (NS). The GDs had a significantly lower glucose sensitivity in pregnancy than the normals after delivery ($p<0.05$). Mean insulin sensitivity was 1.65 ± 0.25 x $10^{-4}min^{-1}$/mU/l in pregnancy in GD and 2.06 ± 0.96 x $10^{-4}min^{-1}$/mU/l in the control subjects with values of 4.61 ± 2.12 x $10^{-4}min^{-1}$/mU/l and 8.64 ± 5.08 x $10^{-4}min^{-1}$ /mU/l respectively after delivery (NS). These results show impaired glucose disposal in gestational diabetes and indicate marked individual variation in both glucose and insulin sensitivity.

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PREGESTATIONAL INSULIN - DEPENDENT DIABETIC PREGNANCY : ATTEMPTED NORMALISATION OF DIABETIC CONTROL AND PREGNANCY OUTCOME OVER A DECADE IN 300 PATIENTS.

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Between 1981 and 1991, three hundred patients with pre - gestational insulin dependent diabetes were managed at the National Maternity Hospital. Objectives were to reduce macrosomia and other foetal complications by careful glycaemic control and to manage diabetic patients without complications in the same way as non - diabetic patients. Complex individualised insulin regimes were used and monitored with home blood glucose recording, fructosamines and HbA1c's. There has been a progressive reduction in the incidence of macrosomia (birth weight > 4500 g) from 18% to 5%, with 90% of babies weighing < 4000 g during the last 4 years. The caesarean section rate increased from 24% to 32%, induction of labour halved (33% to 17%) and spontaneous labour increased from 45% to 54% in the last 4 years. Patients delivering after 40 weeks gestation increased from 26% to 44% over the decade and in the last 4 years 83% delivered after 38 weeks. This has been achieved without compromised foetal outcome. One perinatal death (Potters syndrome) and no cases of cerebral irritation were seen in the last 4 years. These results show that meticulous glycaemic control can allow the vast majority of diabetic mothers to anticipate a similar pregnancy outcome to those of non - diabetics.

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OUTCOME OF 445 PREGNANCIES AMONG DIABETIC MOTHERS IN THE COUNTY OF NORTHERN JUTLAND 1976-90

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To ascertain the regional outcome and to determine the degree of centralisation we have recorded data from all diabetic pregnancies in the County of Northern Jutland (550.000 inhabitants). In the 16 year period 445 consequetive, unselected pregnancies among 287 women with diabetes mellitus were admitted. The distribution according to White-classification was: A: 117(26%), AB: 35(8%), B: 79(18%) C: 128(29%), D: 54(12%), F: 32(7%) (AB=insulin-treatment instituted during actual pregnancy in women without previously recognized diabetes). Thirty-two wanted abortion which is legal till the end of 12'th gestational week, thirty-six (8,7%) women had with spontaneous abortions and ten (2,4%) abortions were induced for medical reasons. There were twelve (3,3%) perinatal deaths of which two had lethal malformations giving an adjusted perinatal mortality of 2,7% (95% confidence limits 1,8-3,6%). Five (1,6%) babies had major and additionally nine (2,5%) minor malformations. Total fetal loss was 58/413=14%. Twenty-eight percent of the babies were large-for-gestational age. The rate of Caesarean sections were overall 44% and 59% in White class B-F. Ninety-eight percent of all wanted pregnancies and all pregnancies reaching beyond 28'th gestational week were delivered of at obstetrical Departments. We conclude that the outcome in our county is comparable to the results published from similar regional surveys and that we have achieved an almost complete centralization of the management of pregnant women with diabetes mellitus.

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DECREASED NON-OXIDATIVE GLUCOSE DISPOSAL IN NORMOGLYCAEMIC PREVIOUS GESTATIONAL DIABETICS

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The present study was set to investigate if women with previous gestational diabetes (GDM), a population with increased risk of developing Type 2 diabetes in the years following pregnancy, are insulin resistant. Twelve previous GDM's and 11 women who had a normal glucose tolerance during pregnancy (age 36.6±1.2 vs. 35.0±1.0 years, BMI 21.6±0.5 vs. 21.2±0.5 kg/m^2, mean±SE) were investigated 6-12 years after index pregnancy. All had a normal 75 g OGTT. Fasting plasma insulin was slightly higher in the previous GDM's 48±3 vs. 39±2 pmol/l ($p<0.05$). A 3-hour euglycaemic, hyperinsulinaemic clamp (40 mU/m^2/min) including indirect calorimetry and infusion of ^{3}H-glucose was performed in each subject. Insulin-stimulated glucose disposal was decreased in previous GDM's (11.1±0.6 vs. 13.5±0.5 mg/kg FFM (fat free mass)/min, $p<0.01$) due to a decrease in non-oxidative glucose disposal (6.6±0.5 vs. 9.0±0.6 mg/kg FFM/min, $p<0.01$) while glucose oxidation was normal. Basal hepatic glucose production was increased in the previous GDM's (2.92±0.10 vs. 2.66±0.06 mg/kg FFM/min, $p<0.05$). In conclusion, our study indicates that decreased non-oxidative insulin-stimulated glucose disposal and increased basal hepatic glucose production, both characteristic features of Type 2 diabetes, might be of pathogenic importance for both the previous development of GDM and the subsequent development of Type 2 diabetes in some previous GDM's.

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ISLET-CELL ANTIBODY POSITIVE GESTATIONAL DIABETIC WOMEN:POST-PARTUM INTRAVENOUS GLUCOSE TOLERANCE.

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A decrease in first-phase insulin secretion has been reported in pre-type I diabetic subjects before the clinical onset of the disease. We aimed to assess intravenous glucose tolerance in women with previos gestational diabetes and islet-cell antibody positivity (ICA+) during pregnancy. Nine non-obese ICA+ women with previous gestational diabetes and normal post-partum oral glucose tolerance underwent an intravenous glucose tolerance test 12.2 ± 2.5 (mean ± SD) months after the end of pregnancy. Sera was obtained for insulin (IRI) at 1 and 3 minutes, and for glucose at 5, 10, 15, 20, 25, 30 and 40 minutes after the end of glucose perfusion (0.5gr/kg). Glycemic values were used to calculate the glucose disposal rate (k value). First-phase insulin secretion is expressed as the sum of 1+3 minutes insulinemia (IRI 1+3). Results were compared (difference between means) to those of nine control non-obese ICA- women with known normal oral glucose tolerance, matched for age and BMI. ICA+ women showed lower IRI1+3: 632.8 ± 240.7 vs 1021.4 ± 391.1 pmol/l ($p<0.02$), and lower k value 1.76 ± 0.69 vs 2.7 ± 0.91 ($p<0.02$). We conclude that women with previous gestational diabetes and ICA positivity during pregnancy show relative decrease of first-phase insulin secretion and glucose disposal rate at short term after pregnancy, in the presence of normal oral glucose tolerance.

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OP 16
Modelling of Glucose Metabolism

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SURFACE ADJUSTED PRIMED-CONSTANT 3-^{3}H-GLUCOSE INFUSION FOR COMMON BASAL PLASMA SPECIFIC ACTIVITY.

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Labeled glucose infusates to maintain constant plasma specific activity has been shown to improve assessment of glucose turnover rates in glucose clamp studies. Appropriate labeling of the glucose infusate depends on a priori knowledge of expected individual basal plasma specific activity. Reevaluation of our previous primed-constant 3-^{3}H-glucose infusion studies showed that in normal subjects basal plasma specific activity was tightly correlated to tracer infusion rate per m^2 body surface area ($n=45$, $y = 0.0114x + 20$, $r=0.943$, $p<0.001$). This means that, if the tracer infusion is adjusted for individual body surface area, a common level of basal specific activity should be obtained in all subjects. To test this hypothesis 12 normal subjects (age: 20-35 years, weight: 65-93 kg, BMI: 20.7-28.4 kg/m^2, surface area: 1.80-2.25 m^2) received a 2 h primed-constant 3-^{3}H-glucose infusion (prime: $20 \cdot 10^6$ cpm/m^2, infusion: $20 \cdot 10^4$ cpm·m^{-2}·min^{-1}) expected to result in a basal plasma specific activity level of 2300 cpm/mg. Measured basal plasma specific after 2 h infusion was 99±10% (mean±SD) of the expected value. In conclusion, by adjusting primed-constant 3-3H-glucose infusion for m^2 body surface area a common level of basal plasma specific activity can be obtained and this may facilitate appropriate labeling of the glucose infusate in subsequent clamp studies.

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MEASUREMENT OF GLUCOSE TURNOVER IN NON-STEADY-STATE WITH A NEW TRACER MODEL AND DECONVOLUTION

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Estimation of hepatic glucose production (HGP) in non-steady-state is difficult. A new method is presented. DATA: A hyperinsulinemic euglycaemic clamp was performed in five normal subjects. Insulin was infused (0.6 mU/Kg/min) for 300 min and then discontinued (300-420 min). Two tracers, [6-^{3}H]-glucose and [6-^{14}C]-glucose, were administered with different formats for model validation; [6-^{14}C]-glucose was added to unlabelled glucose. METHODS: A new two-compartment tracer model was developed which describes insulin control on glucose kinetics. Once identified from one tracer, the model performed better than Steele's and Radziuk's models by accurately predicting the known appearance rate of the other tracer. Endogenous glucose concentration, i.e., that fraction of measured glucose concentration due to HGP only, was calculated from the data. The tracer model was used to calculate HGP from endogenous glucose concentration by deconvolution. RESULTS: Despite the different format of tracer administration, the HGP profiles derived from the two tracers in each subject were almost superimposable; furthermore a low dispersion was observed among subjects. Maximal HGP inhibition was 89% at 300 min; half-maximal suppression and resumption times were 33.2±8.2 and 96.8±24.4 min, respectively. The proposed method provides a reliable estimate of HGP in non-steady-state; it is of general applicability and especially useful when specific activity is not successfully clamped.

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EUGLYCEMIC INSULIN CLAMP (EIC) AND NEGATIVE HEPATIC GLUCOSE PRODUCTION (HGP): A REVALUATION

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We tested whether cycling of [3-^{3}H]-D-glucose in hepatic glycogen or insufficient time for tracer equilibration explain HGP negative estimates during EIC. Nine healthy subjects underwent three 40 mU/m^2·min·240 min EIC with final total glucose disposal = 8-9 mg/min·Kg. In the first two studies, [3-^{3}H]-D-glucose was infused during basal 120 min, discontinued during initial 120 min and resumed in final 120 min of the EIC to achieve a 4-fold lower (study A) or higher (study B) plasma specific activity (SA) than in the basal. Glucose incorporated into hepatic glycogen during basal and released during EIC would affect HGP in study A (basal SA = 4284 ± 237 higher than EIC SA = 980 ± 74 dpm/mg). HGP in both studies (-0.02 ± 0.31 vs 0.15 ± 0.21 mg/min·Kg) was similar and not different from zero. As the 120 time-window between basal and EIC tracer infusion could have depleted [3-^{3}H]-D-glucose counts accumulated in liver glycogen during basal tracer infusion, minimizing radioactivity recycling, in the third study (C) [3-^{3}H]-D-glucose was infused as a primed-continuous infusion during the basal and the EIC (basal SA = 3990 ± 356, EIC SA = 1114 ± 100 dpm/mg). Again, HGP (0.26 ± 0.31 mg/min·Kg) was not different from study A or B or from 0. CONCLUSIONS: (1) tracer recycling cannot explain HGP negative estimates; (2) when both metabolic and isotopic steady state is achieved, HGP negative estimates are not observed.

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A MODEL TO MEASURE TRANSMEMBRANE GLUCOSE FLUXES AND INTRACELLULAR PHOSPHORYLATION IN HUMAN SKELETAL MUSCLE

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We propose a model-based method to measure in vivo the effects of insulin upon the individual steps of glucose metabolism in human skeletal muscle. We studied 5 healthy individuals by the euglycaemic insulin (~470 pM) clamp in combination with forearm technic (brachial artery and deep forearm vein catheterisation) and intra-arterial pulse injection of nonradioactive mannitol (non transportable), 3-O-^{14}C-methil-D-glucose (transportable, but not metabolizable) and 3-^{3}H-D-glucose (transportable and metabolizable). Washout curves in deep forearm vein were analysed by a flow-compartmental model describing blood flow heterogeneity, transmembrane glucose transport and intracellular glucose phosphorylation. Transmembrane glucose flux into (Fin) and out of (Fout) the cell, intracellular phosphorylative flux (Fmet), extracellular (Vec) and intracellular (Vic) glucose distribution volumes and intracellular glucose concentration (Ci) were estimated. Insulin increased Fin, Fout, Fmet and Vic/Vec (67.4±18.3 vs 20.3±4.7 µmol/min/kg, $p<0.02$, 22.8±5.8 vs 12.9±2.6 µmol/min/kg, $p<0.1$, 43.5±12.4 vs 7.2±2.4 µmol/min/kg, $p<0.01$, 5.53±1.17 vs 2.83±0.34, $p<0.02$). Insulin stimulated phosphorylation more than transport (8.33±2.1 vs 3.3±0.53 fold, $p=0.05$), whereas Ci decreased (0.77±0.17 vs 1.1±0.25 mM, $p<0.02$), showing that phosphorylation was restrained by transport. CONCLUSIONS. In human skeletal muscle:1. This model-based approach measures the individual steps of glucose metabolism; 2. Glucose transport is rate-limiting during hyperinsulinaemia; 3. Glucose phosphorylation is stimulated by insulin independently from transport and plays an important role in determining the overall response to insulin.

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MINIMAL MODEL ANALYSIS OF THE EFFECT OF HYPERGLYCEMIA IN INSULIN DEPENDENT DIABETES.

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Prolonged hyperglycemia has previously been shown to reduce glucose disposal in type 1 diabetes. Our aim was to examine whether this effect was on glucose-mediated glucose disposal (SG), insulin sensitivity (SI) or on residual B-cell function. Five young non obese type 1 diabetic volunteers were studied, with undetectable fasting plasma C-peptide. Insulin was infused intravenously at 12 mU/kg/hr for 18 hours with glucose infusion adjusted 2 hourly to maintain euglycemia (E) or hyperglycemia (H, approximately 15 mmol/l). An intravenous glucose tolerance test was then performed with insulin being infused to approximate non-diabetic endogenous insulin and analysed by our modification of the Minimal Model, as we previously described and validated. The mean glucose disappearance rate (Kg) was reduced in the hyperglycemic experiments (H vs E, 1.03 ± 0.16 SEM vs 1.59 ± 0.38 min^{-1} X 10^2). This indicated increased "insulin resistance" in the hyperglycemic group because the same insulin was infused in both groups. Minimal Model analysis indicated that this was due to reduced SI (H vs E, 0.59 ± 0.27 vs 5.58 ± 1.40 min^{-1} per mU/L x 10^4, $p < 0.05$), as there was no difference in SG (H vs E, 2.47 ± 0.46 vs 2.57 ± 0.34 min^{-1} X 10^2). In conclusion, the effect of prolonged hyperglycemia in type 1 diabetics with no endogenous insulin secretion is to cause a reduction in insulin sensitivity and has no effect on glucose-mediated glucose disposal.

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Glucose tolerance and Beta-cell function assessed by Continuous Infusion of Glucose and Oral Glucose Tolerance Test.

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The one hour low-dose continuous infusion glucose test (CIG) is an alternative to oral glucose tolerance test (OGTT). Our aim was to compare these tests in 13 non-diabetic, 11 diet-treated Type 2 (non-insulin-dependent) diabetic and 6 impaired glucose tolerance subjects. Each received two CIG tests (5 mg.kg ideal body weight^{-1}.min^{-1}) and two 75g OGTT. One hour CIG glucose had a significantly lower coefficient of variaton (CV) than two hour OGTT glucose (5% and 16%, respectively, $p<0.001$). The two measures correlated, Spearman $r = 0.83$, $p<0.001$. All OGTT defined normal glucose tolerant subjects (WHO criteria) had normal CIG (100% specificity). Of 17 subjects with glucose intolerance by OGTT, 15 had CIG glucose intolerance (89% sensitivity). Beta-cell function (%B) measured from CIG by computer model assessment (CIGMA) was more reproducible than that measured by OGTT from the ratio of the 30 min plasma insulin and glucose increments ($\delta I/\delta G_{30}$) with CVs of 13% and 30%, respectively, $p<0.02$. %B and $\delta I/\delta G_{30}$ correlated $r=0.86$, $p<0.001$). CIGMA also assesses glucose sensitivity (CV = 20%), while OGTT does not. In conclusion, the one hour CIG test is a specific and sensitive test for WHO defined abnormal glucose tolerance, and measures of glucose tolerance and Beta-cell function more precisely than the OGTT.

OP 17
Insulin Secretion I

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CALCITONIN GENE-RELATED PEPTIDE (CGRP) AND AMYLIN INHIBITION OF INSULIN RELEASE IS REVERSED BY THE 8-37 CGRP FRAGMENT.

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The 8-37 fragment of human calcitonin gene-related peptide [hCGRP(8-37)] has been shown to antagonise the effects of both CGRP and amylin in a number of tissues. Thus, we have investigated the influence of hCGRP(8-37) (1 μmol/l) on the inhibition of insulin release (IRI) induced by both CGRP and amylin 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). Rat CGRP (75 pmol/l) inhibited the insulin response to 9 mmol/l glucose (incremental areas, glucose alone: 55±14,SEM, ng/10 min; glucose+CGRP: 14±4 ng/10 min; $p<0.025$). This inhibition was not observed when hCGRP(8-37) was simultaneously infused (incremental area: 64±30 ng/10 min). Rat amylin (75 pmol/l) also blocked the insulin response to 9 mmol/l glucose (incremental areas, glucose alone: 52±12 ng/10 min; glucose+amylin: 15±5 ng/10 min; $p<0.025$). Addition of hCGRP(8-37) to the perfusate counteracted this blocking effect (incremental area: 41±10 ng/10 min; $p=0.55$ vs. glucose experiments). Finally, infusion of hCGRP(8-37) alone had no effect on glucose-induced insulin secretion. In conclusion, in the rat pancreas: 1) Homologous CGRP and amylin, at the picomolar level, exert comparable inhibitory effects on glucose-induced insulin release. 2) These effects are antagonised by hCGRP(8-37), thus suggesting that both peptides act on the B-cell, at least in part, through a common receptor.

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INHIBITION OF GLUCOSE INDUCED INSULIN SECRETION BY AMYLIN IN RATS IN VIVO

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Amylin, like insulin, is secreted from pancreatic B cells and some studies suggest an inhibiting effect on insulin release in vitro. We investigated the effect of amylin exposure on insulin secretion in vivo during hyperglycemic clamp tests in freely moving rats. Short term amylin infusion (2h) dose-dependently decreased the infusion rate of glucose required to maintain plasma glucose at 200 mg/dl (mg/kg*min: control, 36.5±5.5; 2ug/h, 27.8±3.2; 20ug/h, 21.5±3.5; n=7-8; $p<0.05$ vs. control). That effect, however, faded during long term (24h) amylin infusion (20ug/h: 38.2±4.4; n=7). Decreased glucose uptake was reflected by lower glycogen content in various muscles after the clamp test (glycosyl units/g m. soleus: control, 22.8±3.4; 2h-20ug/h, 6.3±6.0; 24h-20ug/h, 22.5±7.9; n=5-6; $p<0.05$ vs. 2h). Preliminary data on plasma insulinemia during the clamp clearly suggest that glucose induced insulin secretion is strongly inhibited by amylin (mU/l: control, n=4, 60.8±10.1; 2h-20ug/h, n=4, 24.5±7.6; 24h-20ug/h, n=3, 66.7±35.0; control vs. 2h: $p<0.05$). Assuming that inhibition of insulin release is due to a direct effect of amylin on B cells, high local concentrations should lead to a strong paracrine effect inhibiting insulin release under physiological conditions.

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INSULIN SECRETION PROFILE OF MODY PATIENTS CARRYING MUTATIONS IN THE GLUCOKINASE GENE

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Linkage between the glucokinase (GCK) gene and diabetes in families with MODY was reported. We report beta-cell secretory profile of 4 diabetic patients carrying mutations in the GCK locus (SSCP analysis). Patients aged 26±11 years, BMI was 20.4±2.1; age of onset of diabetes was 16±6 years; 3 were male. Seven healthy individuals were used as control group. Statistics are Mann-Whitney's U test and data expressed as mean±SD. Fasting plasma glucose and insulin were respectively 6.9±0.6 mM and 5±2 mU/l. First phase insulin secretion (t1+t3 min), during intravenous glucose test, was subnormal but not delayed: 69±37 vs. 151±96 mU/l insulin ($p=0,09$) and 2.7±0.8 vs. 4.4±1.7 nM C-peptide ($p=0,04$). During glucose clamp (10 mM glucose) the mean values of insulin and C-peptide (t60-120 min) were reduced: 15±10 vs 40±11 mU/ml ($p=0,01$) and 1.4±0.2 vs. 2.6±0.4 nM ($p=0,008$) respectively. Responses to intravenous arginine test performed at the end of the clamp were equally subnormal: 45±16 vs. 194±117 mU/l insulin ($p=0,008$) and 1.3±0.5 vs 2.6±1.5 nM C-peptide ($p=0,13$). However, 3 out of 4 patients had C-peptide values in the mean-1SD range of control group. In conclusion, mild insufficiency of beta-cell secretory capacity in response to glucose characterize these patients with mutations in GCK gene.

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GLUCOKINASE INHIBITION DIFFERENTIALLY AFFECTS INSULIN RELEASE BY GLUCOSE AND ARGININE *IN VIVO*.

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A prominent defect early in type 2 (non-insulin dependent) diabetes is an impaired acute insulin response to glucose (AIR_G) while the response to non-glucose secretagogues remains intact, suggesting a specific defect in glucose-sensing. Beta-cell glucokinase is a prime candidate "glucose sensor" since its activity *in vitro* correlates tightly with insulin secretion. However, metabolic effects of inhibiting glucokinase *in vivo* are unknown. We examined the effect of glucosamine (glucokinase inhibitor) on insulin responses to glucose and arginine in conscious rats. Male rats with jugular vein catheters received glucosamine (200mg, iv) or saline 10min prior to infusion of glucose or arginine (10%x0.1ml/minx15min). Since glucosamine alone reduced [insulin], it is expressed as Δ% from prestimulus level. Control rats displayed normal 1st (AIR_G) and 2nd phase ($area_{3\text{-}15min}$) responses (+237±23%, +2722±533%xmin, n=6). In glucosamine-treated rats AIR_G was markedly reduced (+71±31%, $p<0.01$), but the 2nd phase was unchanged (+2184±424Δ%xmin, n.s., n=6). Concomitantly, [glucose] increased more after glucosamine ($AUC_{0\text{-}15min}$=+676±56 *vs* +396±13mg/dlx min, $p<0.01$). Arginine-induced insulin release (AIR_{Arg}=+496±39%, 2nd phase= +5968±832Δ%xmin, n=7) was not altered by glucosamine (AIR_{Arg}=+396±29% n.s., 2nd phase= +5005±270Δ%xmin n.s., n=6). Arginine-induced hyperglycemia was enhanced by glucosamine ($AUC_{0\text{-}15min}$: 251±82 *vs* 71±29mg/dlxmin, $p<0.05$). In summary, glucokinase inhibition by glucosamine resulted in islet function characteristic of type 2 (non-insulin dependent) diabetes, ie, markedly reduced AIR_G with intact response to arginine. These findings strengthen the hypothesis that glucokinase is a crucial mediator of islet glucose sensing that may be defective in type 2 (non-insulin dependent) diabetes.

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NITRIC OXYDE PRODUCTION IS NOT A PREREQUISITE FOR ARGININE INDUCED INSULIN SECRETION.

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Two mechanisms have been proposed for arginine-induced insulin secretion : a direct charge-related depolarizing effect and/or metabolization to nitric oxide (NO). The purpose of this study was to evaluate in isolated perfused rat pancreas the respective importance of these mechanisms. First, we compared the effects of L-arginine and of D-arginine which is not a substrate for NO synthase and second, we studied the effect of L-arginine in presence of an inhibitor of NO synthase, N nitro-L-arginine methyl ester (NAME). In the presence of 5 mM glucose, L-arginine at 5 mM induced a transient insulin response (7.5 $\pm$ 0.8 ng/min) and a biphasic one at 20 mM (56.8 $\pm$ 0.9 ng/min). D-arginine, ineffective at 5 mM, induced only a moderate monophasic increase at 20 mM (3.6 $\pm$ 0.9 ng/min). NAME which alone slightly increased insulin release (3.7 $\pm$ 0.1 ng/min) strongly potentiated (43.7 $\pm$ 14.2 ng/min) the effect of L-arginine (5 mM). In the presence of 8.3 mM glucose, this potentialing effect was not observed. In conclusion, arginine could stimulate insulin secretion via a direct charge mediated effect but only at high concentrations. NO generation does not seem to be involved ; on the contrary, at low glucose, blockade of its generation, potentiates L-arginine effect, which is not observed in presence of a slightly stimulatory glucose concentration.

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Hyperinsulinemia decreases second phase but not first phase arginine-induced insulin secretion in humans.

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Little is known about the feedback effect of insulin on the dynamics of stimulated insulin secretion. Aim of this study was to investigate the effect of hyperinsulinemia on first and second phase arginine-induced insulin secretion in humans. Seven healthy subjects underwent three studies (lasting 360 min) in random order: a control study using saline infusion and two euglycaemic clamps using low-dose (0.3 mU/kg/ min) and high-dose (1.2 mU/kg/min) insulin infusion. At 180 min, arginine (25 g) was infused for 30 min. Insulin secretion profile was calculated from C-peptide concentrations by using C-peptide kinetic modelling and deconvolution. First and second phase insulin secretion were obtained by integrating the secretion profile during 180-185 min and 185-225 min, respectively. Before the arginine administration, steady-state insulin levels (120-180 min) were (Mean±SEM) 10.0±0.4, 27.6±0.3 and 75.9±1.3 µU/ml during saline, low and high insulin infusions, respectively. During the arginine stimulation, first phase was indipendent of any effect induced by both insulin infusions, whereas second phase insulin secretion was reduced ($p<0.01$). First phase was 76.1±11.0, 69.2±14.1, and 70.0±11.2 pmol/kg, whereas second phase was 245.2±44.7, 138.3±35.7, and 131.1±32.5 pmol/kg for saline, low- and high-dose insulin infusions, respectively. In summary, this study shows that second phase, but not first phase, insulin secretion during arginine infusion is modulated by the pre-stimulus insulin levels.

OP 18
Effects of Dietary Lipids I

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IMPAIRED TRIGLYCERIDE HANDLING IN HIGH SUCROSE FED STREPTOZOTOCIN TYPE II DIABETIC RAT: EFFECT OF FISH OIL

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There is evidence (including our previous data) for the assumption that a buildup of lipid in muscle leads to insulin resistance, and that supply via stored triglycerides (TG) is an integral part of it. Therefore, we assessed postprandial values of serum TG, FFA, muscle TG content, liver acetyl CoA carboxylase (A-CoAC) activity in the neonatal streptozotocin model of Type II diabetes (DM). Male Wistar rats were injected at 2 days of age with 90 mg/kg STZ or vehicle (C). At 8 weeks, groups were subdivided and fed either a basal (B) or high (63 cal%) sucrose (HS) diet for 3 weeks, supplemented or not with FO (30 wt% n-3 PUFA). All diabetic rats were equally hyperglycemic at relative insulin deficiency. Diabetes did not alter the liver A-CoAC; HS feeding raised (C-B: 1.28±0.07 vs C-HS: 2.07±0.04 or DM-HS: 1.85±0.32 nmol min^{-1} mg^{-1}, $p<0.001$) and FO normalized A-CoAC activity. Diabetes did not alter serum TG levels (C-B: 3.2 ± 0.2 vs DM-B: 3.0 ± 0.3 $mmol.l^{-1}$), but HS diet led to a higher ($p<0.025$) TG increment in DM (8.0±1.0) than C rats (5.0±0.4) at equal A-CoAC activity. Data suggest a defect in clearance of circulating TG and/or saturation of muscle TG deposition in HS fed STZ-DM rats, correctable by FO.

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GLUCOSE TRANSPORTER FUNCTION AND MEMBRANE LIPIDS IN THE INSULIN RESISTANT HIGH FAT FED RAT.

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High saturated fat diets lead to insulin insensitivity; the mechanisms are unclear. We compared insulin sensitivity (75 mU insulin/h euglycaemic clamp in 24h fasted rats) and tissue glucose transporters in Wistar rats pair-fed for 4 weeks on a) a high fat diet (55% calories as fat, primarily lard), b) control diet (10% calories as fat). Fasting blood glucose and 3-3H-glucose turnover were similar. The area under the 2h blood glucose curve after 400 mg oral glucose was greater in fat-fed rats (11.74 $\pm$ 0.19 vs 10.87 $\pm$ 0.30 $mmol.l^{-1}.2h$, $p<0.05$). Clamp insulin levels were higher in fat-fed rats (153 $\pm$ 18 vs 100 $\pm$ 7 mU/l, $p<0.05$). Glucose requirement to maintain blood glucose at 4.0 mmol/l from +120 to +150 min of the clamp was 37% lower in fat-fed rats (116 $\pm$ 7 vs 184 $\pm$ 15 µmol/min/kg, $p<0.005$) as was 3-3H-glucose turnover ($p<0.001$); hepatic glucose output did not differ (fat-fed, 5 $\pm$ 10, controls, 8 $\pm$ 6 umol/min/kg). Adipocyte 14C-glucose uptake at 100nM insulin was not significantly lower in fat-fed rats (0.240 $\pm$ 0.018 vs 0.298 $\pm$ 0.028 fmol/h/cell, $t=1.746$, $0.05<p<0.1$); insulin concentration necessary for half-maximal stimulation was similar. Adipocytes from fat-fed rats had 30% fewer glucose transporters (Glut 4); insulin-induced translocation to the plasma membrane was 34% lower. Muscle transporter numbers were normal. Membrane lipid fluidity (heart, muscle) was similar but the cholesterol:phospholipid ratio was reduced ($p<0.05$). The mechanisms for insulin insensitivity may differ in muscle and fat. Glucose transporter depletion may explain insulin insensitivity in fat; altered membrane composition could affect their function in muscle and heart.

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FISH OIL INTO HIGH LARD DIET PREVENTS OBESITY, HYPERLIPEMIA AND ADIPOCYTE INSULIN RESISTANCE, IN RATS.

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High fat diet close to Western human diet induces hyperlipemia, obesity and adipocyte insulin resistance in rat. The aim of this work was to examine whether the inclusion of fish oil into high fat diet prevented these deletorious effects. Rats were fed 16 days with diets containing (by cal.) 10% fat (C) or 50% fat from: lard (HL), lard 35% + corn oil 15% (CO), lard 35% + fish oil 15% (FO), with 20% protein and 70% or 30% starch. In addition to adipocyte insulin responsiveness and the expression of Glut 4 in total membranes, we examined fatty acid synthase (FAS) expression in adipose tissue and liver. As compared to C: 1) Body weight, insulinemia and glycemia were unchanged by high-fat diets; 2) triglyceridemia, cholesterolemia and adipose tissues weights were higher in HL and CO but lower in FO; 3) adipocyte glucose transport responsiveness to insulin was decreased in HL and CO but restored in FO, in good agreement with diet effects on glut 4 expression; 5) adipose tissue FAS was decreased by 70% in HL and CO but unchanged by FO; in contrast, hepatic FAS reduction was larger in FO (85%) than in HL and CO (75%). In conclusion, the replacement of 30% of lard by FO: A) has differential metabolic effects on liver and adipose tissue. B) prevents high fat diet induction of obesity, hyperlipemia and adipocyte insulin resistance.

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ENTEROINSULAR AXIS ACTIVATION BY GLUCOSE AND FAT IN TYPE 2 DIABETICS COMPARED TO NORMAL CONTROLS

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To compare the effect of glucose and fat on the function of the Enteroinsular Axis in diabetics and normals, we studied 4 Groups of 6 subjects each, A and C Type 2 patients, B and D normals. A hyperglycaemic clamp at 17.8mmol/l was done for 90'. The 30' glucose (30g) was given orally in Groups A and B, while olive oil (30g) in C and D. Plasma Glucose, Triglycerides, Insulin and C-peptide were measured every 5'. Between 15'-30' Insulin and C-peptide levels remained stable. In Groups A and B Insulin abruptly increased the 35' and levelled the 50'. Insulin 30'vs50' 210±60vs760±360pmol/l, $p<.05$, in A and 200±60vs 1100±445 pmol/l, $p<.05$, in B. The Insulin incremental area between 30'-90', AvsB, was 210846±66650vs414800±112265pmol/min, $p<.05$. In Groups C and D Insulin increased the 35' but levelled the 75'. Insulin 30'vs75' 105±20vs201±80pmol/l, $p<.05$ in C, 359±170vs950±310 pmol/l, $p<.05$, in D. The incremental area between 30-90', CvsD, 37820±10200vs 303040±101460pmol/min, $p<.05$. C-peptide changes paralleled those of Insulin, while Triglycerides remained stable throughout. Conclusions: Enteroinsular Axis activation after oral glucose is defective in Type 2 diabetics compared to controls. This difference is even greater with fat as the oral stimulus. Enteroinsular Axis hypofunction may contribute to the pathogenesis of Type 2 diabetes.

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EFFECTS OF DIETARY MONOUNSATURATED FATTY ACIDS ON SERUM LIPIDS IN TYPE 2 DIABETES MELLITUS.

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Recent studies in non-diabetic subjects have shown that monounsaturated fatty acids (MUFA) may have a hypolipidaemic effect equal to that of polyunsaturated fatty acids (PUFA) without reducing HDL-cholesterol. We compared the effects on plasma lipids of 3 diets high in MUFA, PUFA and saturated fatty acids (SFA) in 22 patients with Type 2 diabetes (16 experimental, 6 control). After a high carbohydrate/fibre, low fat baseline diet for 2 weeks, all continued on the baseline diet for 18 weeks with the 16 experimental patients isocalorically given fat high in MUFA, PUFA and SFA, each for 6 weeks in random order. LDL-cholesterol levels were similar on PUFA and MUFA (mean±SEM 3.63±0.22 and 3.56±0.22 mmol/l, respectively), and significantly lower than on SFA (4.03±0.20 mmol/l, $p<0.01$). HDL-cholesterol levels did not differ significantly, though highest on MUFA and lowest on SFA. The ratio of HDL-cholesterol to LDL-cholesterol was significantly higher on MUFA (0.40±0.04) than on SFA (0.34±0.02, $p<0.05$). Ratios on PUFA (0.36±0.03) were not significantly different. Control plasma lipids remained unchanged throughout. MUFA appear as effective as PUFA in producing a more favourable lipid profile than SFA in Type 2 diabetes mellitus.

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THE INFLUENCE OF DIETARY FATTY ACIDS ON POSTPRANDIAL LIPID LEVELS IN TYPE 2 (NON-INSULIN-DEPENDENT) DIABETIC PATIENTS.

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Dietary fish oil (FO) and linoleic acid (LA) lower serum triglycerides and cholesterol levels, respectively. Postprandial, meal- derived chylomicrons (CM) are the main triglyceride containing particles converted into chylomicron remnants (CMR). We studied the influence of FO addition to a LA enriched diet on postprandial levels of CM and CMR in 12 Type 2 diabetic subjects. During a randomized double blind trial (4 months) a LA enriched diet (P/S 1.0) with daily either 6 g corn oil (CO = placebo) or 3 g FO capsules(1800 mg EPA, 1200 mg DHEA) was consumed. Before and after 4 months a LA enriched testmeal (P/S 1.0) was given, supplemented with vitamin A (27.000 IE/m2 body surface area) after a 12 hour fast. Chylomicron retinyl palmitate (CM.RP) and chylomicron remnant retinyl palmitate (CMR.RP) were measured at 1-2 hourly intervals for 12 hours. In both groups area under curve (AUC±SD)of CMR.RP and peak(±SD)of CMR.RP were lower at 4 months than at baseline but not significantly different from each other: AUC CMR.RP: FO+LA: -1296 (±3246),LA: -1030(±1329)(nmol/lx12h) p=0.87, Peak CMR.RP: FO+LA: -1387(±745),LA: -1162 (±689)(nmol/l) p=0.61) In the FO+LA group the AUC CM.RP was significantly lower than in the LA group: -3959(±4391)and +1163(±1554)(nmol/lx12h)p=0.03 and the peak CM.RP tended to be lower: FO+LA: -524(±683)and LA: +208(±424)(nmol/l) p=0.06. (two-sample t-test). The chronic addition of FO to a LA enriched diet lowers postprandial chylomicron levels while chylomicron remnant levels decreased in both groups. Thus dietary FO may beneficially influence the cardiovascular risk in type 2 diabetes.

OP 19
Immunology II

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NATURAL COURSE OF PRE-TYPE 1 DIABETES IN HIGH RISK INDIVIDUALS

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To characterize the prediabetic period we observed 58 siblings of newly diagnosed diabetic children, who were found to be positive for islet cell antibodies (ICA) and/or insulin autoantibodies in their initial blood sample taken within 6 months after the diagnosis of the index case. The siblings were followed for an average period of 48 months with sequential blood samples and intravenous glucose tolerance tests (IVGTTs). During that period 17 siblings presented with clinical signs of Type 1 diabetes. The duration of the known prediabetic period ranged from 0.5 to 48 months. The converters were younger than the unaffected siblings (7.1. vs. 9.9 years; $p < 0.05$) and had higher initial levels of conventional ICA and complement-fixing ICA (CF-ICA; $p < 0.01$). In addition they had lower first phase insulin response (FPIR; 1+3 min) in the first IVGTT ($p < 0.001$). Subsequently the converters had on all occasions higher ICA levels and lower FPIR ($p < 0.05$ or less) than the other siblings. In addition the glucose elimination rate was lower in the converters ($p < 0.01$ or less) from the third IVGTT onwards. No sign of further loss of ß-cell function could be observed in the converters after the first IVGTT. In the converters the CF-ICA levels increased up to 18 months ($p < 0.05$). Accordingly those siblings manifesting clinical Type 1 diabetes were characterized by young age, strong and increasing signs of islet cell-specific autoimmunity, reduced insulin secreting capacity and emerging glucose intolerance. The present observations do not support a linearly progressive ß-cell destruction in the prediabetic period.

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HIGH PREVALENCE OF ISLET CELL ANTIBODIES (ICA) IN HEALTHY SCHOOLCHILDREN IN SARDINIA.

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Recently, Sardinia has shown the second highest incidence of IDDM in Europe (Eurodiab survey). To confirm this unexpected trend, we assessed the prevalence of ICA in 2,632 unselected healthy schoolchildren (aged 6-14 yr). They were all of Sardianian ancestry and living in different villages of 3 of the 4 provinces in the island. To date, 1,894 sera have been analysed. Overall, ICA >5 JDF-U were detected in 4.42% and ICA >20 JDF-U in 0.97%. Divided geographically, they all showed differences.

	IDDM 100,000/yr 0-14 yrs	Individuals	ICA >5	(JDF-U) (%) 5-20	>20
CAGLIARI	27.5	1033	42 (4.06)	27 (2.61)	15 (1.45)
NUORO	30.6	470	21 (4.46)	18 (3.82)	3 (0.63)
ORISTANO	44.3	391	4 (1.02)	2 (0.51)	2 (0.51)

The Table shows that the Oristano province, that with the highest incidence of type I diabetes, had the lowest prevalence of ICA. In another 368 healthy individuals (aged 19-29) from the Sassari province (incidence 20.71/100,000/yr), the frequency of ICA was the highest observed in the 4 provinces (9%, ICA >5 JDF-U). The high IDDM incidence rates in Sardinia are consistent with these ICA data in a healthy young population in the island. However, the overall ICA prevalence and IDDM incidence distribution do not geographically overlap.

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FOLLOW-UP OF CHILDREN FROM BACKGROUND POPULATION WITH HIGH ICA TITRES.

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In 1990-91, ICA have been tested in 13 390 unselected school-children (age 6-17 yr) as part of a research program on risk factors for Type 1 diabetes in background populations. The first blood collection was assayed for ICA detection by the immuno-fluorescence technique using a single human pancreas and for HLA-DQ typing. 28 sera were found with ICA ≥20 JDFu (0.2%; group1) and 170 between 4-20 JDFu (1.3%; group 2). A significant ($p<0.01$) enrichment of alleles encoding for an alanine at the position 57 of the DQß chain was observed in the children with high ICA titres in comparison to ICA-negative children (group3 <4 JDFu)

n alleles \ groups ala 57 DQ B1	1 (n =12)	2 (n=76)	3 (n=93)
2	4 (33%)	7 (9%)	9 (10%)
1	7 (58%)	29 (38%)	40 (43%)
0	1(9%)	40 (53%)	44 (47%)

Among the 2 groups 95 (48%) children have been followed for a median duration of 12 mth. ICA titres were remarkably stable in the group 1, among which one boy has become diabetic; in the group 2, 3 children converted into values >20 JDFu, and 12 (15%) became negative among whom 11 were previously measured at the detection limit. This study emphasizes that 1) given the low incidence of the disease in France ICA would probably not be sufficient to identify school-children at risk for Type 1 diabetes 2) according to the DQ B1 alleles distribution, high ICA titres seem to be more informative like in 1rst degree relatives.

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PERSISTENT CELLULAR AND HUMORAL IMMUNE CHANGES IN THE PREDIABETIC PERIOD: A PROSPECTIVE TWIN STUDY.

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To test whether immune changes associated with Type 1 diabetes persist in the prediabetic period, we performed a 10 year prospective study of non-diabetic identical twins of recent onset patients with Type 1 diabetes; twins were followed to diagnosis of diabetes or for at least 6 years. Samples were tested for cellular (activation of T-lymphocytes (ATL)) and humoral (ICA and IAA) changes and compared with 22 controls. Of 19 twins, 9 developed diabetes, the 10 remainder have been followed for a mean of 10.2 years. The positive predictive value of increased levels of ATL'S (>6%), ICA (>4 JDF units) or IAA at referral was 89%, 90% and 100% respectively. All 9 prediabetic twins had either cellular or humoral changes detected in every sample (ATL's 29/32; ICA 38/39; IAA 2/39 samples). In twins remaining non-diabetic ATL's, ICA and IAA were rarely detected (1/41, 6/44, 0/44 samples respectively), and none had immune changes throughout the study-period ($p<0.001$). In summary, once induced the immune process leading to Type 1 diabetes is both predictive and persistent, not intermittent. These observations are consistent with a brief, not a prolonged, period of induction of this process.

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ß-CELL SPECIFIC T-CELL CLONES FROM PERIPHERAL BLOOD OF TYPE I DIABETES PATIENTS.

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T-lymphocytes appear to play an important role in the pathogenesis of type I Diabetes. Recently we have screened several newly diagnosed patients for T-cell reactivity against a preparation containing the ß-cell specific antigen of Mw 38 kDa. We found that reactivity could be detected in the majority of T-cell lines derived from peripheral blood mononuclear cells of these patients. In order to identify the fine-specificity of these T-cell lines, we have isolated T-cell clones from a number of these patients. We obtained T-cell several clones directed against 38 kDa protein present in insulin-secretory granules (ISG). In addition, this study reveals other ß-cell specific proteins that are recognized by $CD4^+$, HLA-DR restricted T cell clones from patients. The exact nature of these proteins remains to be determined. Cloning of ß-cell specific T-cell lines appears to be helpful to identify possible autoantigens and in turn enables us to study the characteristics of T lymphocytes that may be involved in the destruction of ß-cells.

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ELEVATED CIRCULATING ADHESION MOLECULES IN TYPE 1 DIABETES AND SUBJECTS AT RISK OF DIABETES

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We recently described the reduced expression of adhesion molecules on monocytes in Type 1 diabetes. We now have analysed for abnormalities in the production of shortly discovered circulating forms of ICAM-1 (cICAM-1) and L-selectin (cL-selectin). Groups of 14 patients with recent onset Type 1 diabetes and 33 first degree relatives (27 ICA-negative, 6 ICA-positive) were compared with 100 healthy blood donors. cICAM-1 and cL-selectin were measured by specific "sandwich" ELISA. Abnormal high levels of adhesion molecules (above 2 SD of the normal range) were found in 10 of 14 patients with recent onset Type 1 diabetes ($p < 0.0001$) and in all of the first degree relatives with islet cells antibodies (ICA). Most interestingly, elevated adhesion molecules levels in ICA negative relatives identified those with high genetic risk of Type 1 diabetes. I.e., all of 14 relatives with HLA DR 3, 4 had abnormal cL-selectin levels (mean 2.5 ± 0.8 ng/ml) while of the remaining 13 relatives with other HLA types none had abnormal levels (mean 0.8 ± 0.2 ng/ml, $p< 0.0001$). Normal blood donors with HLA DR 3 or 4 did not have elevated cL-selectin levels. We conclude that circulating adhesion molecules represent a new marker of Type 1 diabetes which identifies persons at genetic risk independent of ICA status.

OP 20
Epidemiology II

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MORTALITY EXPERIENCE OF TYPE 1 (INSULIN-DEPENDENT) DIABETES MELLITUS 1940-1991

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A population-based mortality study of 845 (M460:F385) Type 1 (insulin-dependent) diabetics diagnosed aged<17 years from 1940-89 was determined 31 December 1991 representing 14,039 person-years of risk. Trends in relative risk of death by calendar date were investigated using Cox Proportional Hazards Modelling. Age, sex and calendar time adjusted Standardized Mortality Ratios (SMR) were estimated using General Linear Modelling. Median age at diagnosis was 10 years (range 3 months - 16 years); median duration of diabetes of 14 years (range 1-51). Forty patients died (4.7%); median age at death 31 years (range 11-51). A further four deaths occurred at onset from ketoacidosis (excluded from analysis). Adjusting for age there was evidence of a decline in the risk of death with calendar year of diagnosis. The maximum likelihood estimate of the linear decline in risk with calendar date was equivalent to a 40% fall in risk per decade (95% CI, 17-58%, p=0.005). By dividing date of diagnosis into five decades and treating the resulting variables as being categorical, it was shown that between 1940-49 and 1980-89 the risk of death fell by an estimated 93% (95%CI, 34-99%). Between 1940-49 and 1980-89 the SMR fell from 519 (95% CI, 307-876) to 119 (95% CI, 17-843), representing an estimated linear decline in SMR/decade of 24% (95% CI, (1-42%; p=0.035). These findings show that the prognosis for Type 1 (insulin-dependent) diabetes mellitus has improved over this era.

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A DRAMATIC CHANGE OF MORTALITY PATTERN IN JAPANESE IDDM

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The aim of the present study is to monitor a population-based IDDM cohort in Japan to identify the change in the mortality pattern in the recent years. A total of 1428 (male 574, female 854) IDDM with onset age <18 yrs, who were diagnosed between 1965-1979 were followed up and the living status as of 1-1-1990 was determined. Of 1428, 1367 (95.8%) were traced and 90 cases were deceased with the mean age at death of 24 ±6 yrs, and the duration of IDDM of 15 ±4 yrs. The mortality rate was 5.7 per 1,000 person-yrs for both sexes, a 9.1-fold increased risk of dying compared to the general population. The 10-, 15-, 20-, and 25-year survival were 98%, 95%, 91% and 83%, respectively. Of 90 deceased cases, 27 died due to diabetic renal disease, 21 by DKA, 12 by violence/accident, 11 by infection, 5 by CHD/CVD, 4 by hypoglycemic coma, 10 by others/unknown. The risk of all cause and renal disease mortality appeared to decline in the 1980's compared with the 1970's (all cause:2.2 vs 7.1/1,000, renal:0.13 vs 1.13/1,000). After 1985, the number of death due to DKA decreased and accidental/macrovascular deaths increased. The dramatic change in mortality pattern observed among Japanese IDDM may attribute to better medical/social circumstances and adequate access to renal dialyses in the recent years.

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THE MORTALITY OF PATIENTS WITH TYPE 1 DIABETES MELLITUS IN NORWAY, 1973-1991.

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The aim of the study was to determine the mortality in diabetes patients in Norway. The mortality status of all subjects with Type 1 diabetes mellitus diagnosed from 1973 through 1982 and age at diagnosis below 15 years, was determined September 1st, 1991. Of the 1914 cases included in the follow-up, 27 were deceased. A two-fold increase of risk for early mortality was found in this cohort compared to the background population (SMR=238). The crude mortality rate was 107/100,000 personyears. A review of death certificates, patient records and autopsy reports revealed that accidents and suicides accounted for the majority of the deaths (10/27). Acute diabetic complications were the cause in 5/27; one cardio-vascular death was observed; diabetic renal disease did not cause any death. Mors subita was an important cause of death in these young individuals (8/27). In 3 out of these 8 cases, hypoglycemia was noted at the death certificate as a possible underlying cause, and in another 3 cases the circumstances could indicate hypoglycemia. The premature mortality in diabetes patients needs further investigation.

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MORTALITY FROM SUICIDE IN A COHORT OF DANISH MALES WITH TYPE 1 (INSULIN-DEPENDENT) DIABETES MELLITUS.

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We investigated the mortality from suicide in the cohort of all Danish males with Type 1 (insulin-dependent) diabetes mellitus born 1949-1964 (incl), with diagnosis established before age 20 years (n=1682). Follow-up from diagnosis to death or Jan. 1, 1991 was based on record linkage with the national population register and supplemented with information from death certificates obtained from the Danish National Registry of Deaths; from published vital statistics, cause-specific standardised mortality ratios (SMR), adjusted for age an calendertime, were calculated. Among 168 deaths recorded during follow-up, 12 were officially classified as suicide (SMR 12/7.48 = 1.6, $0.05 < p < 0.1$); for the age group 20-24 years SMR was 2.98 $p < 0.005$. Furthermore, all deaths officially classified as due to unknown causes (n=28) and accidents (n=22) were re-classified according to standardised suicidological criteria; for deaths from unknown causes, 3 could be re-classified as probable and 2 as possible suicide, whereas 1 of the deaths due to accidents could be re-classified as possible suicide. We conclude that (1) young males with Type 1 (insulin-dependent) diabetes mellitus may confer a higher risk of committing suicide than expected, and (2) suicide may present an underestimated cause af death among patients with Type 1 (insulin-dependent) diabetes mellitus.

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MORTALITY FROM CORONARY HEART DISEASE IN NON-INSULIN-DEPENDENT DIABETIC AND NON-DIABETIC SUBJECTS

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Our aim was to compare the mortality from coronary heart disease (CHD) in non-insulin-dependent diabetic (NIDDM) and non-diabetic subjects in areas representing a relatively low (West Finland) or high (East Finland) CHD incidence in the general population. CHD and its risk factors were examined in 1982-1984 in 510 NIDDM patients and 649 non-diabetic subjects aged 45-64 yr in East Finland, and in 549 diabetic and 724 non-diabetic subjects of the same age in West Finland. Mortality by January 1, 1990 was analyzed using death certificates, autopsy data and hospital records. Figures for age-adjusted cardiovascular mortality were in men: East Finland, diabetic 20.2%, non-diabetic 6.8% ($p<0.001$); West Finland, diabetic 15.5%, non-diabetic 4.1% ($p<0.001$). The respective figures in women were: East Finland, diabetic 14.0%, non-diabetic 0.6% ($p<0.001$); West Finland, diabetic 8.6%, non-diabetic 0.3% ($p<0.001$). Age-adjusted mortality from CHD was in men: East Finland, diabetic 17.8%, non-diabetic 4.5% ($p<0.001$); West Finland, diabetic 12.7%, non-diabetic 3.2% ($p<0.001$). The respective figures in women were: East Finland, diabetic 8.2%, non-diabetic 0.6% ($p<0.001$); West Finland, diabetic 5.6%, non-diabetic 0.0% ($p<0.001$). The results suggest that NIDDM accelerates CHD independently of possible genetic and environmental factors which are responsible for regional differences in CHD incidence in the back-ground population.

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EXCESS MORTALITY IN TYPE 2 DIABETIC PATIENTS WITH MINIMAL ELEVATION OF ALBUMIN EXCRETION

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In 1983-4 urinary albumin excretion rate (AER)was measured in 525 Type II (non-insulin-dependent) diabetic patients. In 1992 we determined the fate of those with abnormal AER (>10.6 μg/min) in a timed, overnight collection, n=174) compared to patients with normal AER (n=226) matched for age, sex and duration of diabetes. Of the abnormals 43.7% were alive, 51.7% dead and 4.6% unknown at follow-up vs 63.5% alive, 29.7% dead and 6.8% unknown in the control group ($x^2=20.9$, $p<0.001$). In the group with abnormal AER those who died were older at study onset (71±1 vs 63±1 years, $p<0.001$) with longer duration of diabetes (11.8±0.9 vs 7.6±8 years) and higher HbA_1 (11.4±0.4 vs 9.7±0.5%), $p<0.01$) than those alive. There was an increase in mortality in the group with minimal elevation of AER, (10.6-30μg/min; n=92) compared to matched controls with AER <10.6 μg/min (n=123); 48 alive, 41 dead, 3 unknown vs 82 alive, 34 dead, 7 unknown, $x^2=17.02$, $p<0.001$. We conclude that abnormal AER below the accepted level of microalbuminuria is associated with increased mortality in Type II diabetes, suggesting that current definitions of microalbuminuria should be revised.

OP 21
Pregnancy in Animals

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A NEW MODEL FOR IMPAIRED GLUCOSE TOLERANCE DURING PREGNANCY: MACROSOMIA IN STREPTOZOTOCIN-INDUCED DIABETIC RATS WITH ISLET-TRANSPLANTATION

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This study was undertaken to develop a new animal model for gestational diabetes and mild gestational hyperglycemia. Six female Wistar rats were rendered diabetic with streptozotocin. Iso-transplantation was performed intraperitoneally(islets from 13 newborn rat pancreases to one recipient). Their blood glucose was reduced and they showed mild hyperglycemia. The rats were then mated. Eight age-and weight-matched normal rats were used as control. An oral glucose tolerance test was performed at day 10 of gestation. Blood glucose levels in the islet-transplantation group were significantly higher than those of the control group at 60 minutes (209±43 vs 133±15mg/dl, respectively, $p<0.001$) and 120 minutes (147±21 vs 105±10mg/dl, respectively, $p<0.001$). The number of fetuses of the transplanted group was not significantly different from the control. Fetal body weight was significantly heavier in the islet-transplanted group(6.6±0.4 vs 5.9±0.3 g respectively, $p<0.001$). These results suggest that the islet-transplanted rat may serve as a model for impaired glucose tolerance and mild diabetes during pregnancy. This animal model may be useful in investigating the mechanism of macrosomia in fetuses from rats with impaired glucose tolerance during pregnancy.

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MORPHOLOGY OF PANCREATIC ENDOCRINE CELLS IN NEONATAL MACROSOMIA FROM STREPTOZOTOCIN-INDUCED DIABETIC RATS WITH ISLET-TRANSPLANTATION

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Newborns from Wistar rats with streptozotocin-induced diabetes which underwent islet-transplantation exhibited macrosomia(mean birth weight 7.44±0.54g n=9, vs newborns from normal rats 5.8± 0.32g n=8). Sections of pancreatic tissues from the newborns were immunostained for insulin (B cells), glucagon(A cells) and somatostatin (D cells). The B cells were examined for insulin under an immunoelectron microscopy. Quantitative morphological analysis was performed on the endocrine pancreas by automatic image analyzer. The percentages of islets and the fractional area of B and A cells in the pancreatic tissues were significantly higher in macrosomic newborns compared with the controls ($p<0.01$ respectively), but the fractional area of D cells and relative ratio of A/B cells was not significantly different in both groups. The volume density of granules and mitochondria in the B cells of macrosomic newborns was significantly higher than that in controls ($p<0.05$ respectively). These results suggest that hyperplasia and the hyperfunctional state of B cells in macrosomia may accelerate fetal growth. The morphological changes of the endocrine pancreas in macrosomic newborns from mildly hyperglycemic rats during pregnancy were similar to those seen in human diabetic pregnancy.

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GLUCOSE TRANSPORTER GENE EXPRESSION IN RAT EMBRYO AND ITS REGULATION IN THE DIABETIC STATE.

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We have shown that hyperglycemia-induced embryopathy could be mediated by myo-inositol depletion of embryo as a result of increased accumulation of glucose (Diabetologia 33: 597, 1990). The present study was performed to investigate glucose transporter (GLUT) expression during early organogenesis and its regulation in the diabetic conditions. Both GLUT-1 mRNA and protein, by Northern and Western blot analysis, were highly expressed in embryo during early organogenesis, reaching peak levels on day 10 of gestation (the main period of neural tube formation). Immunohistochemical staining showed that GLUT-1 protein was predominantly distributed in the tissue of neural tube and noted to be localized at the plasma membrane of the neuroepithelial cell. High expression of GLUT-1 mRNA and protein levels persisted in the embryo on day 10 even in the diabetic conditions in both streptozotocin-induced diabetic rats (4.6±0.6 mg/ml) and conceptus cultured from day 9 for 24 h with hyperglycemic media (4.2 mg/ml). Furthermore, the diabetic states did not change also the staining intensity and cellular distribution of the GLUT-1 protein in the neural tube. High expression of GLUT-1 in the neural tube during the period of neurulation despite the diabetic environment may play a role in hyperglycemia-induced neural tube defects, by permitting increased entry of glucose into neuroepithelial cells, leading to myo-inositol depletion of embryo.

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GENERATION OF FREE OXYGEN RADICALS IN EMBRYOS OF DIABETIC PREGNANCY.

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The aim of the study was to evaluate the possible role of free oxygen radicals in the embryonic maldevelopment of diabetic pregnancy. Rat and mouse embryos from *in vitro* culture (gestational days 9-11), as well as embryos of diabetic rats (days 11-13) were examined. We found severe malformations and growth retardation in rodent embryos after *in vitro* culture in elevated concentrations of glucose (30-50 mM), β-hydroxybutyrate (2.5-10 mM) and α-ketoisocaproate (1-3 mM), individually as well as combined. The deleterious effects on embryonic development by all these agents was alleviated by addition of superoxide dismutase (SOD) to the culture medium. The findings suggest that the teratogenicity of these compounds is coupled to generation of free oxygen radicals. Further support for this concept was the finding of increased mRNA levels of MnSOD and increased activity of SOD in embryos subjected to elevated glucose concentration in the culture medium, as well as in embryos of diabetic rats. The data suggest that *enhanced substrate-induced production of free oxygen radicals in embryonic tissues mediates the teratogenic effect of the diabetic environment.* The teratogenicity of the diabetic environment may result from the *combined influence of several disturbed metabolic parameters* in the mother and conceptus.

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MATERNAL HYPOGLYCEMIA DURING EARLY ORGANOGENESIS IMPAIRS EMBRYONIC GROWTH IN DIABETIC RATS. **K. Tanigawa, M. Kawaguchi and Y. Kato. Department of Internal Medicine, Shimane Medical University, Izumo, Japan**

The present study was designed to determine whether maternal hypoglycemia alters early organogenesis in normal and diabetic rat embryos. Female Wistar rats were injected i.v. with streptozotocin (45 mg/kg) 2-3 weeks before mating. On day 9.5 after conception, mother rats received saline or actrapid human insulin injection (400 mU) i.p. after fasting for 20 h. Hypoglycemia as low as 3.5 mmol/l was maintained for 60 min. Pregnancy was terminated on day 11.5 and embryos were examined. The number of somites was smaller in diabetic embryos than in normal embryos (25.9±0.2 vs. 26.7±0.1, $p < 0.005$), and hypoglycemia reduced somite number in both rat embryos. Crown-rump length (mm) was smaller in diabetic than in normal embryos (3.69±0.04 vs. 4.03±0.03, $p<0.005$). Maternal hypoglycemia significantly lowered crown-rump length in both embryos: 3.0% in normal and 4.3% in diabetic embryos, respectively. Protein content was also reduced in diabetic embryos compared with normal embryos (264±51 vs. 282±42 µg, $p < 0.005$). In addition, maternal hypoglycemia further lowered protein content in diabetic embryos (205±38 µg, $p < 0.005$), but this effect was less prominent in normal embryos. A teratogenic effect of maternal hypoglycemia was observed in diabetic embryos but not in normal embryos. These data indicate that maternal hypoglycemia during early organogenesis strongly impairs growth in diabetic embryo.

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ISLET TRANSPLANTATION IN DIABETIC PREGNANT RATS REDUCES THE DIABETOGENIC EFFECT IN THEIR OFFSPRING

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Diabetes of the mother during pregnancy induces alterations in the fetus, resulting in impaired glucose tolerance in the offspring. In youngsters of severely diabetic mothers, three hours glucose infusion gives a normal rise in plasma glucose but abnormally high insulin levels. This hyperinsulinemia is associated with hyperresponsiveness of the B-cells and insulin resistance (previous publications). In order to normalize maternal glycemia, isolated islets from neonatal rats were transplanted into the vena porta of severely hyperglycemic (Streptozotocin) rats at day15 of gestation. Strict glycemic control of the mothers was achieved throughout further gestation and lactation. In the adult offspring of these transplanted rats (SDTX) insulin levels during glucose infusion were significantly lower than in the offspring of sham-transplanted diabetic mothers (SD) and were not different from controls (CO).

µU/ml	0min.	30min.	60min.	180min.	180+60min.
CO (11)	18 ± 2	90 ± 7	86 ± 8	112 ± 12	33 ± 5
SD (16)	20 ± 2	164 ± 9*	173 ± 7*	183 ± 10*	52 ± 4*
SDTX (16)	25 ± 3	129 ± 18	103 ± 14	147 ± 25	37 ± 6

The work confirms that hyperglycemia of the mother during late gestation (the period of development of the endocrine pancreas and of the insulin-receptor systhem) is the inducing factor for the diabetogenic tendency in the offspring, since normalisation of this hyperglycemia eliminates its long-term consequences.

OP 22
Glucose Turnover

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REGULATORY ROLE OF BASAL INSULIN LEVELS ON GLUCOSE-MEDIATED GLUCOSE METABOLISM.
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The ability of acute hyperglycemia (HG) to promote glucose utilization and metabolism was studied in 6 normal females (32±3yo; BMI 23.4±1.2 kg/m^2) participating in two 240 min hyperglycemic (13.4 vs 13.7 mM; CV=2.4±.4%) clamps performed with somatostatin (500 µg/h), glucagon (0.5 ng/min·kg), 3-[^{3}H]- and U-[^{14}C]-glucose infusions, and indirect calorimetry. Basal insulin (0.1 mU/min·kg) was replaced only in one study (50 vs 22 pM; $p<0.001$). ^{3}H-glucose (20.5±1.3 vs 18.9±1.4) and ^{14}C-glucose Rd (20.0±1.4 vs 17.2±1.3 µmol/min·kg) were similar with and without basal insulin. With insulin replacement, exogenous glucose infusion (12.3±1.4 vs 6.7±2) was higher and hepatic G production lower (8.0±0.1 vs 11.4±1.3 µmol/min·kg; both $p<0.05$). In spite of similar glucose utilization, hyperglycaemia+insulin, as compared with no insulin replacement, was associated with higher glycolytic rate (^3H_2O generation; 14.4±0.2 vs 9.0±2.1), plasma glucose oxidation ($^{14}CO_2$ production; 11.2±1.6 vs 6.4±0.9), and total carbohydrate (from indirect calorimetry) oxidation (15.1±1.1 vs 7.0±0.6; all $p<0.05$). On the contrary, total non-oxidative carbohydrate metabolism (5.8±1.9 vs 11.6±1.7), non-oxidative plasma glucose metabolism (8.1±2.1 vs 10.8 ±.9), and Cori cycle (1±.3 vs 1.4±.3 µmol/min·kg) were lower ($p<0.05$). Without insulin replacement, hyperglycaemia was associated with higher plasma FFA levels (0.69±0.12 mM) and lipid oxidation (3.0±0.2 µmol/min·kg) than with hyperglycaemia+insulin (0.14±0.04 mM and 0.6±0.4 µmol/min·kg, respectively; $p <0.005$). In conclusion, basal insulin: 1) does not enhance promotion of glucose disposal by hyperglycemia; 2) is associated with lower HGP and Cori cycle; 3) diverts glucose disposal to glucose oxidation rather than non-oxidative glucose metabolism.

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GLUCOSE FLUXES AFTER AN ORAL GLUCOSE LOAD IN PATIENTS WITH TYPE 2 DIABETES OF VARIABLE SEVERITY.

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Since the relative contribution of the liver and peripheral tissues to postprandial hyperglycaemia in type 2 diabetes remains controversial, we reevaluated this issue using a dual isotope technique combined with indirect calorimetry. Glucose (G) kinetics was measured before and during 5 h following the ingestion of 75 g of G in 3 groups of subjects: Controls (C) (n=11), mildly diabetic (mD) (n=7) and severely diabetic (sD) (n=12) patients. The basal and mean 0-5 h G concentrations averaged respectively 5.5±0.2 and 6.8±0.2 mM in C, 6.5±0.2 and 10.2±0.6 mM in mD and 10.8±0.6 and 15.7±0.7 mM in sD. The amount of oral G appearing in systemic circulation was identical (88% of the load) in the 3 groups. Hepatic glucose production (HGP) was similar in C and mD in the basal state (80±5 *vs* 76±4 mg/m^2.min) and during the 5 h following G ingestion (38±3 *vs* 30±3 mg/m^2.min) but higher in sD under both conditions (108±5 and 54±3 mg/m^2.min; $p<0.001$). Mean 0-5 h tissular uptake was comparable in the 3 groups (~150 mg/m^2.min) whereas metabolic clearance rate was lower in sD (54±3 ml/m^2.min) and mD (78±4 ml/m^2.min) than in C (127±6 ml/m^2.min). In conclusion, the exaggerated postprandial G response characterizing type 2 diabetes results only from a removal defect in mD and from a combination of excessive HGP and impaired removal in sD.

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NORMAL SPLANCHNIC UPTAKE OF DIETARY GLUCOSE IN DIABETIC CIRRHOTIC PATIENTS.

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To evaluate the role of splanchnic uptake of dietary glucose on carbohydrate intolerance in liver cirrhosis, we have studied 6 diabetic cirrhotics withdrawn from their hypoglycaemic therapy, and 5 controls, both in the fasting state and during a 260-min mixed-meal continous administration (≈11 Cal/kg of BW, 50% glucose, 18% amino acids, and 32% fat). D-[6,6-^{2}H]glucose was infused i.v. to trace total glucose Ra as well as systemic appearance of both D-[2-^{3}H]-labelled and unlabelled glucose. In the cirrhotics fasting glucose concentration (11.4±1.3 mmol/L) and Ra (17.5±2.8 μmol/kg.min) were greater ($p<0.001$ and $p<0.04$) than in controls (4.4±0.2 mmol/L and 10.1±0.5 μmol/kg.min). After the meal, steady-state glucose levels were greater ($p<0.01$) in the cirrhotics (16.6±1.6 mmol/L) than in controls (7.5±0.4), but total glucose Ra was comparable in the two groups (20.1±1.6 vs 21.5±2.7 μmol/kg.min, respectively). Insulin increased from 16±3 to 45±10 μU/ml in the patients, and to a similar level in controls, i.e. from 10±3 to 69±21 μU/ml. Percent splanchnic uptake of dietary glucose was comparable in the cirrhotics (66±6%) and in controls (71±8%) (P = NS). Thus, peripheral insulin-resistance appears to be the major cause of post-prandial carbohydrate intolerance in liver cirrhosis.

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REGULATION OF HEPATIC GLUCOSE PRODUCTION BY INSULIN AND HYPERGLYCAEMIA IN DENERVATED HUMAN LIVER.

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To assess the role of hepatic innervation on the regulation of hepatic glucose production (HGP) during euglycemic hyperinsulinemia, hyperglycemia/hyperinsulinemia and hyperglycemia alone, 6 stable patients after Orthotopic Liver Transplantation (OLT: age=44±5 y; IBW=101±1%; transplant age=8±2 mo; prednisone=10 mg·day^{-1}; cyclosporin=5 mg·kg^{-1}·day^{-1}) 6 patients with Chronic Uveitis (CU) on the same immunosuppressive drugs and 6 controls (CON) were studied three times with the infusion of [3-^{3}H]glucose. Study 1: 1 mU·kg^{-1}·min^{-1} euglycemic clamp (SS glucose≈4.8 mM); Study 2: +4.5 mM hyperglycemic clamp (SS≈9 mM); Study 3: +5.5 mM hyperglycemic (SS≈11.5 mM) clamp plus somatostatin infusion (337.5 μgr/hr), plus basal glucagon (0.4 ng·kg^{-1}·min^{-1}) and insulin (0.1 mU·Kg^{-1}·min^{-1}) replacements. Basal IRI (85±11 vs 56±6 vs 48±6 pM), C-PEP (0.96±0.3 vs 0.68±0.1 vs 0.67±0.3 nM) and glucagon (132±18 vs 93±12 vs 79±4 pg/ml) were higher in OLT vs CU and CON resp. ($p<0.01$). Basal HGP (1.9±0.1 vs 1.9±0.2 vs 2.0±0.2 mg·Kg^{-1}·min^{-1}) was similar in the 3 study groups. During the last hour of Study 1 plasma IRI (442±14 vs 430±15 vs 456±21 pM), C-PEP (0.27±0.2 vs 0.25±0.1 vs 0.24±0.1 nM) and glucagon (110±12 vs 91±7 vs 89±7 pg/ml) were similar in all groups; the suppression of HGP was defective in OLT (0.8±0.2) vs CU and CON (0.3 and 0.2±0.1)($p<0.01$). In Study 2 the insulin (FP: 286±25 vs 292±18 vs 250±18; SP: 280±16 vs 259±14 vs 262±12 pM) and C-PEP secretion (FP: 1.88±0.3 vs 1.75±0.2 vs 1.95±0.3; SP: 3.07±0.2 vs 3.31±0.2 vs 2.64±0.5 nM) were similar in OLT, CU and CON. Glucagon concentration decreased in OLT [139±7->75±28 pg/ml ($p<0.01$)], to the same value of CU and CON (p=NS). HGP was normally suppressed in all groups (60-120 min: 0.2±0.1 vs 0.1±0.1 vs 0.3±0.2 mg·kg^{-1}·min^{-1}). During Study 3 (60-120 min) plasma IRI (45±5 vs 40±4 vs 42±3 pM) and glucagon (85±7 vs 94±10 vs 85±5 pg/ml) were comparable (p=NS) and HGP was normally suppressed [0.5±0.2 vs 0.2±0.1 vs 0.3±0.1 mg·kg^{-1}·min^{-1}(p=NS)] in all groups. In conclusion, denervated liver show an altered regulation by insulin at euglycemia. In contrast, the modulation of HGP by hyperglycemia is maintained. These results further support the existence of intrinsic hepatic autoregulation by glucose in humans.

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INSULIN ADMINISTERED INTRAPERITONEALLY AND INTRAVENOUSLY SUPPRESS IDENTICALLY HEPATIC GLUCOSE PRODUCTION AT MATCHED SYSTEMIC LEVELS.

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The major potential advantage of intraperitoneal (IP) insulin administration is the restoration of a porto-peripheral insulin gradient. However, specific action of the latter on hepatic glucose production (HGP) is still debated. Four adult type I insulin-dependent diabetic patients underwent 2 randomly ordered, sequential, euglycemic, moderately hyperinsulinemic glucose clamps. The intravenous (IV) clamp consisted in 2h-insulin rates of 0.2, 0.4 and 0.6 mU/Kg/min, and the IP clamp in 3h-rates of 0.4 and 0.6 mU/Kg/min given into the chronic catheter of an implanted pump, via puncturing its sideport. HGP was measured isotopically using 6.6^{2}H glucose IV infusion. Peripheral insulin levels were 2-3 times higher with IV insulin, matching only for the 0.2 IV and 0.4 IP sequences : 16.5 ± 2.6 and 15.3 ± 1.8 mU/l respectively. Peripheral glucose uptake was 0, 1.0 ± 0.4 and 3.8 ± 0.9 mg/Kg/min during the IV clamp vs 1.0 ± 0.6 and 1.7 ± 0.3 mg/Kg/min during the IP clamp. HGP was 2.2 ± 0.2, 0 and 0 mg/Kg/min IV, vs 1.9 ± 0.2 and 0.7 ± 0.5 mg/Kg/min during IP sequences. Thus, glucose uptake as well as HGP were equivalent during IP and IV infusions when systemic insulin levels matched, rather than when insulin rates matched. These data suggest the primacy of systemic insulin over portal insulin in its restraint of HGP, and thus no specific advantage of IP over IV insulin administration.

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SUSTAINED RESPONSE OF HEPATIC GLUCOSE PRODUCTION (HGP) TO GLUCAGON IN TYPE 2 DIABETIC SUBJECTS

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To establish whether increased HGP in NIDDM can be due not only to hepatic insulin resistance but also to increased hepatic sensitivity to glucagon we infused 7 NIDDM and 8 matched non diabetic subjects with glucagon at 4 different infusion rates (0.2, 1, 6 and 8 ng/kg/min) while endogenous insulin and glucagon secretion were blocked by somatostatin infusion. Insulin was infused throughout the study at a rate able to maintain euglycemia before glucagon infusion. Each subjects was studied on 4 occasions: glucagon was infused at a constant rate for 4 hrs and HGP was measured by 3-^{3}H-glucose infusion. HGP increased sharply in both groups during the first 2 hrs of glucagon infusion (maximum HGP integrated during 0-120 min = 1.3±0.1 vs 1.5±0.2 mg/kg/min in NIDDM and non diabetic respectively, p=n.s.). No difference was observed in the ED_{50} glucagon dose for HGP response during 0-120 min. (0.78±0.13 and 0.88±0.19 ng/kg/min, p=n.s.). However, during the last 2 hrs of the study, in NIDDM HGP remained elevated above baseline at all the 4 glucagon infusion rates (integrated HGP =0.38±0.14, 0.63±0.22, 0.79±0.25 and 0.80±0.25 mg/kg/min) while in the non diabetic subjects it returned back to baseline (integrated HGP= 0.03±0.01, -0.06±0.15, -0.39±0.41 and -0.19±0.23 mg/kg/min). We conclude that in NIDDM HGP fails to return to baseline during prolonged glucagon infusion as it happens in non diabetic subjects. This ability of glucagon to stimulate a sustained increase in HGP might play a role for the increased HGP observed in NIDDM.

OP 23
Insulin Secretion II

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ARACHIDONIC ACID-INDUCED PROTEIN PHOSPHORYLATION IS NOT REQUIRED FOR ITS EFFECT ON INSULIN SECRETION.

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We have previously reported that arachidonic acid (AA) induces the phosphorylation of an 18kDa protein in electrically permeabilised rat islets of Langerhans. This protein is not a substrate for calcium calmodulin-dependent protein kinase (PK), PKA or PKC. We have now investigated whether this effect can be attributed to unmetabolised AA and whether AA-induced phosphorylation is required for insulin secretory responses to AA. 100μM AA induced the phosphorylation of the 18kDa protein in the presence of both the lipoxygenase inhibitor nordihydroguaretic acid (NDGA,100μM) and the cyclooxygenase inhibitor indomethacin (10μM) suggesting that this phosphorylation was a result of AA itself. 50nM staurosporine had no effect on AA-induced ^{32}P incorporation into the 18kDa protein but, this concentration of staurosporine markedly inhibited PKC activity extracted from islets (control, 100.4$\pm$6.7; +50nM staurosporine, 20.8$\pm$2.1 fmol/islet/min, n=3, $p<0.01$). Complete inhibition of the AA-induced phosphorylation was observed with a concentration of staurosporine (200nM) which is known to inhibit other protein kinases. However, 200nM staurosporine had no effect on AA-induced insulin secretion in the presence of a substimulatory glucose concentration (2mM glucose, 0.13$\pm$0.07; +100μM AA, 1.42$\pm$0.19; +200nM staurosporine, 1.74$\pm$0.19 ng/islet/h, mean$\pm$SEM, n=9, $p>0.2$). Furthermore, other fatty acids which are known to be present in islets such as oleic acid (OA, 100μM) and linoleic acid (LA, 100μM), also stimulated the phosphorylation of an 18kDa protein. However, these fatty acids had no effect on insulin secretion (+100μM OA, 99.1$\pm$12.3; +100μM LA, 68.2$\pm$9.0 %control, $p>0.1$) in experiments where AA clearly stimulated insulin secretion (+100μM AA, 291.3$\pm$64.7 %control $p<0.01$). These results suggest that AA and some other fatty acids can promote ^{32}P incorporation into the 18kDa protein, independently of PKC activation, and that the AA-induced phosphorylation is not required for insulin secretory responses to AA.

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MOLECULAR INTERACTION OF GLIMEPIRIDE (HOE 490) WITH THE SULFONYLUREA RECEPTOR

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Glimepiride (HOE 490) is a new orally active sulfonylurea for the treatment of Typ 2 (non-insulin-dependent) diabetes. The interaction of glimepiride with the sulfonylurea-receptor being part of an ATP-sensitive K^+-channel was investigated using ß-cell membranes prepared from rat ß-cell tumors. Glimepiride competed with the binding of [^{3}H]glibenclamide to ß-cell membranes showing an IC_{50}-value of 81$\pm$2 nM compared with 16 $\pm$ 5 nM for unlabeled glibenclamide and 12 $\pm$ 2 μM for tolbutamide. Photoaffinity labeling of the sulfonylurea - receptor of M_r 140 000 by [^{3}H]glibenclamide was inhibited by glimepiride in a concentration-dependent manner; half-maximal inhibition of labeling of the 140 kDa-protein was achieved with 10^{-8} M glimepiride compared with 3 x 10^{-8} M for glibenclamide and 6.5 x 10^{-5} M for tolbutamide. [^{3}H]Glimepiride can be used as a direct photoaffinity probe as shown by covalent labeling of albumin after irradiation with UV-light. Photoaffinity labeling of ß-cell membranes with [^{3}H]glimepiride resulted in a predominant labeling of a 65 kDa polypeptide. After solubilisation with CHAPS both, [^{3}H]glimepiride and [^{3}H]glibenclamide were highly incorporated only into the 65 kDa-protein upon UV-irradiation. The labeling of the 65 kDa-protein was strongly inhibited by the presence of furosemide and tolbutamide whereas diazoxide showed no effect. These results suggest that two polypeptides of M_r 140 000 and M_r 65 000 are constituents of the sulfonylurea-receptor. In intact ß-cell membranes, glimepiride preferably binds to the 65 kDa-protein.

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GLUCOSE INGESTION CAUSES INCREASED FREQUENCY AND AMPLITUDE OF INSULIN PULSATILITY

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It is well established that in the fasting state insulin secretion is derived in part from intermittent secretory bursts (pulsatile secretion). However, the role of pulsatile insulin secretion towards the postprandial insulin response is unknown. We sought to address this question by studying dogs with chronically implanted portal vein catheters. Portal vein blood was sampled at one minute intervals for 60 minutes both before and following ingestion of 30 g of glucose. Time series data were analyzed for pulses by cluster analyses. During fasting portal vein insulin profiles demonstrated secretory bursts with an amplitude of 385 ± 63 pmol and frequency of 5.8 ± 0.3 pulses/hr. Following meal ingestion there was a dramatic increase ($P < 0.01$) in both amplitude (931 ± 217 pmol) and frequency (12 ± 1 pulses/hr) of insulin secretory burst activity. Deconvolutional analyses indicates that basal (nonpulsatile) insulin secretion was unaltered following meal ingestion, while the contribution of pulsatile insulin secretion increased from ~ 40% when fasting to ~ 85% postprandially. We conclude that increased postprandial insulin secretion is achieved mainly by both increased frequency and amplitude of insulin secretory bursts. Since pulsatile insulin secretion is impaired in patients with Type 2 diabetes mellitus, it is possible that loss of insulin pulsatility contributes to carbohydrate intolerance in this disease.

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SOMATOSTATIN INDUCTION OF PULSATILE INSULIN SECRETION IN TYPE 2 DIABETES MELLITUS: EFFECT ON INSULIN SENSITIVITY

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Eight Type 2 diabetic subjects were studied to determine whether restoration of regular pulsatile insulin secretion could improve insulin sensitivity and glycaemic control. A random, double-blind study comprised i.v. administration of either 20μg somatostatin (Study P) or 0.9% NaCl (Study C) pulse, over 10s, repeated at 16min intervals, for 14h overnight, followed by a 1h basal period, 15min insulin tolerance test (ITT) and 2h hyperglycaemic clamp. Somatostatin inhibited insulin output and induced regular endogenous pulsatile insulin secretion. Mean plasma insulin waveform obtained was identical to that of normal subjects. Maximal suppression of plasma insulin (45%) occurred at 6min 17s after injection and the short half-life of somatostatin allowed recrudence of insulin secretion and recovery to baseline within the pulse-administration interval of 16min. Oscillation amplitude was 2.65 mU/l (±31% mean). No regular cyclical oscillations of plasma insulin were observed after saline delivery. Somatostatin administration impaired overall β-cell function (Homeostasis model assessment, HOMA %B; Study C vs. Study P, 74 +7.2 -6.5 vs. 47 +6.9 -6.1 %, $P<0.005$) (mean ±SEM), and glycaemic control (fasting plasma glucose; 7.02 ±0.67 vs. 8.58 ±0.83 mmol/l, $P<0.005$). No significant reversal of insulin resistance was observed following induction of insulin pulsatility (HOMA %S; 36 +8.4 -6.8 vs. 39 +7.9 -6.5 %, NS), (ITT, glucose disappearance rate; 0.09 ±0.02 vs. 0.10 ±0.02 mmol/l/min, NS), (M/I ratio; 0.19 ±0.04 vs. 0.22 ±0.10 mg/(kg.min) per mU/l, NS). In Type 2 diabetic subjects, somatostatin induced regular cyclical oscillations of plasma insulin. Repeated delivery of a 20μg somatostatin pulse i.v. for 14h impaired β-cell activity, so that, despite restoration of pulsatile insulin secretion no reversal of insulin resistance was effected.

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PRESERVED INCRETIN ACTIVITY OF GLP 1 [7-36 AMIDE] BUT NOT OF SYNTHETIC HUMAN GIP IN PATIENTS WITH TYPE 2-DIABETES MELLITUS

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In Type 2-diabetes, the overall incretin effect is reduced. The present investigation was designed to compare insulinotropic actions of exogenous incretin hormones (GIP and GLP-1 [7-36 amide]) in 9 Type 2-diabetic patients (fasting plasma glucose 7.8 mmol/l; HbA_{1c} 6.3 ± 0.6 %) and in 9 age- and weight-matched normal subjects. Synthetic human GIP (0.8 and 2.4 pmol/kg/min over 1 h each), GLP-1 [7-36 amide] (0.4 and 1.2 pmol/kg/min over 1 h each), and placebo were administered under hyperglycaemic clamp conditions (8.75 mmol/l) in seperate experiments. Plasma GIP and GLP-1 [7-36 amide] concentrations (RIA) were comparable to those after oral glucose with the low, and clearly supraphysiological with the high infusion rates. Both GIP and GLP-1 [7-36 amide] dose-dependently augmented insulin secretion (insulin, C-peptide) in both groups ($p < 0.05$). With GIP, the maximum effect in Type 2-diabetic patients was significantly lower (by 54 %; $p < 0.05$) than in normal subjects. With GLP-1 [7-36 amide] Type 2-diabetic patients reached 71 % of the increments in C-peptide of normal subjects (difference not significant). Glucagon decreased during hyperglycaemic clamps in normal subjects, but not in Type 2-diabetic patients. GLP-1 [7-36 amide], but not GIP, lowered glucagon ($p < 0.05$) in both groups. In conclusion, in mild Type 2-diabetes, GLP-1 [7-36 amide], in contrast to GIP, retains much of its insulinotropic activity and reduces glucagon concentrations.

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EFFECTS OF CYCLOSPORINE ON INSULIN SECRETION AND GLUCOSE METABOLISM

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Type I diabetes being an autoimmune disorder is experimentally treated with cyclosporine(Cy) at its early stages.The data gathered on its effects on insulin secretion and glucose homeostasis are contradictory.To assess the potential effects of Cy on ß-cell function we studied insulin and C-peptide secretion during a mixed meal load in nondiabetic subjects who were renal transplant recipients on immunosuppressive treatment with cy (5mg/kg/day) +azathioprine (Az)+prednisone(P) (group I:7 patients aged 27.7±11.2 years) or Az+P(group II:11 patients aged31±10 years). The differences of daily doses of P and Az between the two groups were nonsignificant. Blood samples were taken at 0,15,30,45 and 60 minutes for glucose, insulin and C-peptide measurements.In group I glucose values were 4.1±0.9, 5.6±0.3, 6.2±1.0, 6.9±1.9, 6.8±1.6 mM, C-peptide values 0.25±0.06, 0.28±0.12, 0.31±0.12, 0.35±0.13, 0.34±0.18 nM and insulin values 81±47.4, 492±414 738±762 , 624±486 , 354±264 pM respectively. In group II glucose values were 4.4±0.8 ,6.2±0.8, 6.2±1.1, 5.4±0.4, 5.6±0.6 mM, C-peptide values 0.37±0.16, 0.5±0.2, 0.5±0.3, 0.57±0.26, 0.5±0.2 nM and insulin values 168±60, 582±612, 618±342, 480±264, 516±354 pM respectively. There were no statistically significant differences between the group receiving Cy treatment and the group not receiving Cy regarding glucose($p>0.05$), C-peptide($p>0.05$) and insulin($p>0.05$) at respective time points except the 0 time point of insulin measurement ($p=0.01$).We conclude that despite the data that cy impairs insulin secretion in islets and HIT cells, it appears that its longterm use does not result in disturbed glucose homeostasis.

OP 24
Effects of Dietary Lipids II

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THE ROLE OF DIETARY FATTY ACID CLASSES IN THE DEVELOPMENT OF OBESITY

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Modifications in membrane fatty acid (FA) composition, and insulin action, are possible through dietary intervention. Using diets of different FA compositions, with identical amounts of ω3 FA, we aimed to examine the metabolic fate of ω3 FA in male Wistar rats. Despite isocaloric feeding, weight gain was lower ($p<0.001$) on a more highly saturated (sat/ω3; 69±8 g) diet than on either a high oleic (ω9/ω3; 93±2 g) or high linoleic (ω6/ω3; 108±4 g) diet. Analysis of red quadricep FA composition revealed phospholipid ω3 levels in the sat/ω3 group (21.63±0.78%) to be significantly higher than in either the ω9/ω3 diet (17.68±0.62% $p<0.05$) or ω6/ω3 (15.34±0.65% $p<0.05$) groups. A similar pattern was observed in other muscles and white adipose tissue. A follow-up study using ^{14}C-ω3 in the diet showed increased ω3 FA incorporation in the sat/ω3 group and conversely decreased $^{14}CO_2$ production. These results demonstrate that metabolic fate of dietary FAs is strongly influenced by the overall FA profile of the diet. The functional consequences are seen in the differing rates of weight gain despite equal intakes, with ω3 FAs apparently protective against weight gain. Since obesity is a powerful predictor of insulin resistance these results have implications for dietary treatment of diabetes.

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BENEFICIAL EFFECTS OF FISH OIL-ENRICHED HIGH FAT DIET ON OBESITY AND HYPERLIPEMIA IN ZUCKER RATS.

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Fish oil inclusion into high fat diet has been shown to have beneficial effects on adiposity and lipemia in normal rats. We addressed here the question of the effects of fish oil-enriched high lard diet in Zucker rats which are genetically obese and hyperlipemic. Female Zucker rats, 6 weeks old were fed 8 weeks with diets containing (by cal.): 10% fat (C) or 50% fat from: lard (HL), lard 35% + fish oil 15% (FO), lard 35% + corn oil 15% (CO), with 20% protein and 70% or 30% starch. Insulin, triglycerides, cholesterol and adipose tissue weights in 4 anatomical depots were determined. Hyperinsulinemia of obese Zucker rats was not affected by any of the diets. Both hypercholesterolemia and hypertriglyceridemia were reduced by FO to the non obese rat values. Body weights were similar in the four groups. In contrast, compared to C adipose tissue weights were: increased by 10% in HL, unchanged in CO, and decreased by 20% in FO, with a differential effect in subcutaneous (-10%) and visceral (-30%) fat depots. In conclusion, in rats, genetically induced hyperlipemia and obesity, two risk factors frequently associated with non-insulin-dependent diabetes are markedly improved by high fat diet containing fish oil.

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POSITIVE EFFECT OF W-6 POLYUNSATURATED FATTY ACIDS ON GLUCOSE TOLERANCE IN HEALTHY SUBJECTS

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The effects of a diet high in w-6 polyunsaturated fatty acids (PUFA-diet) on glucose tolerance and serum lipids and lipoproteins were studied with a cross-over study design in 9 young healthy subjects randomized either to a PUFA-diet or a control diet high in saturated fat. The diet periods lasted 3 weeks and there was a 2 weeks' wash-out period between the diets. Both diets comprised 40 % fat. The polyunsaturated to saturated fatty acid ratio was 1.5 during the PUFA-diet and 0.25 during the control diet. Otherwise the diets were identical and they were composed of normal food items. Before and at the end of the diets an intravenous glucose tolerance test (glucose dose 300 mg/kg, venous blood samples at 10 min intervals for 90 min) was performed. Glucose tolerance remained unchanged during the control diet (glucose area 508 $\pm$ 63 vs. 524 $\pm$ 63 mmol/l·min, before vs. after, mean $\pm$ SD, N.S.) but it improved during the PUFA-diet (543 $\pm$ 71 vs. 497 $\pm$ 74 mmol/l·min, p = 0.037) without changes in the respective insulin areas. Glucose area correlated positively with the fasting level of free fatty acids before and after the PUFA-diet (r = 0.83, p = 0.003 and r = 0.57, p = 0.053, respectively). During the PUFA-diet there were significant decreases in serum total cholesterol (15 %, p = 0.012), LDL-cholesterol (20 %, p = 0.028) and total triglycerides (35 %, p = 0.010). HDL-cholesterol did not change. In conclusion, diet rich in w-6 polyunsaturated fatty acids improves glucose tolerance in young healthy subjects.

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Beneficial effects on blood pressure, lipid and carbohydrate metabolism of a high-monounsaturated fat diet compared with a high-carbohydrate diet in non-insulin-dependent diabetic (NIDDM) subjects.

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We compared the influence on blood pressure, glucose and fat metabolism of a diet rich in monounsaturated fatty acids (MUFA) with a high-carbohydrate diet (HCHO-diet) in a cross-over study in fifteen NIDDM subjects. The energy composition of the HCHO-diet was 50% carbohydrate and 30% fat (10% MUFA) and of the MUFA-diet 50% fat (30% MUFA) and 30% carbohydrate.
As compared with the HCHO-diet, the MUFA-diet reduced 24-hour systolic and diastolic blood pressure (130±10 vs 126±8 and 78±5 vs 75±6 mmHg, respectively (2P<0.03)).
Mean and peak blood glucose concentrations of the day-profiles following a standard carbohydrate rich diet during the MUFA-diet (7.6±0.5 and 9.9±0.6 mmol/L, respectively) were lower than during the HCHO-diet (8.4±0.6 and 11.3±0.7 mmol/L, respectively). In contrast, similar mean insulin, FFA, glucagon and triglyceride levels were found. Fructosamin levels decreased significantly during the MUFA-diet (from 353±10 to 334±10 µmol/L) but were unchanged during the CHO-diet (323±10 vs 323±10 µmol/L).
Both diets reduced total cholesterol and LDL-cholesterol, whereas HDL-cholesterol was unaltered. The LDL/HDL-ratio decreased during the MUFA-diet from 3.4±0.2 to 3.05±0.2 (p<0.05) but was not significantly influenced by the CHO-diet (3.37±0.2 vs 3.19±0.2).
Conclusions: A MUFA-diet has beneficial effects on blood pressure, glucose metabolism and lipoprotein composition in NIDDM subjects.

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OMEGA-3-FATTY ACIDS INCREASE LIPOLYSIS AND GLUCONEOGENESIS FROM GLYCEROL BUT NOT HEPATIC GLUCOSE PRODUCTION OR BLOOD GLUCOSE IN NIDDM

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Previous uncontrolled studies have suggested that dietary supplementation with omega-3-fatty acids may impair glycemic control in NIDDM although the incidence of NIDDM is remarkably low in populations where the use of fish oil is high. We performed a double-blind, placebo (6 grams of corn and 6 grams of olive oil daily for 6 weeks) controlled study in 9 NIDDM patients (age 53±4 years, BMI 30.7±1.8 kg/m^2) using cross-over design. Omega-3-fatty acid supplementation consisted of 2.16 grams EPA, 1.44 grams DHA and 8.16 grams other fish oils daily for 6 weeks. Lipids, serum fatty acid composition and in vivo rates of lipolysis, gluconeogenesis from glycerol and total hepatic glucose production (HGP) were determined before and at the end of placebo and omega-3-fatty acid supplementation. Serum EPA of cholesterol esters increased 5-fold from 1.3±0.3 to 6.5±0.8% (p<0.001) and DHA ~2-fold from 0.6±0.1 to 1.2±0.2% (p<0.001). VLDL-triglycerides decreased by 1.06±0.41 mM (~30%, p<0.05) during omega-3-fatty acid and by 0.24±0.18 (NS) during placebo supplementation. Serum HDL- and LDL-cholesterol concentrations remained unchanged. Plasma glucoses were similar basally (9.9±1.1 mM) and after omega-3-fatty acids (10.2±0.9 mM) and placebo (10.3±1.1 mM). After omega-3-fatty acids compared to placebo lipolysis (3.6±0.9 vs 2.7±0.4 µmol/kg.min) and gluconeogenesis from glycerol (2.8±0.6 vs 2.1±0.4 µmol/kg.min) were significantly increased whereas rates of HGP were similar. We conclude that omega-3-fatty acids produce favourable alterations in serum lipids without adversely affecting glycemia in patients with NIDDM. Although use of glycerol for gluconeogenesis increases in the liver (presumably because of inhibition of its use for triglyceride synthesis), HGP remains unchanged either because this increase is too small to affect HGP or because of hepatic autoregulation of glucose production.

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DIETARY FISH OIL ENHANCES PEROXIDATION OF SERUM LIPIDS IN TYPE 2 DIABETES MELLITUS.

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Lipid peroxides may be important in the development of vascular disease, a common cause of morbidity in Type 2 Diabetes Mellitus. We studied serum lipid peroxides in 22 Type 2 diabetic patients before and after dietary fish oil. At baseline fasting venous blood samples were obtained from patients and 21 matched controls for fluorimetric measurement of serum lipid peroxides. Patients received 5 capsules twice daily of fish oil or placebo (olive oil) in a double blind crossover fashion for 6 weeks, with a 6 week washout period. Lipid peroxides were reassessed following each treatment period. Results, expressed as malondialdehyde equivalents, were analysed at baseline using an unpaired T-test, and thereafter using analysis of variance. Mean serum lipid peroxides at baseline (with 95% confidence intervals) were significantly elevated in patients versus controls being 1.15 µmol/l (0.92 to 1.38) and 0.71 µmol/l (0.62 to 0.79) respectively (p < 0.01). Values following placebo were similar to baseline. After fish oil, lipid peroxides measured 1.77 µmol/l (1.41 to 2.13) being significantly greater than baseline or placebo results (p < 0.001). Thus elevated lipid peroxides in Type 2 diabetic patients are increased further by dietary fish oil. This potentially adverse effect may limit the therapeutic use of fish oils in such patients.

OP 25
Immunology III

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GLUCOSE REGULATION OF THE AUTOANTIGEN GAD IN HUMAN PANCREATIC ISLETS

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Glutamic acid decarboxylase (GAD) is a possible primary autoantigen in type 1 diabetes mellitus. In this study, we determined the effects of different glucose concentrations on the synthesis of GAD in human islet cells. Human pancreatic islets, isolated from adult cadaveric organ donors, were cultured for 72 h at 5.6, 11 or 28 mM glucose. Following culture, the islets were labelled with [^{35}S]methionine. Immune-complexes were formed by incubating lysates of islet cells with serum from a patient with newly diagnosed type 1 diabetes or with a polyclonal sheep anti-GAD serum. SDS-polyacrylamide gel electrophoresis was carried out and fluorography accomplished on dried gels. A single 65 kDa/GAD band was identified. The biosynthesis was strongly stimulated at the higher glucose concentrations, whereas the synthesis of the HLA class I proteins was not affected. In conclusion, increased activity of human islet cells seems to increase their antigenic expression. Possibly, such an increase exacerbates the auto-immune destruction of the β-cells in the early stages of insulin-dependent diabetes.

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GLUTAMIC ACID DECARBOXYLASE IN HUMAN ISLET : EVIDENCE OF ITS EXPRESION IN THE CYTOPLASM OF ALFA, BETA AND DELTA CELLS.

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The enzyme GAD is a major islet cell autoantigen whose expression was believed to be restricted to the beta cells. We have re-assessed the cellular distribution of GAD in human islets using double immunofluorescence staining of pancreatic cryostat sections and cytosmears and monolayer cultures of purified islet cells. GAD-6 MoAb (Developmental Studies Hybridoma Bank), polyclonal antibodies C-38 and 308 to GAD (kindly provided by Dr. Wu, University of Kansas) and antibodies to insulin, glucagon and somatostatin followed by the corresponding rhodamine or fluorescein labelled antisera were used. The staining of the three types of substrate demonstrated that GAD is expressed not only in the beta cells but also in alpha and in some delta cells. We identified 2019 islet cells from 37 smears; 86.0±8.4 (mean of percentage±SD) of the beta cells, 69.3±15.2 of the alpha cells and 27.2±4.6 of the delta cells were GAD positive. In conclusion, GAD is not a beta cell specific autoantigen in human islets. It remains to be established if one of the molecular forms of GAD is beta cell specific

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ABSENCE OF IMMUNE ABNORMALITIES IN THE PANCREASES OF NON-DIABETIC PATIENTS WITH LONG-STANDING GAD-ANTIBODIES

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Type 1 (insulin-dependent) diabetes is characterized by antibodies to islet cells (ICA) and glutamic acid decarboxylase (GAD-antibodies). Both ICA and GAD-antibodies can be detected several years before clinical onset of the disease, but not all individuals with these antibodies develop type 1 (insulin-dependent) diabetes. Lymphocytic infiltration and immune histological abnormalities of pancreatic islets are detected at and after clinical onset of disease. To determine whether these immune abnormalities are present in patients with long-standing ICA positivity, we examined pancreases from three ICA-positive patients with endocrine autoimmunity who died without developing type 1 (insulin-dependent) diabetes. All the islets analysed from each of the three pancreases contained normal content of the pancreatic hormones (pro)insulin, glucagon and somatostatin. There was no evidence of increased HLA class I or 'de novo' class II molecule expression on islet cells, and infiltration of T- or B-lymphocytes or macrophages were not detected. Characterization of antibodies in sera from the patients at death, and up to 10 years before death, revealed a β-cell-selective pattern of ICA, typical of GAD-antibody staining, and high levels of antibodies immunoprecipitating GAD from brain extracts. Two patients had antibodies to the 50 kD tryptic fragment of the 64 kD islet antigen but no antibodies to the 37/40 kD fragments. The third patient has not yet been tested. Thus, long standing GAD-antibodies can be detected in the absence of type 1 (insulin-dependent) diabetes and of cellular immune abnormalities in pancreatic islets.

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IMMUNOHISTOPATOLOGIC AND MOLECULAR STUDIES ON THE PANCREAS OF A NEWLY DIAGNOSED TYPE-1 DIABETIC PATIENT

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A pancreas from a diabetic patient who died two days after diagnosis was available for immunologic and molecular studies. Ricordi's digestion technique was applied and 128.000 islets were obtained. Snap-frozen tissue blocks were also prepared. Monolayer cultures and cryostat sections were stained by immunofluorescence to estimate the number of insulin containing cells, characterize the infiltrate and assess HLA class I, class II, ICAM-1 and GAD expression.
12.2±14.2 (mean % ± SD) of the islets contained insulin; 7.9±2.3 showed perinsulitis, lymphomonocyte phenotype frequency: $CD45^+$> $CD14^+$> $CD3^+CD8^+$ > $CD3^+CD4^+$. TCR V segment usage is under investigation using MoAbs, PCR and in situ hybridization. Preliminary results do not indicate preferential usage of any V segments though in one example one islet was surrounded by $V\beta5b^+$ lymphocytes. 40.3%±4.8 of the islets hyperexpressed HLA class I and all islets expressed GAD irrespective of insulin content. HLA class II and ICAM-1 "aberrant" expression is still under study but appears to be very sporadic. These results confirm most of the features of the pancreas in initial Type I Diabetes but HLA class II aberrant expression seems to be rare in this case while GAD is expressed even in islets devoid of β cells.

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BIOCHEMICAL CHARACTERIZATION OF A PROPOSED ISLET TARGET ANTIGEN: THE GM2-1 ISLET GANGLIOSIDE.

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The role as target antigen of the GM2-1 islet ganglioside is suggested by the observation that it specifically inhibits the ICA binding on pancreatic frozen sections and its expression parallels the ICA antigen(s) one in animal islets. We aimed to biochemically characterize the hydrophylic portion of this molecule exposed on the cell surface to immunological recognition. The GM2-1 fraction, extracted from human pancreas and isolated by preparative TLC, was digested with different neuraminidases to determine the sialic acid position. The sugars and the sialic acid constituing the hydrophylic portion were identified by Gas Chromatography after acidic methanolysis. The sialic acid type was identified as N-acetyl neuramic acid located in terminal position as suggested by the sensitivity of GM2-1 to the neuraminidases. The sugars were glucose, galactose and N-acetyl glucosamine. In conclusion these results, first evidence on the biochemical structure of GM2-1 show that it shares with LM1, a major target antigen in autoimmune demyelinating syndrome, the presence of a single neuramic acid in terminal position and glucosamine in addition to glucose and galactose. This particular structure may confere to these molecules a particular charge and conformation with antigenic potentiality.

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ISOLATION OF A HUMAN MONOCLONAL SECRETING ANTI-ISLET CELL AUTOANTIBODIES FROM CD5- B CELLS IN TYPE 1 DIABETES.

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To analyze the repertoire of several antibodies produced by *in vitro* stimulation of B lymphocytes in basis to the presence of CD5 antigen, we transformed with Epstein Barr Virus sorted CD5 + CD20 +, CD5-CD20 + and non-T cells from nine newly diagnosed type 1 diabetic patients and cultured in microtiter wells for 4-6 weeks. Culture supernatants from growing clones were screened by indirect immunofluorescence on cryostat sections of human pancreas. We isolated one human monoclonal autoantibody producing ICA, derived from CD5-CD20 + cells from a ICA positive type 1 diabetic child 5 days after the diagnosis. The isotype of this monoclonal (MB91) was IgM. Double immunofluorescence on both fixed cryostat sections and cytocentrifuged mechanical cell dispersions of normal human islets revealed that MB91 was positive for insulin and glucagon but negative for somatostatin containing cells. MB91 didn't stain for human thyroid, brain, adrenal gland and rat stomach, liver and kidney. It was also negative for monkey, rat and mouse pancreas. Binding of MB91 disappeared when human pancreas was treated with chloroform methanol, but this binding was unaffected when treated with pronase.This human monoclonal antibody will be useful for the identification of the yet unknown nature of the autoantigen/s in type 1 diabetes mellitus.

OP 26
Hypoglycaemia

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SHORT-TERM REVERSIBILITY OF HYPOGLYCAEMIA UNAWARENESS IN TYPE 1 (INSULIN-DEPENDENT) DIABETES MELLITUS.

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To test the hypothesis that meticulous prevention of hypoglycaemia (H) may reverse hypoglycaemia unawareness (HU), 5 patients with long-term type 1 (insulin-dependent) diabetes mellitus (18±3 yrs), no autonomic neuropathy (AN), 3-5 yrs history of HU and recurrent mild/severe H, HbA_{1c} 5.3±0.4%, were studied at baseline (T0), after 2 weeks (T1) and 3 months (T2) of changed insulin program strictly aiming at pre-prandial blood glucose (BG) >8 mM based on 4 BG measurements/day. Threshold/magnitude of H symptoms, counterregulatory hormone responses (CRH) and cognitive function (CF) were assessed during a hyperinsulinaemic-hypoglycaemic clamp (90-min sequential steps of BG at 5, 4.3, 3.6, 3, 2.3 mM) (AJP 260:E67, 1991). At T0, H symptoms barely appeared at BG 2.27±0.03 mM, CF did not deteriorate, CRH responses were severely blunted. At T1 (daily BG 9.2±1.1 mM, no H) H symptoms reappeared at BG thresholds of 2.94±0.02/2.88±0.02 mM (neuroglycopenic/neurogenic), both threshold/magnitude CRH improved, and CF markedly deteriorated vs. T0 ($p<0.001$). At T2, (daily BG 9.1±0.9 mM, no H, HbA1c 6.9±0.8%), magnitude of H symptoms (score 11.1±1.9 vs. 6.2±0.9), epinephrine (1.41±0.13 vs. 0.81±0.1 nM), cortisol (16±1.5 vs. 11±0.3 µg/dl), growth hormone (20±4 vs. 7±1.9 ng/ml), not glucagon, further improved and CF deteriorated (T2 vs. T1, $p<0.05$). Conclusion. Meticulous prevention of H may acutely reverse HU in type 1 (insulin-dependent) diabetes mellitus, at least in subjects without AN.

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ROLE OF LIVER INNERVATION IN DEFENSE AGAINST HYPOGLYCAEMIA IN HUMANS.

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In order to assess the role of liver innervation in the hepatic response in defense against insulin-induced hypoglycemia, 6 stable patients after Orthotopic Liver Transplantation (OLT: age=46±8 y; IBW=101±1%; transplant age=7±1 mo.; prednisone=10 $mg \cdot day^{-1}$; cyclosporin=5 $mg \cdot kg^{-1} \cdot day^{-1}$), 6 patients with chronic uveitis on the same immunosuppressive therapy (CU) and 6 control subjects (CON) were studied with a bolus plus a continuous infusion of [3-^{3}H]glucose. After 120 min of basal equilibration period, an hypoglycemic (SS glucose≈3.0 mM), hyperinsulinemic (0.3 $mU \cdot kg^{-1} \cdot min^{-1}$) clamp was performed. Basal plasma IRI (80±11 vs 55±1 vs 48±2 pM), C-PEP (0.89±0.3 vs 0.69±0.2 vs 0.64±0.3 nM) and glucagon (119±21 vs 91±9 vs 72±7 ng/ml) were higher in OLT vs CU and CON respectively ($p<0.01$). Basal HGP (2.0±0.2 vs 1.8±0.2 vs 2.0±0.1 $mg \cdot kg^{-1} \cdot min^{-1}$) was similar in the three groups. During hypoglycemia, plasma insulin levels were comparable in OLT, CU and CON (202±10 vs 182±17 vs 183±5 pM respectively). Plasma C-pep was adequately suppressed in the three study groups (0.08±0.03 vs 0.07±0.01 vs 0.09±0.02 nM). Glucagon increased similarly (100 %) in the three groups during the second hour of hypoglycemia to 187±21 vs 108±12 vs 95±4 pg/ml in OLT, CU and CON resp. During the first 40 min of hypoglycemia HGP was reduced in OLT (0.8±0.1) vs CU and CON [1.3±0.2 and 1.3±0.1 $mg \cdot kg^{-1} \cdot min^{-1}$ respectively ($p<0.01$)], while during the 40-120 min period it was similar (1.3±0.2 vs 1.4±0.2 vs 1.3±0.1 $mg \cdot kg^{-1} \cdot min^{-1}$) in the 3 study groups. In summary: OLT demonstrate: 1. basal hyperinsulinemia and hyperglucagonemia, with normal HGP; 2. inhibition of C-PEP concentration and stimulation of glucagon secretion during insulin-induced hypoglycemia similar to control groups; 3. in contrast, OLT demonstrated an early defect in the hepatic response to hypoglycemia, presumably due to the lack of neuroadrenergic innervation. Such defect is reverted by the normal raise of counterregulatory hormones in the second hour of hypoglycemia. In conclusion, hepatic innervation plays a pivotal role in the early defense against hypoglycemia.

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FOREARM SUBSTRATE EXCHANGE DURING HYPERINSULINEMIC HYPOGLYCEMIA IN HUMANS; LACK OF EVIDENCE FOR MUSCLE LACTATE RELEASE.

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To assess forearm substrate exchange in general and any possible muscle source for hypoglycemia induced hyperlactataemia in particular, 5 healthy young male subjects were each studied twice during 2 h of hyperinsulinaemic euglycemia followed by 4 h of 1) hypoglycemia (plasma glucose < 50 mg/100ml) and 2) euglycemia. Insulin was infused at a rate of 1.5 mU/kg/min throughout.
When compared to euglycemia, hypoglycemia was associated with: 1) Increments in circulating glucagon (54.8 ± 9.9 vs 22.2 ± 2.2 ng/l, $p < 0.05$), growth hormone (21.4 ± 6.9 vs 5.2 ± 3.4 ng/ml, $p < 0.05$) and catecholamines and increased suppression of C-peptide together a modest lowering of insulin values. 2) Decreased plasma glucose (54.8 ± 0.8 vs 90.4 ± 2.9 mg/100ml, $p < 0.05$), forearm glucose uptake (3.1 ± 1.3 vs 19.9 ± 4.4 mg/100 ml, $p < 0.05$) and requirements for exogenous glucose (M-value - 5.0 ± 2.4 vs 12.4 ± 0.7 mg/kg/min, $p < 0.05$) and impaired suppression of isotopically determined endogenous glucose production (0.35 ± 0.7 vs -2.04 ± 0.20 mg/kg/min, $p < 0.05$). 3) Exaggerated increase in blood lactate (1820 ± 220 vs 1320 ± 160 μmol/l, $p < 0.05$) and decrease in alanine (216 ± 26 vs 261 ± 22 μmol/l, $p < 0.05$). Forearm releases of both lactate (54 ± 89 vs 7 ± 63 μmol/l, $p > 0.65$) and alanine were however indistinguishable. Total forearm blood flows increased during both studies.
These data suggest that forearm muscle is neither a primary site for glucose uptake nor for lactate release during hypoglycemia. The decrease in alanine concentrations is not likely to be muscle dependent either.

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EFFECTS OF ANTECEDENT GLYCAEMIC CONTROL ON COGNITIVE FUNCTION DURING HYPOGLYCAEMIA IN TYPE 1 (INSULIN-DEPENDENT) DIABETIC PATIENTS

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To determine whether the degree of previous glycaemic control may modify cognitive responses to hypoglycaemia, the glycaemic thresholds for and magnitude of cognitive dysfunction as assessed by P300 event-related potentials as well as subjective and hormonal responses during hypoglycaemia were evaluated. Hypoglycaemia was induced by intravenous insulin infusion in 18 Type 1 diabetic patients, 7 of whom were strictly controlled (HbA_{1c}: 6.3±0.3%; mean±SEM; Group 1) and 11 of whom were poorly controlled (HbA_{1c}: 9.1±0.4%; Group 2). Within 60 min, mean blood glucose declined from 5.6 and 5.7 mmol/l (baseline) to a nadir of 1.6 and 1.8 mmol/l followed by an increase to 5.6 and 4.3 mmol/l after 120 min in Group 1 and 2, respectively. There was no significant difference between both groups in regard to P300 latency at baseline, but between 50 and 70 min a significant prolongation of this component was noted in Group 2 as compared with Group 1 at blood glucose levels between 1.6 and 2.3 mmol/l ($p<0.05$). The glycaemic thresholds at which a significant increase of P300 latency over baseline was first noted were 1.6±0.2 mmol/l in Group 1 and 3.5±0.2 mmol/l in Group 2 ($p<0.05$). The glucose threshold at which the P300 amplitude was first significantly reduced was 2.2 mmol/l in Group 2, whereas no such a reduction was observed in Group 1. The glycaemic thresholds for the perception of subjective symptoms were 1.7±0.2 mmol/l in Group 1 and 2.5±0.2 mmol/l in Group 2 and those for the first significant rise of epinephrine 1.6±0.2 mmol/l in Group 1 and 2.8±0.1 mmol/l in Group 2 ($p<0.05$). Thus, the glycaemic thresholds for and magnitude of cognitive dysfunction during hypoglycaemia are reduced in strictly controlled as compared with poorly controlled Type 1 diabetic patients. These findings support the concept of cerebral adaptation to antecedent low blood glucose levels.

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REGIONAL CEREBRAL BLOOD FLOW IN INSULIN-TREATED DIABETIC PATIENTS EXPOSED TO RECURRENT SEVERE HYPOGLYCAEMIA.

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During acute hypoglycaemia EEG studies and cognitive function tests suggest that the frontal lobes are particularly affected in non-diabetic and diabetic humans. Regional cerebral blood flow was studied in 20 (insulin-dependent) diabetic patients, 10 of whom had never experienced severe hypoglycaemia (Group A) while 10 had a history of 5 or more episodes of severe hypoglycaemia (Group B). This latter group were recruited from a cohort previously shown to have significant cognitive impairment associated with recurrent severe hypoglycaemia. The two groups were matched for age, sex, social class and years of education. Brain imaging was performed at rest using a single slice multi-detector dedicated head-scanner after bolus injection of 250 MBq of ^{99m}Tc-Exametazime. Regional cerebral blood flow was compared between the two groups in 12 regions derived from a standard neuro-anatomical atlas on 2 parallel slices at 35 and 55mm above the orbital meatal line. The results showed that in 21 of the 24 regions studied cerebral blood flow was increased in Group B v group A, although univariate analysis demonstrated a significant increase in basal cerebral blood flow only to the dominant (left) frontal region ($P=0.027$) in Group B. This relative increase in basal blood flow to the frontal lobe(s) may be an adaptive mechanism to mitigate the cumulative effect of recurrent severe hypoglycaemia on cerebral function in patients with insulin-treated diabetes.

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THE RECOVERY OF BRAIN FUNCTION AFTER HYPO-GLYCAEMIA IN NORMAL MAN

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The aim of the present study was to evaluate the recovery of brain function after moderate hypoglycaemia in normal man. Hypoglycaemia was induced by an intravenous infusion of insulin (2.5 mU/kg) in seven healthy right-handed men aged 25.4+1.1 years (Mean ± SD). The brain function was evaluated with P300-amplitude after auditory stimulus, reaction time measurements and EEG before, during (2.4+0.44 mmol/l for 70 min) and three times in the recovery period following hypoglycaemia. Hypoglycaemia caused a reduction in the P300-amplitude, a prolongation in reaction time and minor changes in the EEG-activity. 15 min after normalisation of the blood glucose level, the P300-amplitude was lower than during hypoglycaemia and still 1,5 hrs after normalisation of the blood glucose level, there was a marked reduction in the P300-amplitude. 4 hrs after normalisation of the blood glucose, the P300-amplitude was restituted. The reaction time was shorter 15 min after normalisation of the blood glucose compared to hypoglycaemia, but was not normalised until 1,5 hrs after of recovery following hypoglycaemia. The EEG-changes were normalised 15 min after hypoglycaemia.

We conclude that moderate hypglycaemia causes marked effects in P300 and reaction time and that brain function measured as P300 is not restored after 1,5 hrs but at 4 hrs after normalisation of hypoglycaemia.

OP 27

Insulin Action I

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HUMAN α2-HS GLYCOPROTEIN INHIBITS MITOGENIC BUT NOT METABOLIC EFFECTS OF INSULIN

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Insulin binding to its specific receptors triggers the intrinsic tyrosine kinase activity (IR-TKA). Availability of natural regulators of IR-TKA enhances our understanding of signaling mechanisms. We have cloned and sequenced a **human** homolog of the rat hepatic IR-TKA inhibitor, pp63. It corresponds to **α2-HS glycoprotein**. We find α2-HSG to: (a) inhibit insulin-dependent autophosphorylation of IR from the rat liver and human placenta; (b) inhibit TKA of purified liver and placenta IR in a dose-dependent manner. At physiologic concentrations, insulin-stimulated TKA was inhibited ~ 90% in human IR preparations. α2-HSG did not affect basal IR-TKA. Inhibition was abolished by dephosphorylation of α2-HSG with alkaline phosphatase; (c) have no effect on insulin binding; (d) specifically prevent (half-maximal effect at 50 μg/ml, maximal at 500 μg/ml) growth-promoting activity of insulin in rat H-35 hepatoma cells; (e) have no effect on induction of tyrosine aminotransferase by insulin. Human α2-HSG and rat pp63 thus share both structural homology and functional characteristics as inhibitors of insulin-mediated IR autophosphorylation, IR-TKA and mitogenesis. Naturally secreted tyrosine kinase inhibitors provide an exciting tool for dissection of the signal transduction pathways triggered by insulin.

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THE IMPAIRED GENE EXPRESSION OF LIVER GLYCOLYTIC AND GLUCONEOGENIC ENZYMES IS REVERSED IN DIABETIC RATS GIVEN VANADATE.

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The trace element Vanadium is a potent insulinomimetic agent in vitro. Oral administration of Vanadate (V) to rats made diabetic by streptozotocin (45 mg/kg i.v.) caused a 65% fall in plasma glucose levels without modifying low insulinemia. We studied whether the hypoglycemic effect of V was associated with altered expression of genes involved in key steps of hepatic glucose metabolism. Pyruvate kinase (PK-L) and glucokinase (GK) mRNA levels were decreased by 70% and 90% in diabetic rats where PK and GK activities were lowered accordingly. Eighteen days of V treatment totally restored PK mRNA and activity and partially (40% of control levels) restored GK parameters. In contrast to the glycolytic enzymes, mRNA levels of the gluconeogenic enzyme, phosphoenolpyruvate carboxykinase (PEPCK) were increased by 1500% in diabetic rats with correlated change in activity. V administration normalized both PEPCK mRNA and activity in liver of treated rats. The 2-fold increase in hepatic glucose transporter (GLUT2) mRNA and protein, produced by diabetes, was also corrected. In conclusion, oral V given to diabetic rats induces a shift of the predominating gluconeogenic flux, with subsequent high hepatic glucose production, into a glycolytic flux by pretranslational regulatory mechanisms.

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PHOSPHOPEPTIDES CORRESPONDING TO IRS-1 INHIBIT ASSOCIATION AND PHOSPHORYLATION OF INSULIN RECEPTOR SUBSTRATES.

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To define structural requirements of insulin receptor substrate interaction we studied the association and phosphorylation of rat liver cytosolic substrates in the presence of synthetic non-phosphorylated or phosphorylated peptides corresponding to putative phosphorylation sites of IRS-1 (pp185) and to several receptor domains. Insulin receptors were immobilized on antibodies and incubated *in vitro* with rat liver lysate obtained either at low non-ionic detergent concentration or after SDS extraction and renaturation. Insulin stimulated receptors were found to associate with protein substrates of 185, 120, 85 and 45 kDa which were phosphorylated in the presence of ^{32}P-ATP. Synthetic peptides corresponding to receptor domains Tyr^{960} and to previously described basic "cluster A" poorly affected this interaction. By contrast phosphopeptides corresponding to sites Tyr^{608}, Tyr^{628}, Tyr^{658} and Tyr^{727} of IRS-1 with the motif *Met-X-Met*, or to site Tyr$^{46-47}$ selectively inhibited phosphorylation of pp185 substrate. Some peptides also inhibited receptor autophosphorylation. It is concluded that these domains of IRS-1 are involved in the association with and phosphorylation by the receptor and that synthetic peptides or phosphorylated analogues are useful tools in defining functional domains of receptor-substrate interaction.

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Regulation by insulin and glucose of Glut 2 mRNA in liver: in vivo and in vitro studies.

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Glut2 is the glucose transporter specifically expressed in the liver and tbat might be implicated in the control of glucose metabolism. Its regulation was studied in vivo, using euglycemic-hyperinsulinemic and hyperglycemic-hyperinsulinemic clamps. In the liver, Glut2 mRNA concentration was decreased 90% during a 6 hours euglycemic (1g/l) hyperinsulinemic (660μU/ml) clamp when compared to 24 hours starved control rats. After hyperglycemic (2,4g/l) hyperinsulinemic (620μU/ml) clamps, Glut2 mRNA concentration was only decreased 50 % when compared to controls. Thus, in vivo, hyperinsulinemia inhibited Glut2 gene expression whereas hyperglycemia partially prevented this effect. To study the respective role of insulin and glucose on Glut2 expression, primary culture of hepatocytes were perfomed during 24 hours in the presence of various concentrations of glucose (0 to 20 mM) and insulin. Glucose induced a dose-dependent stimulatory effect on Glut2 mRNA. Insulin (10^{-7}M) inhibited Glut2 expression when hepatocytes were cultured between 0 to 10 mM glucose but was without effect in presence of 20 mM glucose. In conclusion, Glut2 expression was regulated conversely by glucose and insulin both in vivo and in vitro, suggesting a role in the control of glucose metabolism in liver.

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IMPACT OF DIABETES ON THE EXPRESSION OF GLUCOSE TRANSPORTERS IN RAT CARDIAC MUSCLE.

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While enhanced glucose utilization potentially improves cardiac function during hypoxia, the molecular defects at the glucose transporter (GTer) expression cascade affecting glucose uptake in the rat diabetic heart are not yet clearly understood. Streptozotocin-induced diabetic rats (DR), before and after 8-day insulin therapy (8-IT), and control rats were used to obtain total cardiac cellular membranes and total RNA. To determine the number of GTer, the membranes were immunoblotted against specific GLUT-1 & 4 antibodies, using western blot analysis. The readings were adjusted to a known GTer number, as measured by Cytochalasin B binding assay in basal low density microsomes prepared from rat adipocytes. The level of specific GLUT mRNA's was determined by using Northern blots and respective radiolabled cDNA probes. Compared to control, in DR, GLUT-4 mRNA was decreased to 52±11% (mean±SEM) and reversed to 133±22% upon 8-IT. GLUT-4 protein followed the same pattern, in these models. GLUT-1 mRNA and protein levels were too small to be detected. In conclusion, diabetes induces a rapid derangement in the rat cardiac glucose transporter system that can be reversed by insulin therapy. These defects potentially expose the diabetic heart to a major impairment during ischemia.

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INSULIN RECEPTOR TYROSINE KINASE ACTIVITY AND GLUT 4 LEVELS IN MUSCLE OF HYPERTENSIVE RATS

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We determined insulin receptor kinase activity and Glut 4 level in hindlimbs of spontaneous hypertensive and control rats to elucidate whether in analogy to other insulin resistant animal models an inactivity of the insulin receptor kinase or an alteration of the glucose transporter (Glut 4) level in the skeletal muscle might contribute to the pathogenesis of insulin resistance. Normotensive normoinsulinaemic Lewis and Wistar rats were used as insulin sensitive controls, obese Zucker rats as an insulin resistant control. Binding of ^{125}J-insulin, crosslinking of ^{125}J-B26-insulin, autophosphorylation in vitro with ^{32}P-ATP and phosphorylation of the synthetic substrate Poly(Glu 4:Tyr 1) were performed after partial purification of solubilized receptors on wheat germ agglutinin columns. Glut 4 levels were determined by Western blotting of subcellular muscle membranes. Insulin receptors from spontaneous hypertensive rats compared to the controls showed no difference of the binding characteristics or the in vitro auto- and substratephosphorylation activity of the receptor, while in Zucker rats insulin receptor kinase defect was clearly evident. Western blots of skeletal muscle membrane proteins revealed no difference in Glut 4 levels. The data suggest that insulin resistance in SHR is neither caused by an insulin receptor inactivity nor by a decreased number of glucose transporters in skeletal muscle.

OP 28
Retinopathy

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PREVALENCE OF RETINOPATHY IN A BRITISH COHORT WITH DIABETES MELLITUS DIAGNOSED BEFORE AGE 2 YEARS.

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A British cohort of 339 children who had Type I (insulin-dependent) diabetes diagnosed before age 2 years during the years 1972-81 was established during 1989. To verify a retinopathy prevalence in this cohort of 3% reported in a postal survey, 213 clinicians were requested to arrange direct ophthalmoscopy for their patients in this cohort. After an initial mailing, reports for 122(36%) patients, 74(61%) males and 48(39%) females were returned and analysed (a second mailing is now proceeding). Ophthalmoscopy was performed in 121(99%) cases, 40% by ophthalmologists, 52% by diabetologists and 8% by others, with 67% of examinations performed within the last 12 months. Patients' pupils were dilated for 87 (77%) examinations. Ophthalmologists were significantly more likely to dilate pupils than other examiners. (X^2=8.74,df=2,p=0.013). Background retinopathy was detected for 12 patients (10%) and proliferative retinopathy for 1 patient (1%). More males (13%) than females (9%) had retinopathy, but this difference was not significant (X^2=0.482, df=1, p=0.487). These preliminary results indicate a prevalence of retinopathy higher than previously reported for this cohort, but lower than prevalences reported in other cohorts of similar diabetes duration.

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ALDOSE REDUCTASE INHIBITION BENEFITS NEUROPATHY BUT NOT RETINOPATHY IN DIABETIC DOGS.

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To evaluate the role of the polyol path in development of diabetic complications, dogs were made diabetic with alloxan (n=18), randomly divided between a placebo group and a group given the aldose reductase inhibitor Sorbinil (20-40 mg/kg/day), and followed 5 years. Glycemia was managed with insulin to allow moderate hyperglycemia while keeping nonenzymatically glycated plasma protein and HbA1 comparable between the 2 groups (mean % HbA1 = 8.0 ± .4 SD for Sorbinil, 7.9 ± .4 for placebo, 5.6 ± .2 normal). Sorbinil treatment prevented elevation of tissue sorbitol (monitored in erythrocytes), and cataractogenesis in the group was less than in the placebo group. Motor nerve conduction velocity (NCV) declined slowly in the placebo diabetic group, becoming significantly less than that of nondiabetic controls by 5 years, and the decline was inhibited by Sorbinil. By that time, NCV in the Sorbinil group was significantly faster than in the placebo group (63 ± 2 vs 58 ± 3 m/sec), and not significantly less than normal (64 ± 3 m/sec). Retinopathy (e.g. aneurysm count, pericyte loss, hemorrhage) nevertheless was not influenced by Sorbinil. Nondiabetic dogs made galactosemic by a 30% galactose diet also have developed diabetic-like retinopathy, but the NCV has remained normal through 5 years, and aldose reductase inhibition has had no effect on retinopathy or NCV in the galactosemic dogs.

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HUMAN DIABETIC CATARACT: POSSIBLE ROLE OF LIPID PEROXIDATION

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An increased lipid peroxidation and protein damage, due to monosaccharides autoxidation, have been reported in diabetics. Furthermore, antioxidant agents, have been tested in order to delay lens damage. These findings support the hypothesis of an increased oxidative stress as a possible cause of diabetic cataract formation. In this study we examined the lenses of 11 type 2 (non-insulin-dependent) well controlled diabetics (mean age: 62±5; range: 56-71), on oral hypoglicaemic or insulin treatment operated for diabetic cataract and 7 healthy controls (mean age: 57±7; range 49-65), whose lenses were obtained after an ocular trauma. Diabetic patients were divided into 2 subgroups according to integrity or abnormality of the Haemo-Ocular Barrier (H-OB) as evaluated by pre-operative iridography. Malondialdehyde (MDA), marker of lipid peroxidation, measured spectrophotometrically as the TBA-reaction was determined in lenses from all subjects. A significant increase of MDA levels was observed in lenses of diabetics compared to those of controls (4.25±0.46 vs 1.14±0.08 nmol/g, M±SEM, $p<0.001$) and, among diabetic patients, in alterated lenses compared to intact ones (5.44±0.37 vs 2.83±0.22 nmol/g, M±SEM, $p<0.01$). These data confirm the role of lipid peroxidation in inducing diabetic cataract, expecially if an alteration of H-OB is present.

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TOXICITY OF GLYCATED AND GLYCOXIDIZED LOW DENSITY LIPOPROTEINS TO RETINAL CAPILLARY ENDOTHELIAL CELLS.

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Disruption of the inner blood retinal barrier (IBRB), which is formed by tight junctions between retinal capillary endothelial cells (REC), is an early feature of diabetic retinopathy (DR). We aimed to investigate the effects of low density lipoproteins (LDL), modified as in diabetes, on REC. We exposed cultured bovine REC to normal, *in vitro*-glycated and *in vitro*-glycoxidized human LDL (N-LDL, G-LDL, GO-LDL). LDL was prepared by incubation for 3 days at 37°C: for N-LDL, under N_2, with antioxidants (EDTA, DTPA) but without glucose; for G-LDL, under N_2, with antioxidants and 50mM glucose; for GO-LDL, under air, without antioxidants, in 50mM glucose. In 6 experiments, confluent REC were exposed to LDL (100μg/ml) or serum-free medium (SFM) for 3 days, in 5mM glucose. N-LDL had no significant effect on cell numbers, viability or cell protein. G-LDL and GO-LDL reduced cell numbers by 45 and 58% ($p<0.01$ vs day0, paired t-tests, $n=6$), viability by 62 and 76% ($p<0.001$), and cell protein by 28 and 44% ($p<0.05$, $p<0.01$), respectively. Significant increases ($p<0.02$) in Lactate Dehydrogenase release were observed with G-LDL and GO-LDL. Observations with SFM were intermediate between those for N-LDL and G-LDL. G-LDL and GO-LDL are toxic to REC, and may contribute to the development of DR.

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GLYCOXIDIZED LDL MODIFIES PAI-1 RELEASE BY RETINAL ENDOTHELIAL CELLS.

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We tested the hypothesis that glycated (G-LDL) and glycoxidized (GO-LDL) human LDL alter the release of tissue plasminogen activator (t-PA) and plasminogen activator inhibitor type-1 (PAI-1) by bovine retinal capillary endothelial cells (REC) in culture. Pooled LDL was incubated for 3 days at 37°C under 3 different conditions: a) under nitrogen, with antioxidants (DTPA), without glucose (normal LDL, N-LDL); b) under nitrogen, with DTPA and 50mM glucose (G-LDL); c) under air, with 50mM glucose and without antioxidants (GO-LDL). In six separate experiments, confluent REC were incubated for 24h with serum free medium (SFM) alone or containing 100 μg/ml of N-LDL, G-LDL or GO-LDL. GO-LDL stimulated significantly more PAI-1 release than N-LDL (1.25 ± 0.40 v 0.68 ± 0.30 ng/mg cell protein (cp), $p<0.02$). There were no significant differences in PAI-1 release between N-LDL and either G-LDL (0.86 ± 0.35 ng/mg cp) or SFM (0.59 ± 0.28 ng/mg cp). t-PA release by REC was not altered by incubation with LDL: 51 ± 19, 47 ± 18, 63 ± 29 and 50 ± 31 ng/mg cp for N-LDL, G-LDL, GO-LDL and SFM, respectively. In summary, glycoxidized LDL can contribute to increased release of PAI-1 by retinal endothelial cells. This could lead to a local decrease of fibrinolysis in retinal capillaries, thereby favouring thrombus formation.

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THE ROLE OF "HYPERGLYCEMIC (METABOLIC) HYPOXIA" IN THE PATHOGENESIS OF DIABETIC COMPLICATIONS

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Acute hypoxic myocardial injury resulting in (diabetes-like) vascular, electrophysiological, and myocyte contractile dysfunction has been linked to impaired oxidation of NADH to NAD^+. The present studies were undertaken to determine whether acute elevation of glucose levels in normal rat retina and endoneurium mimics effects of O_2 deprivation on NADH/NAD^+ (reflected by increased tissue lactate/pyruvate ratios). Retinal lactate/pyruvate ratios increased from 24.0±4.7(SD) to 40.3±5.7 ($P<0.0001$) in 5 vs 30 mM glucose (2 hour incubation, $n=7$); endoneurial ratios increased from 9.6±0.8 to 12.9±1.9 ($P<0.001$) in 5 vs 50 mM glucose. These glucose-induced redox changes were significantly reduced or prevented by aldose reductase inhibitors (32.2±7.0, $P=0.031$ in retina; 8.6±0.9 in endoneurium). These observations, together with prevention of diabetes-induced vascular and neural dysfunction *in vivo* by aldose reductase inhibitors and acetyl-L-carnitine, suggest that neural and vascular dysfunction developing early after the onset of poorly controlled diabetes may result from glucose-induced, sorbitol pathway-linked increases in NADH/NAD^+ (and associated imbalances in carnitine metabolism) like those observed in hypoxic (nondiabetic) hearts.

OP 29
Autonomic Neuropathy

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DIABETIC AUTONOMIC NEUROPATHY : WHICH TESTS TO USE

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In patients with long-standing diabetes mellitus, dysfunctions of the autonomic nervous system are frequent. To ascertain the diagnosis of diabetic autonomic neuropathy (DAN), various combinations of cardio-vascular tests have been proposed. In the present study variations of heart frequency during deep breathing (ΔR_6) and during a Valsalva manoeuvre (both testing parasympathetic function); the number of sweat glands on hand and feet (both testing sympathetic function); and the response of pancreatic polypeptide to hypoglycemia ($\ln[\Delta hPP+1]$, testing vagal function) were evaluated in 24 consecutive Type 1 (insulin-dependent) diabetics considered as having mild to moderate DAN (based on past history, 2-5 tests outside mean±1SD of normal, diabetes duration >15 years) as well as 10 control subjects (≤1 test abnormal) were analyzed, looking for a model that would optimally discriminate diabetics from controls. 'Stepwise linear regression' analysis gave the following model:

$f = 1.232 - (0.040 \times \Delta R_6) - (0.039 \times \ln[\Delta hPP+1])$

(multiple $r = 0.847$, multiple $r^2 = 0.717$, $SE = 0.254$)

Using this function, 23/24 diabetics, and 9/10 controls were classified correctly (sensitivity 96%, specificity 90%). Inclusion of additional parameters did not improve discrimination between diabetics and controls. We conclude that the combined determination of ΔR_6 (as a test of parasympathetic function) and of $\ln[\Delta hPP+1]$ (as a test of vagal function) allows an excellent diagnosis of DAN.

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LEFT VENTRICULAR MASS IS INCREASED IN NEUROPATHIC DIABETIC PATIENTS WITH NORMAL BLOOD PRESSURE

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Autonomic neuropathy is associated with a reduced nocturnal fall of blood pressure. It has been shown that nocturnal systolic blood pressure strongly correlates with left ventricular mass index (LVMI). The relationship between diabetic autonomic neuropathy, circadian blood pressure changes and echocardiographic parameters was investigated in 27 normotensive diabetic patients (10 with and 17 without neuropathy) who underwent 24-h blood pressure monitoring and M-mode echocardiographic recording. The two groups were comparable for age, sex, duration of diabetes, body mass index and metabolic control. 24-h average and diurnal systolic (SBP), diastolic (DBP) and mean blood pressure (MBP) were similar. The percent changes from day to night of SBP, DBP and MBP were significantly lower in neuropathic patients ($p<0.04$). Increased LVMI (135.4±10.2 vs 102.9 ±6.3 g/m^2, $p<0.005$), septal wall and posterior wall width were observed in neuropathic patients. Fractional shortening (37.4±2.0 vs 37.1±2.1 %), peak velocity of early left ventricular filling (E), of late ventricular filling (A) and their ratio (E/A) were similar in the two groups. In summary, normotensive diabetic patients with neuropathy display a reduced nocturnal blood pressure fall, associated with an increased LVMI. The increased LVMI.may represent a link between diabetic autonomic neuropathy, nocturnal blood pressure levels and high cardiovascular mortality rate.

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QUANTITATIVE CHARACTERIZATION OF CARDIAC AUTONOMIC NEUROPATHY BY POSITRON EMISSION TOMOGRAPHY (PET)

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Cardiac autonomic neuropathy may increase morbidity and mortality. Standardized autonomic function tests utilize indirect methods, which distinguish poorly between sympathetic (S) and parasympathetic (P) dysfunction. PET imaging allows direct quantification of both S and P neurotransmitter dysfunction. We now report our preliminary data using the highly specific noradrenaline analog, C-11 hyroxyephedrine (HED), thus directly assessing cardiac S integrity, in four insulin dependent (type 1) diabetic patients, 1 male, 3 female, mean (SD) age 33(10) years, diabetes duration 18(7) years. All had reduced heart rate variability (HRV)<10 beats/min: two had severe S dysfunction (valsalva ratio (VR)<1.05, systolic blood pressure fall (SBPF)>20mmHg with symptomatic postural hypotension; two were less severely affected, (VR 1.54 and 1.57, SBPF<10mmHg). Imaging was performed with N-13 ammonia for blood flow imaging and C-11 HED for neuronal imaging. Left ventricular (LV) regional distribution volumes (Vd) for HED were estimated to assess severity and homogeneity of neuronal dysfunction and compared to14 normal nondiabetic controls. All patients had homogeneous blood flow images. The Vd was reduced in the all diabetic patients (range 1.1 to 31 mL/g, vs 44.5(3.7) mL/g in controls), and was greatly reduced in the two patients with postural hypotension, Vd range 1.9(1.1) mL/g to 19(18)mL/g. The extent of the LV affected varied from 2-83%, with distal cardiac segments more severely affected. **Conclusions.** PET using C-11 HED allows quantitative and sensitive characterization of cardiac S dysfunction. Our studies suggest a nonuniform denervation, confirmation of which with subsequent studies may help elucidate its pathophysiology.

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MECHANISMS OF ARTERIAL HYPOTENSION AFTER SUBCUTANEOUS INSULIN IN DIABETIC AUTONOMIC NEUROPATHY.

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To assess the hemodynamic effects of prandial therapeutic doses of insulin in diabetic autonomic neuropathy (DAN), lying, 3-5 min standing mean blood pressure (MBP), plasma norepinephrine, plasma volume (^{125}I-,^{131}I-albumin), and leg total peripheral resistance (mean BP/leg blood flow, pletismography) were measured in 9 patients with (DAN+, score 6.6±0.6) and 6 without DAN (DAN-, score 0.16±0.16), before and after subcutaneous injection of placebo and insulin (0.2 U/kg) on the same day, and on separate days. Plasma glucose was clamped at 8.5±0.3 mM throughout studies. After insulin, MBP did not change in DAN-, but decreased in DAN+ vs. placebo, both lying (94±0.3 vs. 99±0.3 mmHg, 0.5-2 h after insulin/placebo) and standing (74±3 vs. 85±3 mmHg, 2 h after insulin)($p<0.01$). Two h after insulin, standing increments in norepinephrine (0.63±0.19 vs. 1.03±0.18 nM) were lower, but the decrease in plasma volume (3.17±0.43 vs. 1.21±0.44 ml/kg) was greater in DAN+ vs. DAN- ($p<0.05$) and correlated with standing MBP decrements in both groups ($r=-0.69$ and 0.64, NE and PV, $p<0.05$). Leg total peripheral resistance increased similarly in DAN- (from 19±2 to 27±3) and DAN+ (from 22±2 to 31±3) (mmHg/ml/100ml/min, p=NS). Conclusions. Subnormal standing increments of norepinephrine and lower plasma volume after insulin, not compensated by appropriate increases in peripheral resistance, are the mechanisms of hypotension after prandial insulin in DAN+.

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IMPAIRED NOCTURNAL DECLINE IN ARTERIAL BLOOD PRESSURE IN TYPE 2 (NON-INSULIN-DEPENDENT) DIABETIC PATIENTS WITH NEPHROPATHY

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Non-diabetic hypertensive patients lacking the normal nocturnal decline in blood pressure have an enhanced cardiovascular morbidity. Since cardiovascular morbidity is increased in Type 2 diabetic patients we performed 24-hour non- invasive ambulatory blood pressure monitoring with the Takeda TM 2420 device in 53 Type 2 diabetic patients with diabetic nephropathy compared to a matched group of 53 normoalbuminuric Type 2 diabetic patients. Prevalence of hypertension was 81% and 38% in the two groups, respectively. All antihypertensive drugs were withdrawn for at least 2 weeks. Autonomic nerve function was evaluated by beat-to-beat variation and orthostatic blood pressure measurements. Normal nocturnal decline in blood pressure ("dippers") was defined as an average reduction in systolic and diastolic blood pressure ≥ 10% during sleep (23-07) compared to daytime (07-23). The nocturnal decline in blood pressure was 6.5 ± 11.5% in the nephropatic group versus 11.7 ± 10.6% (mean ± SD) in the normoalbuminuric group ($p<0.05$). The prevalence of dippers was reduced in the nephropatic compared to the non nephropatic group, 42% vs 62% respectively ($p<0.05$). In a regression analysis the nocturnal blood pressure decline was associated to beat-to-beat variation ($p<0.02$) but not to blood pressure level. Our study suggests that impaired nocturnal decline in blood pressure is more prevalent in Type 2 diabetic patients with nephropathy and autonomic neuropathy.

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DOES NERVE GROWTH FACTOR PLAY A ROLE IN DIABETIC AUTONOMIC NEUROPATHY?

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There is evidence for an autoimmune component to the pathogenesis of autonomic neuropathy in Type 1 diabetes. Nerve growth factor (NGF) is required for development and maintenance of autonomic nervous function, is highly expressed in the iris and known to have structural homology to insulin. We studied the presence of autoantibodies to NGF in: 20 patients with long-standing Type 1 diabetes (mean age 46.45±11.33 years, duration of diabetes 33.1±10.27 years) with abnormal autonomic function tests, of whom 14 had symptomatic autonomic and peripheral neuropathy, 3 had an episode of iritis and 6 had no autonomic symptoms; 9 age-matched patients with Type 1 diabetes but with shorter duration of disease (21.78±16.7) and no complications; 10 healthy subjects (mean age 34.6±5.96 years). We also assayed insulin antibodies (IA) to evaluate a possible cross-reaction of IA with NGF. Insulin and NGF antibodies were affinity purified and measured by ELISA, using human insulin and mouse 7S NGF as antigens. IA were present in 6 (30%) patients with neuropathy and in 5 (55%) without complications. We could not show evidence for the presence of antibodies to NGF, by comparing the three groups using the Mann-Whitney U test. Thus it remains speculative whether antibodies to NGF or the presence of insulin antibodies cross-reacting with NGF play a role in autonomic neuropathy.

OP 30
Animal Diabetes

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A TRANSGENIC MODEL OF CHRONIC HYPERGLYCEMIA IN MICE WITHOUT PRIMARY ALTERATION OF β-CELL FUNCTION.

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Our aim was to develop a transgenic model of chronic hyperglycemia in which ß-cell function was not primarily altered. We exploit the fact that the extracellular domain of the insulin receptor (ectodomain) can be expressed as a secreted soluble protein that binds insulin selectively. A transgene comprising 921 amino acids of the human ectodomain, a 3.0 Kb segment of the mouse transferrin promoter and 3' SV40 polyadenylation signal was used for the generation of transgenic mice. Two lines were selected for accumulation of the ectodomain in the plasma, detected by the binding of ^{125}I-insulin in a solid phase assay with a monoclonal antibody. The tissues expressing and secreting the transgenic protein were in a decreasing order of intensity : white fat, lung, brain, adrenal glands and liver. Postabsorptive transgenic mice were hyperglycaemic, 143±11 versus 94±6 mg/dl ($P<0.01$), had a higher total plasma insulin concentration, 59±9 versus 21±4 µU/ml ($P<0.001$) and a higher glucose turnover rate, 23±2 versus 18±1 mg/min/kg ($P<0.09$). During an hyperglycaemic clamp (200mg/dl), total plasma insulin was also higher in transgenic mice,193±47 versus 95±11 µU/ml ($P<0.05$). It suggests that plasma insulin is effectively "buffered" by the ectodomain. This represents a model of hyperglycemia without primary alteration of insulin secreting and insulin sensitive tissues.

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NEONATAL TOLERIZATION WITH GAD FROM RAT BRAIN IN NOD MICE ALTERS THE INCIDENCE OF DIABETES.

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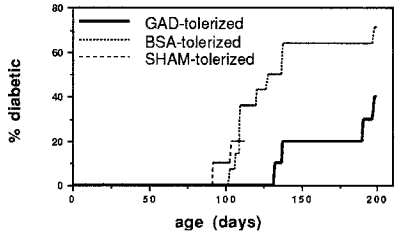
NOD mice share many pathologic features with Type 1 (insulin-dependent) diabetes in man. One of these is autoantibodies against GAD preceding the onset of the disease. In man approx. 80% of newly diagnosed individuals have GAD-Ab's. GAD molecules may serve as the primary target antigen or they may represent secondary antigens released after initial ß-cell destruction. To test the role of GAD in diabetes pathogenesis in NOD mice, we purified GAD from rat brains using affinity chromatography with a monoclonal antibody (GAD6). Following dialysis against PBS the purified material was injected intraperitoneally into neonatal female NOD mice (n=10). Control female NOD mice received either PBS (n=10) or BSA (n=14). So far the cumulative diabetes incidence curves are as follows:



In conclusion, neonatal GAD tolerization does not prevent diabetes in at least 40% of the mice, however, the onset is significantly ($p<0.05$) delayed compared with that observed in BSA and sham-tolerized animals. These data indicate that GAD associated autoimmunity is involved in the pathology of diabetes in NOD mice. The presence of GAD-Ab's in prospectively sampled sera from all the mice is currently investigated to ascertain the effectiveness of the tolerization.

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LINOMIDE, A NOVEL IMMUNOMODULATING DRUG, PREVENTS DIABETES IN NON-OBESE DIABETIC (NOD) MICE.

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Our aim was to ascertain the effect of linomide, an immunomodulating drug, on the course of diabetes in NOD mice. Linomide 0.5 mg/ml in drinking water was given to five-week old female NOD mice for 40 weeks. At 16 weeks insulitis scores for untreated (n=6) and treated mice (n=9) were 72±2.6% and 11.6±2.7%, (mean±SEM) respectively. By forty weeks 11/18 control animals were diabetic, in contrast to 0/18 in the treated group. I.P. GTT (1.0 gr/kg) revealed basal glucose (mmol/L, mean±SEM) levels of 8.2±0.7, 6.8±0.3, 8.3±0.3 and 6.3±0.2 for surviving non-diabetic untreated (n=7), treated (n=18), 5 week-old male NOD controls (n=11) and normal Balb/c mice (n=9) (untreated vs. Balb/c $p<0.013$, treated vs. Balb/c- n.s.). 60 minute values were 18.3±2.0, 9.8±0.8, 10.7±0.3 and 8.5±0.3, respectively (treated vs. male NOD and Balb/c controls-n.s., untreated vs. male NOD controls $p<0.012$) indicating that at 40 weeks all treated animals had normal glucose tolerance while the untreated group were either frankly diabetic or glucose intolerant. No inter-group differences of T-cell subsets, PHA reactivity or NK-cell number or function was observed. Linomide appears to be a highly effective drug for prevention of autoimmune diabetes in NOD mice through, as yet, undefined mechanism(s).

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INJURY OF ß-CELLS AND HYPERGLYCEMIA FOLLOWING ADMINISTRATION OF PLATINUM ANTICANCER DRUGS

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Several heavy metal ions are known to impair glucose homeostasis by altering islet cell functions. To examine anticancer platinum compounds for their effects on the endocrine pancreas, male Wistar rats were treated with an i.v.dose of 6mg/kg oxoplatin (trans-dihydroxy-cis-dichlorodiammine-platinum IV) or 2mg/kg cisplatin (cis-dichlorodiammineplatinum II) for 5 consecutive days. Glucose tolerance was evaluated 1, 2 and 80 days following termination of treatment by serially measuring plasma glucose before and after an i.v. glucose load (0.4g/kg).Both drugs, oxoplatin more severely than cisplatin, impaired glucose tolerance and produced deficient insulin response relative to the degree of hyperglycemia.Plasma glucagon was paradoxically increased only by cisplatin.Pancreatic insulin content already 1d after oxoplatin and cisplatin treatment was reduced by 57% ($p<0.01$) and 27% ($p<0.05$), respectively.Early histopathological changes seen in the islets, following oxoplatin but not cisplatin administration, included pyknotic cell nuclei, cytoplasmic vacuolization and a 42% ($p<0.001$) decrease in the ß-cell mass.Glucose tolerance and insulin secretory response to glucose, initially impaired after cisplatin treatment, recovered during the 80-day-observation period.However, oxoplatin induced progressive loss of ß-cells and persistent hyperglycemia (>20mmol/l).In conclusion, anticancer drugs are capable of initiating toxic reactions against the pancreatic ß-cells.

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CHARACTERIZATION OF A STRUCTURALLY NOVEL, HIGHLY POTENT, ORALLY ACTIVE ALDOSE REDUCTASE

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WAY-121,509, a spiro[isoquinoline-4(1H),3'-pyrrolidine]-1,2',3,5'(2H)-tetrones, is one of the most potent orally active aldose reductase (AR) inhibitors yet identified. In a spectrophotometric assay with bovine lens aldose reductase and DL-glyceraldehyde as substrate this compound was shown to be a mixed type noncompetitive/ uncompetitive inhibitor with high intrinsic activity ($IC_{50}=1.4X10^{-8}M$). In STZ-diabetic and galactosemic rats WAY-121,509 exhibited exceptional oral potency, preventing polyol accumulation (quantitated by GC) in the sciatic nerve with ED_{50} values of 0.08 and 0.3 mg/kg/day, respectively. The enantiomers of WAY-121,509 were indistinguishable in both in vitro and in vivo aldose reductase assays suggesting that there is rapid racemization around the C-4 asymmetric center. Quantitation by HPLC of the enantiomeric ratio in plasma samples confirmed this suggestion. The plasma half-life, 3-4h in rats and 10-12h in dogs was estimated from plasma drug level measurements and from an ex vivo assay, in which erythrocyte AR activity was estimated at selected times, following a single oral dose of inhibitor. Together, these data demonstrate that WAY-121,509 is a highly potent and orally effective aldose reductase inhibitor with a pharmacokinetic profile compatible with once daily dosing.

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INSULIN RESISTANCE AND POSTRECEPTOR CHANGES OF LIVER METABOLISM IN FAT-FED MICE.

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Postreceptor insulin resistance was studied in liver tissue from NMRI mice after 3 months' fat-feeding. Intravenous glucose tolerance tests showed impaired glucose tolerance ($p < 0.001$) and increased plasma insulin concentrations consistent with insulin resistance and reduced peripheral and hepatic uptake of glucose. This could also be inferred from the observation that pre-noon the fat-fed mice were normoinsulinemic (66.0 ± 5.9 µU/ml vs 85.3 ± 12.3 µU/ml for controls, NS) and hyperglycemic (plasma glucose 10.3 ± 0.35 mM vs 8.0 ± 0.30 mM, $p<0.01$). In livers of fat-fed mice the glycogen content was reduced (91 ± 8 mM vs 192 ± 19 mM, $p<0.0001$). Likewise glucokinase activity was reduced (2,28 ± 0.10 U/g vs 2.71 ± 0.17 U/g, $p<0.05$). On the contrary, lactate dehydrogenase was increased (417 ± 11 U/g vs 285 ± 20 U/g, $p<0.0001$). Of the lipogenic enzymes fatty acid synthase was decreased (0.30 ± 0.02 U/g vs 0.50 ± 0.04 U/g, $p<0.0001$), whereas glucose 6-phosphate dehydrogenase and the rate limiting enzyme in fatty acid synthesis, acetyl CoA carboxylase, both were unaffected. It is concluded that insulin resistance, unchanged capacity for liver fatty acid synthesis and capability of greatly increased glucose production from lactate in liver are similar to what has been reported for liver metabolism in type 2 (non-insulin-dependent) diabetic subjects and may be studied in more detail and with more ease in this particular animal model.

OP 31
Immunology IV

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AUTOANTIBODIES TO GLUTAMIC ACID DECARBOXYLASE VARY IN THEIR MHC CLASS II ALLELE ASSOCIATIONS.

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The autoantibody responses to GAD are heterogeneous: GAD-Abs are detected in a proportion of individuals with also whole islet ICA, while in the absence of whole islet ICA, some GAD-Abs appear as a beta cell selective pattern. To determine whether heterogeneity was linked to MHC, we examined HLA DR and DQ alleles in a cohort of 32 GAD-Ab positive non-diabetic individuals with also whole islet ICA or with the beta cell anti-GAD pattern. Those with GAD-Abs and whole islet ICA had a predominance of diabetes susceptible alleles, while those with GAD-Abs producing a beta cell pattern did not, but rather had protective alleles. All those with GAD-Ab and whole islet ICA were DR3 or 4; 9 (69%) were DR 3/4. Only 1 (7%) individual without whole islet ICA but with the beta cell anti-GAD pattern was DR 3/4 ($p<0.001$). HLA DR2 was found in 8 (42%) of those with a beta cell anti-GAD pattern, and in none of those with also whole islet ICA ($p<0.01$). All those with whole islet ICA had DQA1 and DQB1 alleles encoding at least 2 DQ$\alpha\beta$ heterodimers with Arg-52 in DQα and non Asp-57 in DQβ, while DQ alleles in 36% of individuals with beta cell anti-GAD pattern yielded no diabetes susceptible DQ$\alpha\beta$ heterodimers. The results support the hypothesis that MHC class II allelic variation influences the specificity of the autoimmune response in type 1 diabetes. Protection and susceptibility to disease associated with class II genes may result from variations in binding and presentation of different autoantigenic peptides. Only some of these response may be pathogenic.

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DECREASED ISLET CELL-SPECIFIC AUTOIMMUNITY AT CLINICAL MANIFESTATION OF TYPE 1 DIABETES IN CHILDREN WITH STRONG GENETIC SUSCEPTIBILITY

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Clinical and autoimmune characteristics of 72 children with Type 1 diabetes were registered at diagnosis and during the initial 2 years of the disease in order to investigate whether subjects with the high risk HLA-DQB1 genotypes differ from those with neutral or low risk DQB1 combinations. DQ genotyping was performed using sequence specific oligonucleotide probes derived from the region coding for the 57th and the surrounding amino acids of the DQ-β-chain. At diagnosis circulating levels of complement-fixing islet cell antibodies (CF-ICA) were lower in subjects homozygous for the non-Asp57 encoding DQB1 alleles (n=55) as compared to those with at least one Asp57 encoding allele (n=17) [24.3 JDF-U (95% CI 14.8-33.8) vs. 54.7 JDF-U (95% CI 28.0-81.3); $p<0.05$]. A similar trend was observed for conventional ICA [53.9 JDF-U (95% CI 43.8-64.0) vs. 68.3 JDF-U (95% CI 49.1-87.5); $p=0.13$]. During the 2-year observation period subjects with the high risk DQw2/DQw8 combination (n=30) had lower serum C-peptide concentrations ($p<0.05$ in two-way analysis of variance) and higher daily insulin doses ($p<0.05$) than the other subjects (n=42). Our novel observation of decreased islet cell-specific autoimmunity in non-Asp57 homozygous children at diagnosis of Type 1 diabetes supports the concept of genetically determined heterogeneity within the disease and suggests that β-cell damage results in weaker signs of autoimmunity in subjects with strong genetic susceptibility. The more rapidly decreasing endogenous insulin secretion and higher requirement of exogenous insulin in DQw2/DQw8 heterozygotes indicate that these patients are characterized by an accelerated β-cell destruction during the early course of the disease.

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Reactivity against glutamic acid decarboxylase (GAD) in type I diabetes mellitus is detected by a rapid precipitation assay.

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The authors present a method which permits screening for GAD reactivity in sera of large number of type I diabetes mellitus patients. Recombinant forms of rat GAD-65 and GAD-67 were produced by a *in vitro* eukaryotic expression system, labeled with ^{35}S-methionine and partialy purified by ion exchange and affinity chromatography. The tracer was then incubated with patient and control sera, immunocomplexes were collected by precipitation with protein A-Sepharose and radioactivity was counted. All the patients and controls in the study had their sera previously titrated for the presence of islet cell antibodies (ICA) accordingly to the Juvenile Diabetes Foudation (JDF). Comparisson of titers of ICA and reactivity against GAD showed a important correlation (r^2=0,74; p=0,0001), eventhough patients with low titers of ICA and high reactivity to GAD-65 as well as the opposite could be observed. This supports the fact that the autoantigen recognized by ICA has a different identity than glutamic acid decarboxilase. Concerning comparissons ICA versus GAD-67 and GAD-65 versus GAD-67, no correlations were found. Very few patients had reactivity against GAD-67 supporting the fact that this form of the enzyme is not as important as GAD-65 as a autoantigen in IDDM.

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INCIDENCE OF AUTOANTIBODIES TO GAD_{64} AND GAD_{67} IN NEWLY DIAGNOSED TYPE 1 DIABETIC PATIENTS AND PREDIABETIC INDIVIDUALS.

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Glutamic acid decarboxylase (GAD) has two forms GAD_{64} and GAD_{67}, which are encoded by two distinct genes. Previous studies have mainly focussed on incidence of antibodies to the major islet cell form, GAD_{64}. To analyze the incidence of autoantibodies to both GAD_{64} and GAD_{67} in newly diagnosed type-1-patients we developed baculovirus and COS cell expression systems for human GAD_{64} and GAD_{67}. ^{35}S-methionine labelled GAD_{64} and GAD_{67} from Sf9 or COS cells was immunoprecipitated with sera from 26 newly diagnosed IDDM patients, 14 prediabetic individuals and 10 controls. The expression of GAD in baculovirus infected Sf9 cells and COS cells varied between 20-50% and 2-5% of total protein respectively. There was a complete correlation between GAD_{64} antibodies and the islet 64kD protein antibodies. The analysis of sera is summarized in the table:

	New type 1	pre-type 1	controls
GAD_{64} antibody **or** GAD_{67} antibody positive	92%	86%	0%
GAD_{64} antibody positive	81%	79%	0%
GAD_{67} antibody positive	65%	57%	0%
GAD_{64} antibody **and** GAD_{67} antibody positive	54%	50%	0%
GAD_{64} antibody **and** GAD_{67} antibody negative	8%	14%	100%

The results show that both GAD_{64} and GAD_{67} are targets of autoantibodies in type 1 diabetes and that they contain both shared and distinct autoantigenic epitopes.

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HUMAN MONOCLONAL AUTOANTIBODIES TO GLUTAMATE DECARBOXYLASE SHARE FEATURES OF ICA AND 64KD-ANTIBODIES

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The first human monoclonal islet cell antibodies (MICA 1-6) obtained from an individual with Type 1 (insulin-dependent) diabetes mellitus were all shown to recognize the Type 1 diabetes-specific 64kD autoantigen glutamate decarboxylase (GAD). We here investigated whether typical features of ICA are represented by these monoclonals. We show that binding of MICA 1-6 to pancreatic sections is blocked by preincubation of sections with ICA-positive sera but not with normal human sera. In addition, MICA 1-6 reveal the typical features of epitope sensitivity to biochemical treatment of the target tissue which has been demonstrated for ICA, and which has been used to argue for a lipid rather than protein nature of target antigens. Analysis of staining patterns on islets by double immunofluorescence testing with a proinsulin-specific antibody however revealed that MICA 1-6 predominantly stain pancreatic ß-cells in agreement with the ß-cell specific expression of the target antigen GAD. In contrast an islet-reactive IgM monoclonal antibody obtained from a prediabetic individual stained all cells of the islet and lacked the tissue specificity of the IgG antibodies MICA 1-6. Our results provide direct evidence that MICA 1-6 share typical features of ICA and 64kD-antibodies and show that GAD is amongst the target antigens of ICA.

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SEQUENTIAL ANALYSIS OF ANTIBODIES TO GLUTAMATE DECARBOXYLASE DURING PREDIABETES AND RELATIONSHIPS WITH ISLET CELL ANTIBODIES

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Autoantibodies to Glutamate decarboxylase (GAD) were determined with an immunotrapping enzyme activity assay using rat brain homogenate in 282 siblings of 241 type 1 diabetic patients. Among 19 islet cell autoantibody (ICA) positive siblings, antibodies to GAD were found in 7 of 8 (87.5%) siblings with ICA titres above 20 JDF units, in 2 of 263 (0.7%) ICA negative siblings. High titre ICA and autoantibodies to GAD were tightly associated (x^2=182, p=0.0001). None of the siblings with low genetic risk i.e. HLA-different to the diabetic proband (n=64), was found antibody positive. Antibodies to GAD were present only in those relatives sharing at least one haplotype with the diabetic proband, including two ICA negative but HLA-identical siblings. Autoantibodies to GAD were present in 6 of 8 siblings who developed the disease, including one ICA negative sibling. Altogether, the simultaneous presence of autoantibodies to GAD and high titre ICA increases the positive predictive value for the disease from 66% to 75%. This study indicates therefore that autoantibodies to GAD are additional predictive markers for future development of Type 1 diabetes and should be now prospectively studied in high risk individuals as well as other autoantibodies to Beta-cell autoantigens.

OP 32
Health Care Delivery

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CARDIOVASCULAR DISEASE AND RISK FACTORS IN INSULIN DEPENDENT (TYPE 1) DIABETES IN EUROPE.

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The distribution of cardiovascular risk factors has been assessed by standardised methods in 3296 insulin-dependent (Type 1) diabetic subjects from 31 diabetes centres in 16 countries throughout Europe. For the age-ranges:- 15-29, 30-44 and 45-59 years, the proportions with Minnesota-coded ECG abnormalities (possible or probable ischaemia) or a history of myocardial infarction, angina, coronary artery bypass grafting or stroke was 6.%, 8% and 25% respectively. Overall, 10% of patients were on antihypertensive drugs, with a between-centre range from 3% to 28%. Excluding those on therapy, the proportion of patients with systolic blood pressure (BP) $\geq$ 140mmHg or diastolic BP $\geq$ 90mmHg was 19%, with a between-centre variation of 10% to 37%. Serum cholesterol concentrations were measured centrally and 14% of all patients had levels exceeding 6.5mmol/l, with a between-centre variation of 5% to 22%. On average, 32% of patients were current cigarette smokers, with a variation between centres of 22% to 42%. For insulin dependent (Type 1) diabetic patients in Europe, about 28% have blood pressure or serum cholesterol levels which exceed the St. Vincent Action Programme targets and the frequency of smoking is disappointingly high for a group at high risk for cardiovascular disease.

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DIABCARE - CONTINUOUS QUALITY IMPROVEMENT FEASIBILITY PHASE

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The WHO-IDF Steering Committee for the implementation of the St. Vincent Declaration established the DIABCARE Monotoring Group to provide the methodology for Continuous Quality Improvement(CQI) into Diabetes Care and the monitoring of the agreed targets.
This study was designed to test the feasibility of a Europe-wide activity on CQI.
For this purpose and also based upon previous European consensus activities a Diabetes Data Set was proposeed as DIABCARE Basic Information Sheet: 118 items in 10 main fields; a computer program was developed to facilitate feedback communication.
From 77 invited Diabetes centres in 21 European countries 47 centres(17 countries) participated. Within three months it was possible to aggregate data from 3969 diabetic patients, 71% on Basic Information Sheet, 12% on DIABCARE disk, 17% from different databases. The data were evaluated according to indicators for process and outcome quality. Within 4 weeks the centres were provided with this evaluation enabling them to compare their data with the average of all participating centres and those of their country .
The results indicated the feasibility of feedback-oriented communication among European centres as prerequisite for continuous quality improvement. Representatives of European Health Authorities considered the programme suitable for further implementation at national level.

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RESULTS OF A NATIONAL PROJECT ON DIABETES CARE

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The aim of the study was to collect data for the national implementation of the Saint Vincent Declaration. Of the total population of 10,364,599 of the Czech Republic in 1991, there were 479,125 known diabetics. The number has gone up by 42% during the past 10 years. The number of diabetic children and adolescents up to age 18 was 1,592 in 1991, representing an annual increase of 4.5%.

Diabetic complications: benign diabetic retinopathy occurs in 7.7% of diabetics, malign diabetic retinopatihy in 1.3%, blindness in 0.2%; nephropathy without renal insufficiency in 4.3%, with renal insufficiency in 0.7%. Health care for patients with diabetes is provided in 315 diabetes clinics, 12 regionals centers and 69 clinics for diabetic children and adolescents. 80% of diabetic children are on intensified insulin regimens, do their own blood glucose monitoring and adjust insulin dosage. With the aim to reach optimal and individualized management, a diabetes program was started in the Czech Republic in 1984. The results obtained serve as a basis for developing a project of integrated diabetes care within the intentions of the Saint Vincent Declaration.

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A NATIONAL PROFILE OF KNOWLEDGE AND PSYCHOSOCIAL STATUS IN 2239 INSULIN REQUIRING DIABETICS

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The aim of this study was to assess the prevalence of knowledge deficit and the psychosocial and attitudinal status of insulin requiring patients throughout the UK. Methods: 74 diabetes clinics were approached and agreed to participate in the survey in which consecutive attending insulin requiring patients were invited to complete the Diabetes Health Profile (DHP), a recently developed reliable and valid self-completion questionnaire measuring knowledge and four diabetes-related psychosocial problem domains: depression, anxiety, eating problems and attitudes to diabetes management. Results: 54 (73%) diabetes clinics throughout the UK returned 2239 DHP's Major knowledge deficits were identified and included failure to recognise that poor control leads to infections (31%), the need to continue carbohydrates (44%) and insulin (40%) when ill. Main psychosocial findings identified in the four diabetes-related domains were: depression (9%), eating problems (15%) and anxiety (14%). Positive attitudes to diabetes management correlated with knowledge of management principles, footcare, diet, glucose monitoring, less lowered mood and fewer eating problems ($r=0.16$ to 0.48: $p=0.0001$). Conclusion: This study confirms serious knowledge deficits and significant psychosocial dysfunction in this large sample. Systematic evaluation of clinic populations represents an important audit function.

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DIABETES EDUCATION IMPROVES METABOLIC CONTROL

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The goal of diabetes education is to stimulate self care by increasing the knowledge how to manage the disease in order to improve metabolic control. "Routine"-education at our out-patient department by physician, diabetic nurse and dietician resulted in low scores of knowledge (28(0-76)%; median (extremes)) and self care (0(0-100)%) in 20 type II diabetics, tested by an interview (group 1). Therefore, we developed an educational progamme (K.J. Jager et al., Diabeteseducatie: de patiënt met type II diabetes mellitus (1991). University Hospital Leiden), consisting of protocols for educators and information for patients. The dietician takes care of education for non-insulin-requiring patients; the education of insulin-requiring patients mainly is placed in the hands of the diabetic nurse. 29 Patients who followed the programme (group 2) were tested by the same interview as group 1. The groups did not differ with respect to age, sex, BMI and diabetes duration. The scores of knowledge (75(32-100)%) and self care (100(17-100)%) of group 2 were higher than those of group 1 ($p<0.001$); in contrast to group 1, in group 2 self care was related to HbA_{1c} ($p=0.05$). In group 2 (but not in group 1) HbA_{1c} decreased in one year (from 7.8±0.9%(mean±s.d.)to 6.8±0.6%($p<0.001$)).

Conclusion: In contrast to "routine"-education, a structured educational programme improves knowledge, self care and metabolic control of the diabetes.

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Long term effects of health education on health behavior, metabolic control and morbidity in newly detected type 2 diabetes

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One major purpose of the Diabetes Intervention Study (DIS) was to analyse the efficacy of intensified health education (IHE) on health behavior, metabolic control and inability to work. DIS is a controlled study with newly detected type 2 diabetics classified as diet controlled. 378 patients were randomly allocated to "conventional treatment" in diabetes clinics and 382 were included in a multiintervention trial with IHE.

IHE consisted of a structured program for diet recommendations for cessation of cigarette smoking and increased physical activity assisted by physiotherapists. IHE was continuously performed for five years with visits every three months in the DIS units.

After five years follow-up health behavior in the IHE group vs. controls was as follows: fat intake 107 g/d (43 %) in both groups, p/s ratio 0.39 vs. 0.26 ($p < 0.01$), alcohol consumption 60 g/wk vs. 70.3 (n.s), cigarette smoking (3.1 vs. 2.5 g (n.s) and physical activity score 328 vs. 174 ($p < 0.01$). The level of metabolic target parameters was improved by IHE: fasting blood glucose 9.38 vs. 8.6 mmol/l ($p < 0.05$) triglycerides 2.6 vs. 2.1 mmol/l ($p < 0.01$) and systolic blood pressure 154 vs. 143 mmHg ($p < 0.01$).

The patients in the IHE group were significantly less frequently absent from work. This was due to significantly lower frequency of cardiovascular illness and diabetes decompensation resp.

Our data convincingly demonstrate that IHE not only improves health behavior but also metabolic control, blood pressure and working disability.

OP 33
Insulin Action II

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TISSUE-SPECIFIC CHANGES IN GLUCOSE TRANSPORTER LEVELS FOLLOWING *IN VIVO* β_2-ADRENERGIC STIMULATION

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The association of hypertension, obesity and insulin-resistance is well-established. The role of the sympathetic nervous system in the pathogenesis of insulin-resistance is unclear. *In vitro* studies show inhibitory effects of catecholamines on insulin-mediated glucose transport. We studied the effect of an infusion of the β_2-adrenergic agonist fenoterol (F), on erythroid (GLUT1) and muscle/fat (GLUT4) glucose transporter levels in several insulin-sensitive tissues of the rat.

F(bromide) was infused at either 270 (high) or 27 (low) $\mu g.kg^{-1}.h^{-1}$ in freely moving male wistar rats for 1 or 6 days. Pair-fed and solution fluid infused rats served as controls. GLUT protein levels were measured in total membrane fractions of Tibialis anterior (TIB), Soleus (SOL), Epididymal fat (EPI) and Brown Adipose Tissue (BAT) using affinity-purified polyclonal antibodies.

F-infusion during 1 day at low dose, caused a decrease (25%) in GLUT4-levels in TIB. The high-dose increased GLUT1-levels in SOL (202%). Long-term infusions did not cause any change in GLUT-levels in these muscles.

F-infusions did not affect GLUT-levels in EPI. BAT GLUT1 levels were increased by short and long high-dose infusions (230 and 202%). BAT GLUT4 levels decreased after long low-dose infusion (20%).

We conclude that β_2-adrenergic stimulation caused tissue-specific, dose related changes in GLUT-levels. These changes could contribute to an alteration in basal and insulin-stimulated glucose disposal.

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ENHANCED INSULIN SENSITIVITY IN THE ATHLETES: INCREASED MUSCLE BLOOD FLOW AND GLUT-4 PROTEIN CONTENT

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The aim of the study was to examine the mechanisms of enhanced insulin sensitivity in well trained male athletes (n=9, age 25.4±1.2 yrs, body mass index BMI, 23.2±0.6 kg/m^2, VO_2max 57.6±1.0 ml/kg/min, mean±SE) as compared to untrained control subjects (n=10, VO_2max 44.1±2.4 ml/kg/min). Total body glucose disposal rate was 32% greater in the athletes (75.8±2.9 µmol/kg LBM/min, ($p<0.002$) than in the controls, as determined during 4 hour euglycemic insulin clamp with similar hyperinsulinemia (682±29 pM vs 670±19 pM, respectively). The difference was due to a 62% higher ($p<0.001$) nonoxidative glucose disposal in the athletes (50.8±2.4 µmol/kg LBM/min). Glucose oxidation rate (indirect calorimetry) was similar in the two groups. Whole body ($r=0.60$, $p=0.03$) and nonoxidative glucose disposal rate ($r=0.64$, $p<0.001$) correlated with VO_2max. In the basal state, forearm blood flow (pletysmography) was 64% ($p<0.05$) greater in the athletes (0.033±0.004 ml/ml forearm/min) and this difference remained during the clamp. Insulin infusion did not change the flow significantly. The glucose disposal rate correlated with forearm blood flow ($r=0.67$, $p<0.002$). Blood flow was inversely related to A–V glucose difference in the athletes ($r=-0.59$, $p<0.001$) and controls ($r=-0.46$, $p<0.001$). The amount of GLUT-4 transport protein (m. quadriceps femoris, western blot, densitometric units) was in the basal state 45% ($p<0.02$) and after the clamp 100% greater ($p<0.01$) in the athletes than controls. In conclusion: 1) In the athletes enhanced glucose uptake is due to increased nonoxidative glucose metabolism. 2) Glucose disposal rate is related to VO_2max and blood flow. 3) Increased blood flow and GLUT-4 protein content could be reponsible for increased glucose disposal in the athletes.

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Glucose transporter protein (GLUT 4) and glucose uptake is increased in human skeletal muscle after endurance training.

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Training increases insulin stimulated glucose uptake in whole body. The responsible tissue and mechanism are unknown. Seven, young, healthy men trained one leg for 10 weeks. The day after last training bout, a 3-step hyperinsulinemic, euglycemic clamp was performed, and glucose uptake in the legs were measured (arterio-venous differences x blood flow) at basal and at insulin levels of 54 ± 3, 174 ± 5, and 2323 ± 80 (mean ± SE) $\mu U \cdot ml^{-1}$. Muscle biopsies taken at basal were analyzed for content of GLUT 4 (Western blot) and insulin binding. Maximal oxygen uptake during one-legged bicycling increased in trained (T) compared with untrained (UT) leg (52 ± 2 vs 44 ± 2 $ml \cdot min^{-1} \cdot kg^{-1}$ ($P<0.05$), respectively). Glucose uptake was always increased in T compared with UT leg (Basal: 1.0 ± 0.2 vs 0.4 ± 0.1 ($P<0.1$); step I: 10 ± 2 vs 7 ± 2 ($P<0.05$); step II: 19 ± 3 vs 14 ± 2 ($P<0.05$); step III: 23 ± 2 vs 19 ± 2 ($P<0.05$) $mg \cdot min^{-1} \cdot kg^{-1}$). GLUT 4 content was 26 % higher in T compared with UT leg (15 ± 2 vs 12 ± 1 (arbitrary units)($P=0.078$)) and positively correlated with maximal insulin stimulated glucose uptake ($r = 0.85$ ($P<0.001$) $n = 14$). Number and affinity of the insulin receptors were identical in T and UT legs. Conclusion: Physical training increases insulin stimulated glucose uptake in human skeletal muscle. This can be explained by an increased amount of GLUT 4, whereas insulin binding is not influenced by training.

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HYPERGLYCEMIA ENHANCES SKELETAL MUSCLE GLYCOGEN SYNTHASE IN DIABETIC BUT NOT IN NORMAL CONTROL RATS.

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To define the impact of hyperglycemia on intracellular glucose disposal we performed glycogen synthase analysis on rectus muscle. Control and diabetic conscious rats were studied under four experimental condition: 1) Basal insulin (SRIF infusion) - Basal glucose; 2) Basal insulin - High glucose; 3) High insulin-Basal glucose; and 4) High insulin-High glucose. Under basal insulin (130 pmol/L) and high insulin(2,500pmol/L), hyperglycemia (15mmol/L) similarly increased glucose uptake and muscle and liver glycogen synthesis in control and diabetic rats. Hyperglycemia resulted in a significant ($p<0.05$) decline in the muscle glucose-6-phosphate in diabetic more than in control rats, thus suggesting activation of intracellular glucose metabolism. The diabetic skeletal muscle glycogen synthase, expressed as Fractional Velocity (FV) and Km, was severely resistant to insulin stimulation compared to controls ($FV_{0.1}$=0.31±0.04 vs 0.49±0.03; Km= 0.19±0.05 vs 0.10±0.01 mM; $p<0.01$), but was markedly responsive to glucose stimulation under basal ($FV_{0.1}$= 0.38±0.03 vs 0.21±0.03; Km= 0.10±0.01 vs 0.35±0.08) and high insulin ($FV_{0.1}$= 0.65±0.07 vs 0.31±0.04; Km= 0.11±0.02 vs 0.19±0.05 mM). By contrast, in control rats, hyperglycemia did not exert any stimulatory effect on skeletal muscle glycogen synthase. Thus some metabolic alteration associated with the diabetic state renders the skeletal muscle glycogen synthase selectively responsive to glucose stimulation. This may represent a compensatory mechanism for the impairment in insulin's activation of this enzyme in diabetes.

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IS EXPRESSION OF THE GLYCOGEN SYNTHASE GENE IN SKELETAL MUSCLE ALTERED IN TYPE 2 DIABETES?

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To examine whether altered expression of the glycogen synthase (GS) gene contributes to impaired skeletal muscle glucose metabolism in Type 2 diabetes, we measured glucose storage (euglycemic clamp), and GS protein (Western blot with polyclonal C-terminal antibody JT90) in 16 patients with Type 2 diabetes (age = 55 ± 3 yrs; BMI = 28 ± 1 kg/m^2; FPG = 10.2 ± 0.7 mmol/l) and in 16 healthy control subjects (age = 55 ± 3 yrs; BMI = 26.4 ± 0.6 kg/m^2; FPG =5.5 ± 0.1 mmol/l). GS mRNA was measured with dot blot analysis in part of the subjects. Insulin-stimulated glucose disposal (2.52 ± 0.26 vs 6.12 ± 0.36 mg/kg.min) and glucose storage (1.08 ± 0.22 vs 3.7 ± 0.29 mg/kg.min) was reduced in Type 2 diabetic patients vs controls (both $p<0.001$). Basal GS mRNA did not differ between diabetics and controls (291 ± 9 vs 327 ± 16 pg/μgRNA). The basal (42 ± 6 vs 53 ± 4 densitometry units/μg muscle) and insulin-stimulated (45 ± 8 vs 57 ± 5 d.u./ug muscle). GS protein content was not significantly different between Type 2 diabetics and controls. Basal GS protein content correlated positively with glucose disposal ($r = 0.76$; $p<0.01$) and glucose storage ($r = 0.64$;$p<0.05$) in control subjects but not in Type 2 diabetic patients ($r = -0.39$ and $r=-0.46$). Conclusion: 1) In healthy man, glucose storage is proportional to muscle GS protein content 2) This relationship is disturbed in Type 2 diabetes. 3) Thus, functional abnormalities of GS may contribute to insulin resistance in Type 2 diabetes.

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MUSCLE PROTEIN ABUNDANCE OF GLYCOGEN SYNTHASE AND PHOSPHOFRUCTOKINASE IN INSULIN RESISTANT TYPE 2 DIABETIC PATIENTS.

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Insulin resistant glucose metabolism (gm) in muscle is a major pathogenetic feature of Type 2 diabetics and their first degree glucose tolerant relatives. We have studied protein abundance and activity of 2 key enzymes in gm: glycogen synthase (GS) and phosphofructokinase (PFK). For immunoblotting, antipeptide rabbit antibodies (abs) specific for human muscle GS and PFK were raised. In muscle homogenate GS and PFK abs recognized single dominant bands with Mr of 84 kDa and 80 kDa, respectively. Biopsies of quadriceps muscle were taken in the basal state from 16 Type 2 diabetics and 14 matched controls and analysis showed a 28% decrease ($p < 0.05$) in total GS activity whereas the activity of PFK and the protein levels of both GS (controls: 86 ± 6 vs diabetics: 86 ± 5 arb.units/100 μg protein) and PFK (controls: 85 ± 5 vs diabetics: 83 ± 3 arb.units/100 μg protein) were similar. In a subgroup of 9 diabetics and 7 controls a 4 h euglycemic hyperinsulinemic clamp plus indirect calorimetry were performed. In diabetics insulin stimulated nonoxidative gm was decreased by 47% ($p < 0.005$) and the relative activation of GS by glucose-6-phosphate was 33% lower ($p < 0.02$), whereas the GS protein level was unaltered. During insulin clamp, glucose oxidation and the activity and protein level of PFK remained normal. In conclusion: Reduced GS activity in muscle of Type 2 diabetics occurs in the presence of normal GS protein abundance.

OP 34
Glycation

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ALTERED FEATURES OF HUMAN VASCULAR SMOOTH MUSCLE CELLS ON GLYCOSYLATED FIBRONECTIN.

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Human vascular smooth muscle cells (hVSMC) play an essential role in vascular function, that could be altered in diabetes mellitus as a consequence of increased nonenzymatic glycosylation. To evaluate whether glycosylation of matrix components impairs the relationship between matrix and cells, we studied the influence of fibronectin glycosylation on adhesion and morphological features of cultured hVSMC derived from microcirculation. Cells were allowed to adhere for 2 hours to polystyrene plates coated with different concentrations of glycosylated (GF) or nonglycosylated fibronectin (F). GF was obtained by a 4 week incubation in a medium containing 200 mmol/l D-Glucose, F was incubated in the same medium without D-Glucose. hVSMC, passage 5-10, showed reduced adhesion GF compared to F: in particular, the number of cells (m±sem) per microscopic field at 320x was 132±10 vs 233±18 at 10 μg/ml (n=4, $p<0.01$). Number of hVSMC adhering to F was stable with time, while it decreased progressively with GF: 3rd hr, 69±6.6 ($p<0.01$) vs the previously described value at the 2nd hr. On glass coverslips coated with F, hVSMC were numerous and appeared flat, with a large cytoplasm, whereas on GF they were few, small and mainly round. Thus, hVSMC showed impaired adhesion and different morphologic features when adhering to nonenzymatically glycosylated fibronectin. This phenomenon may be relevant for the pathogenesis of diabetic angiopathy.

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HYPERGLYCAEMIA ALTERS THE PHYSICO-CHEMICAL PROPERTIES OF PROTEINS IN ERYTHROCYTE MEMBRANES OF DIABETIC PATIENTS

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The dynamic properties of erythrocyte membranes in children suffering from diabetes and of control erythrocyte membranes subjected to *in vitro* glycation have been investigated by means of fluorescence quenching of membrane tryptophan residues and ESR spectroscopy. It has been revealed that the apparent distance separating the membrane protein tryptophan and the bound 1-anilino-8-naphthalenesulphonate (ANS) molecules is decreased in erythrocyte membranes from children with diabetes, which results in a significant increase of the maximum energy transfer efficiency. Likewise, the above parameters appeared to become successively altered due to the nonenzymatically attached glucose. The decrease in the ratio h_w/h_s of maleimide (MSL) bound to membrane protein -SH groups of erythrocytes in diabetes may ensue the lowered membrane protein immobilization in the plane of lipid bilayer. In turn, the relative rotational correlation time (τ_c) of iodoacetamide spin label (ISL) was increased in the membranes of diabetic subjects. When membranes were incubated *in vitro* with the increasing concentrations of glucose, the parameter h_w/h_s was reduced respectively by 35% and by less than 5% at the glucose concentrations of 16.1 mM and 5.4 mM. These alterations were accompanied by the corresponding elevation of the relative rotational correlation time (τ_c) of iodoacetamide spin label (ISL) (increased by over 150% and 10%, respectively), thus suggesting that the conformational changes in membrane proteins may occur at both the intrinsic and exposed thiol groups. We conclude that the altered erythrocyte membrane fluidity in diabetes underlies the changed dynamics of diabetic red blood cells. The coinciding of the *in vivo* as well as the *in vitro* data supports the idea that nonenzymatic glycosylation of membrane proteins may be the major factor attributable to these alterations in the dynamic properties of erythrocyte membranes.

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AMINOGUANIDINE INHIBITS THE NONENZYMATIC MODIFICATION OF PROTEINS BY LIPOPEROXIDATION DERIVED ALDEHYDES

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Aim: Lipoperoxidation derived aldehydes, present at increased levels in diabetic patients, form Schiff bases with protein amino groups, a reaction which has much in common with glycation and has pathological consequences, specially in atherogenesis. Since Aminoguanidine has been shown to react with Amadori product-derived aldehydes and is thought to inhibit advanced glycation through scavenging of carbonyl intermediates, we investigated its effect on the modification of proteins by lipoperoxidation derived aldehydes.
Materials and Methods: Human Low Density Lipoprotein (LDL) was incubated with copper sulphate in phosphate buffer at 37 °C for 24 hours; Bovine Serum Albumin (BSA) was incubated with malondialdehyde under the same conditions. Differnt concentrations of Aminoguanidine (5, 10, 25, 50 mM) were added to some samples. Samples were analyzed by agarose gel electrophoresis, TBA reaction and spectroscopy.
Results: Aminoguanidine inhibited in a dose dependent way the increased electrophoretic mobility and TBRAS generation of oxidatively modified LDL. It also inhibited in adose dependent way the increased electrophoretic mobility and 260-400 nm absorbance of malondialdehyde-modified BSA.
Conclussions: Aminoguanidine is a powerful inhibitor of the nonenzymatic modification of proteins by lipoperoxidation derived aldehydes. The effects of both added or in-situ generated aldehydes were inhibited.

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THE MECHANISM OF LOWERING OF NONENZYMATIC GLYCATION BY VITAMIN C

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Previous experiments have documented that vitamin C inhibits nonenzymatic glycation of serum proteins, collagens and renal basement membranes in vitro and in vivo. In present work we investigated molecular mechanisms of vitamin C effects in vitro. We study the incorporation of D(U-^{14}C)glucose, L(1-^{14}C)ascorbic acid and L(1-^{14}C) dehydroascorbic acid into serum albumin and collagen IV during incubation for 1-28 days. We used different concentrations of glucose (5-33mmol/l) and vitamin C (0.1-5.0mmol/l), different molar ratios and three different pH. Simultaneously we measured the reaction with nitrobluetetrazolium, thiobarbituric acid and fluorescence. Results: Ascorbic acid significantly reduced glycation by 18-30%, while dehydroascorbic acid enhanced glycation (plus 8-14%). Effects of ascorbic/dehydroascorbic acids were dependent on their concentrations (molar ratio ascorbic acid: glucose from 1:200). Inhibitory/stimulatory effects depended on pH: both were more expressed in pH 7.8 than 6.8. Incorporation of ascorbic acid into proteins was higher than incorporation of dehydroascorbic acid (plus 20-60%). Binding of radioactive ascorbic and dehydroascorbic acids was almost independent on glucose concentration in range 5-33 mmol/l during the first 7 days of incubation.
We conclude that several molecular mechanisms could be involved in the effects of vitamin C on nonenzymatic glycation.

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INHIBITION OF GLYCATION BY A FLAVONOID AGENT IN EXPERIMENTAL DIABETES.

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Glycation is considered as a pathogenetic factor for the development of diabetic complications. Since prevention of this phenomenon must be started from the onset of diabetes and sustained for the whole life of the patient a useful drug must be effective, cheap and without side-effects. Diosmin (DS), a commercially available flavonoid meets with all this criteria and was tested in Wistar rats made diabetic with Streptozotocin (Dia). Half the animals (Dia+DS) received 70mg/kg/day Diosmin. After 8 months blood was taken and skin samples were collected. All parameters were different between controls and Dia.

	Contr.	Dia.	*	Dia+DS	**
Body weight (g)	357±62	208±63	0.05	271±26	0.01
Glycemie (mmol/l)	5.4±1.9	29.7±2.9	ns	28.5±3.9	0.000
Insulin. (µU/ml)	10.9±3.6	4.4±0.7	0.05	3.78±0.4	0.050
HbA1 (%)	3.0±0.6	6.2±0.9	0.05	4.9±1.1	0.01
Skin fluo. (U/mg)	9.1±2.2	21.4±4.9	0.05	13.2±5.3	ns

* p vs Dia ** p vs C

Glycemia was not different in the diabetic animals. In the treated group the body weight was higher. Although the insulinemia was lower in the Dia+DS group, the HbA1 value was significantly lower. This decreased glycation is confirmed by the skin collagen fluorescence (parameter of collagen glycation) which is not significantly different from the controls. Conclusion: in STZ- rats the flavonoid Diosmin inhibits the glycation of different proteins. Clinical implications in humans remain to be investigated.

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REDUCED FORMATION OF THE EARLY AMADORI PRODUCTS INDUCED BY D-LYSINE TREATMENT IN EXPERIMENTAL DIABETES.

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Diabetic hyperglycemia has been associated with increased nonenzymatic glycation (NEG) of proteins, possibly contributing to the development of late diabetic complications. D-lysine, the inactive isomer of L-lysine, has been shown to competitively reduce protein NEG in vitro. To study D-lysine effect in vivo, 5 streptozotocin (60 mg/kg b.wt.) diabetic Sprague-Dawley rats were treated at diagnosis for 45 days with two daily (mid-morning and mid-afternoon) subcutaneous injections of D-lysine (0.5 g/ml/die). Five diabetic rats were injected with placebo (equal volumes of physiological saline). At study completion, percent glycation was measured in hemoglobin, serum and eye lens proteins by boronic acid affinity column chromatography. Serum creatinine was evaluated by the Jaffe reaction. Blood glucose and serum creatinine levels were similar in both diabetic groups (>390 mg/dl and <1.2 mg/dl). However, a significant reduction in glycated hemoglobin (2.98%±0.75% vs 4.02%±0.46%; $p<0.05$), glycated serum proteins (1.00%±0.40% vs 2.52%±1.15%; $p<0.02$) and glycated eye lens proteins (0.64%±0.40% vs 1.52%±0.94; $p<0.05$) was found in D-lysine treated vs placebo treated diabetic animals. These results demonstrate that D-lysine, for the time and at the dose used, is not nephrotoxic and shows a significant inhibitory effect on the formation of early NEG Amadori products. Thus D-lysine, by interfering with NEG, could affect pathogenetic mechanisms leading to late diabetic complications.

OP 35

Na+ Counter Transport

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ISOLATION OF THE Na+/H+ ANTIPORTER GENE EXPRESSED IN HUMAN PROXIMAL TUBULE CELLS

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The gene coding for the ubiquitously expressed Na+/H+ antiporter (NAH1) has recently been cloned. Increased activity of the antiporter has been found in platelets and lymphocytes of patients with diabetic nephropathy and essential hypertension. Recently, evidence has accumulated from animals that tissue specific forms of the antiporter may exist. The aim of this study was to isolate and characterise the Na+/H+ antiporter from human proximal tubule cells (PTC). Highly purified PTC were isolated from renal transplant and nephrectomy biopsies. From these, cytoplasmic RNA was prepared and first strand cDNA transcribed. Oligonucleotide primers to specific regions of NAH1 were used to amplify the cDNA using the polymerase chain reaction. A 700 base pair fragment was amplified from the 3' end of the cDNA. Oligonucleotide primers to NAH1 were not able to amplify any other regions of cDNA derived from PTC whilst these regions could be amplified from cDNA obtained from lymphocytes. Preliminary sequencing data of the 700 bp fragment demonstrates a <50% homology with NAH1. These results suggest that PTC express a tissue specific form of the Na+/H+ antiporter. Its importance in the pathogenesis of diabetic nephropathy or hypertension is unknown.

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SODIUM-HYDROGEN ANTIPORT ACTIVITY IS ELEVATED IN TYPE 2 DIABETIC PATIENTS WITH MICROALBUMINURIA AND HYPERTENSION

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Na^+/H^+ antiport plays a central role in intracellular pH(pH_i) homeostasis and cell growth control as well as in renal sodium reabsorption. An increased activity of leucocyte and fibroblast Na^+/H^+ antiport and of red blood cell Na^+/Li^+ countertransport(Na^+/Li^+ CT) has been reported in Type 1 diabetic patients with nephropathy. No information is available in Type 2 diabetic patients. We therefore measured Na^+/Li^+ CT in red blood cells and Na^+/H^+ antiport activity in serially passaged cultured skin fibroblasts from 5 Type 2 diabetic patients with microalbuminuria and hypertension(Group 1), 5 normotensive and normoalbuminuric Type 2 diabetic patients(Group 2) and from 5 matched controls (Group 3). Na^+/H^+ antiport activity (measured by a microfluorimetric technique using the pH indicator BCECF as the rate of amiloride-sensitive intracellular alkalinization after acid-loading with NH_4Cl) was significantly elevated in Group 1 compared to Group 2 and Group 3 (6.24±1.95 mmol H^+/l/min vs 2.79±0.66 vs 4.03±0.38; p<0.01 for both) when $pH_i$was 6.5, while no differences were observed at pH_i = 6.7 (0.71±.08 vs 0.38±.74 vs 0.46±.60). Basal pH_i and buffering power capacity were similar in the three groups. Na^+/Li^+ CT was higher in Group 1 (498±123 mmol/l/min) than in Group 2 (309±33;p<0.01) and Group 3 (245±56;p<0.01). There was a significant correlation between these two transport system activities (r = 0.565;p<0.05). These findings suggest that cultured fibroblasts from Type 2 diabetic patients with microalbuminuria and hypertension have intrinsic abnormalities in cation cell handling, independently of the metabolic disturbances of diabetes.

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INTRACELLULAR pH AND Na^+/H^+ ANTIPORT ACTIVITY OF CULTURED SKIN FIBROBLASTS IN DIABETIC NEPHROPATHY.

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In essential hypertension and diabetic nephropathy, blood leucocytes show increased Na^+/H^+ antiport activity. It is unclear if these effects are dependent on plasma factors such as glucose and growth factors. We therefore investigated the intracellular pH (pH_i) and Na^+/H^+ antiport activity of cultured skin fibroblasts from 12 controls (Co), 12 Type 1 diabetic patients with nephropathy (DN) and 10 normoalbuminuric diabetic patients (D), using the pH sensitive probe, bis-carboxyethyl-carboxyfluorescein. The pH_i was more alkaline in DN (6.91 ± SD 0.07) compared to Co (6.82 ± 0.05, P<0.002) or D (6.82 ± 0.05, P<0.002). This was due to increased Na^+/H^+ antiport activity at pH_i 6.5, since ethylisopropyl-amiloride sensitive H^+ efflux via the Na^+/H^+ antiport was higher in DN (8.31 ± 2.9 mmol l^{-1} min^{-1}) compared to Co (3.69 ± 1.79 mmol l^{-1} min^{-1}, P<0.001) or D (4.22 ± 1.13 mmol l^{-1} min^{-1}, P<0.001). The V_{max} of the antiport was similar in all three groups. Therefore, the increased resting pH_i of fibroblasts from DN was attributable to an increased affinity of the antiport for intracellular H^+ rather than altered V_{max}. Phosphorylation could lead to changes in antiport H^+ affinity, and may be implicated in cellular changes of DN. Furthermore, using discriminant analysis on pH_i and Na^+/H^+ antiport activity, DN was separated from the other 2 groups with a sensitivity of 92 % and a specificity of 100 %.

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SODIUM-HYDROGEN ANTIPORTER ACTIVITY IS ELEVATED IN OBESITY AND TYPE 2 DIABETES ONLY IN THE PRESENCE OF ARTERIAL HYPERTENSION.

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It is unclear whether obesity and type 2 diabetes show an elevated prevalence of arterial hypertension because they have high insulin levels or because they share with arterial hypertension some genetic markers , as the Na+/H+ antiporter. To evaluate whether obesity and type 2 diabetes present an elevated activity of this ion exchanger we studied its characteristics in peripheral blood lymphocytes from 10 healthy controls, 6 normotensive obese patients, 10 normotensive obese type 2 diabetics, 4 hypertensive obese patients and 4 hypertensive diabetic patients. The activity of the Na+/H+ antiporter was assayed by acid loading the 6-carboxyfluorescein-loaded cell monolayers with nigericin in mannitol solution. The rate of Na+-induced, EIPA-sensitive alkalinization depended upon starting intracellular pH and external Na+ concentrations. No difference was observed in external Na+- and inner H+-dependence of the antiporter in normotensive patients and in healthy controls, whereas the activity as a function of inner proton concentration was higher in hypertensive obese and type 2 diabetic patients than in normotensive controls and patients (p=0.000): in particular, at pH 6.2 it was 19.9±1.4 mmol H+/l/min (m±sem) in healthy controls, 17.6±1.6 in normotensive obese patients, 17.6±1.8 in normotensive type 2 diabetics, 44.6±4.1 in hypertensive obese patients and 33.5±1.6 in hypertensive type 2 diabetics. In conclusion, Na+/H+ antiporter hyperactivity is not a marker of obesity and type 2 diabetes independently of arterial hypertension.

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RED CELL SODIUM/LITHIUM COUNTERTRANSPORT ACTIVITY REFLECTS A PARENTAL COMPONENT IN TYPE I DIABETES.

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Elevated red blood cell sodium/lithium countertransport activity (RBC Na/Li CNT) is an intermediate phenotype of essential hypertension predominantly explained by inheritance in the general population, and has been associated with nephropathy in Type I (insulin-dependent) diabetes mellitus. Whether the metabolic abnormalities of Type I diabetes mellitus may blur the genetic component in RBC Na/Li CNT is controversial. We investigated whether parental RBC Na/Li CNT may associate with RBC Na/Li CNT in 43 Type I (insulin-dependent) diabetic probands (21 men, 22 women; mean±SD age = 17±7 years, duration of diabetes = 4.3±2.8 years; HbAIc = 8.7±2.2%) without clinical proteinuria. RBC Na/Li CNT ranged between 0.01 and 0.93 mmol/l_{rbc}/hr among parents, with higher values in men than in women (median: 0.39 vs 0.33 mmol/l_{rbc}/hr, p=0.011). RBC Na/Li CNT activity was not related between spouses (r=0.087; p=0.58). RBC Na/Li CNT was similarly distributed in Type I diabetic offspring, ranging between 0.05 and 0.84 (median: 0.36) mmol/l_{rbc}/hr, and was positively associated with midparental RBC Na/Li CNT (r=0.363; p=0.016), but not with gender, duration of diabetes, insulin dose, glycated haemoglobin and blood pressure. Consistently with findings in pedigrees from the general population, RBC Na/Li CNT may retain a predominant genetic component independently of the concomitance of Type I diabetes mellitus.

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ERYTHROCYTE TRANSMEMBRANE SODIUM TRANSPORT IN TYPE 1 (INSULIN-DEPENDENT) DIABETES MELLITUS.

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In order to study possible differences in erythrocyte transmembrane sodium transport between type I diabetics and controls and its relation with clinical and analytical features in the formers, the Na^+-K^+ pump activity, Na^+-K^+cotransport activity, passive Na^+ permeability, sodium-lithium countertransport activity (Na^+-Li^+CT) and intracellular Na^+ ($[Na^+]_i$) have been measured in erythrocytes proceeding from 101 type 1 diabetics and 47 healthy controls by means of basal Na^+ efflux determination, its inhibition by ouabain or bumetanide and its stimulus by extracellular Li^+. $[Na^+]_i$, Na^+-K^+ pump activity and Na^+-K^+cotransport activity were significantly (p<0.05) lower in diabetics than in controls (6.39±1.25 vs. 6.84±1.04 mM/l cell, 1421±250 vs. 1516±281 µM/L cells/h, 105.1±73.6 vs. 130.8±66.3 µM/L cells/h, respectively). Microalbuminuric diabetics with duration of diabetes greater than 190 months had CT Na^+/Li^+ values greater than those free from this abnormality (115.33±47.91 vs. 73.33±35.27 µM/L cells/h, p=0.062). Diabetics with HbA_{1c}>9.5% had CT Na^+/Li^+ values significantly (p<0.05) greater than those with HbA_{1c}<9.5% and controls (127.77±52.27 vs. 97.97±45.78 and 98.43±33.58 µM/L cells/h, respectively). According these data, type 1 diabetes is associated with several abnormalities in erythrocyte transmembrane sodium transport. Particularly CT Na^+/Li^+, considered a vascular and diabetic kidney disease risk factor, holds an important relation with glycemic control.

OP 36
Islets II

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MOLECULAR CLONING OF A NOVEL CADHERIN FROM THE PANCREATIC BETA CELL

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Calcium-dependent cell adhesion molecules (cadherins) play a key role in organogenesis and regulation of cell growth and cell-cell interaction. The diversity of the cadherins in pancreatic islet cells was evaluated using a polymerase chain reaction procedure using 8- and 128-fold degenerate oligonucleotide primers based on the conserved carboxy-terminal domain of (N)eural, (E)pithelial and (P)lacental-cadherin cDNA sequences. A novel cadherin sequence was identified among the 22 clones obtained from pancreatic ß-TC3 cDNA. Also present were N-CAD (n=8) and E-CAD (n=6). Specific PCR primers for the new cadherin were constructed and used in conjunction with the cloned cDNA fragment to obtain clones from a ß-TC3 cDNA library. The nucleotide sequence of the novel cadherin was most closely related to N-CAD (69% sequence identity) and the encoded protein appeared to have a similar structure with a corresponding pro-sequence cleavage site, extracellular, transmembrane and intracellular domains. Its mRNA (6.8kb) was present in endocrine but low in exocrine pancreatic tissue indicating that this molecule may serve a specific function in the islet.

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REGULATION OF AMYLIN IN HUMAN PANCREATIC ISLETS

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Amylin is expressed in human pancreas and cosecreted with insulin in response to different secretagogues. **Aims:** To investigate the regulation of amylin expression and secretion in normal and diabetic human islets. **Methods:** Groups of 24 normal and diabetic islets were incubated for 90 min at 5.5 and 16.7 mM glucose and 5µM forskolin. In other experiments amylin response was analyzed after 1 and 7 days culture. Amylin and Insulin were determined by RIA. Northern blot analysis for amylin and insulin were proceed from cultured islets. **Results:** Amylin and insulin secretion from diabetic islets didn't increase significatively at high glucose stimulus (65 ± 5 *f*mol Amy/islet, 61 ± 1 *f*mol/islet) and (15 ± 1 pmol IRI/islet, 38 ± 1 pmol/islet). Forskolin produced a significatively increase of amylin more than insulin (344 ± 147 *f*mol/islet versus 61 ± 10 pmol/islet). In normal islets amylin didn't increase at 16.7mM glucose in opposite to insulin (131 ± 30 *f*mol Amy/islet, 176 ± 27 *f*mol/islet) and (70 ± 1 pmol IRI/islet, 149 ± 1 pmol/islet). The effect of culture at low and high glucose concentration produced an increase of amylin response independently of the acute glucose stimulus. The mRNA expression of amylin increased at high glucose concentrations. **Conclusions:** Amylin is differentially regulated than insulin in normal and diabetic islets. The chronic exposure of islets to high glucose concentrations would result in overproduction of amylin and consequently to play a physiopathological role in non-insulindependent diabetes.

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DIRECT DEMONSTRATION THAT cAMP POTENTIATES CALCIUM-DEPENDENT EXOCYTOSIS IN SINGLE B-CELLS.

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Exocytosis of insulin requires an elevation of the cytoplasmic Ca^{2+}-concentration ($[Ca^{2+}]_i$). Little is known about the molecular processes that link changes in $[Ca^{2+}]_i$ to activation of the exocytotic machinery. By using microfluorimetry, the patch-clamp method and circuit-analysis techniques to measure changes in membrane capacitance ($C_m \propto$ membrane area), we have monitored the correlation between Ca^{2+}-currents, $[Ca^{2+}]_i$ and insulin release in single NMRI-mouse B-cells. Voltage-clamp depolarizations (200-500 ms to 0 mV) evoked Ca^{2+}-currents, increased $[Ca^{2+}]_i$ by ≈500 nmol/l and stimulated exocytosis (detected as an increased C_m). Application of 80 µmol/l cyclic AMP (cAMP), by photorelease from a caged inactive precursor, markedly (≥10-fold) potentiated the exocytotic response without affecting basal $[Ca^{2+}]_i$ or depolarization-induced $[Ca^{2+}]_i$-transients. The effect of cAMP was rapid and manifest within 1 s. By contrast, cyclic GMP (80 µmol/l) lacked effects on exocytosis, suggesting the effect is specific for cAMP. This represents the first *direct* demonstration that cAMP potentiates $[Ca^{2+}]_i$-dependent exocytosis at the single-cell level. In biochemical studies it is not possible to distinguish between this possibility and the alternative explanation that cAMP leads to the recruitment of previously non-secreting B-cells.

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CYCLIC AMP SYNCHRONIZES CYTOPLASMIC Ca^{2+} OSCILLATIONS IN CLUSTERS OF PANCREATIC ß-CELLS

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The cytoplasmic Ca^{2+} ($[Ca^{2+}]_i$) response to glucose has been studied in clusters of 5-14 pancreatic ß-cells with the indicator fura-2 using digital image analysis. Raising glucose from 3 to 11 mmol/l often induced large-amplitude oscillations of $[Ca^{2+}]_i$. The oscillations were initially synchronized within microdomains. During glucose stimulation the entire cluster became functionally coupled within 15 min and oscillated with a frequency of 0.3-0.7/min. Increasing cyclic AMP by addition of its dibuturyl derivative or glucagon resulted in an immediate synchronization of adjacent microdomains. It was tested whether the coupling could be reversed by n-heptanol, a compound known to block intercellular transfer of dye molecules between ß-cells. When this alkanol was introduced at a concentration of 0.5-2 mmol/l for 8-15 min, the oscillatory pattern became irregular although with a persistent coordination of $[Ca^{2+}]_i$ oscillations. At higher concentrations of heptanol, the large-amplitude oscillations disappeared both in aggregates and in individual ß-cells. Similar results were obtained with the junctional uncoupler 18-α-glycyrrhetinic acid. Apart from interfering with the glucose-induced $[Ca^{2+}]_i$ oscillations these agents were not sufficiently effective to break the electrical coupling between ß-cells, which may require fewer connexons than effective dye transfer. The results provide direct evidence that cyclic AMP induces electrical coupling in ß-cell clusters.

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SERUM FROM INSULIN-DEPENDENT DIABETICS INCREASE L-TYPE Ca^{2+}-CHANNEL ACTIVITY AND CYTOPLASMIC FREE Ca^{2+} IN INSULIN-PRODUCING CELLS.

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Mouse pancreatic ß-cells and RINm5F cells, incubated for 24 h with serum from patients with newly diagnosed insulin-dependent diabetes mellitus (IDDM), revealed increased voltage-dependent Ca^{2+} currents, an effect more pronounced in the cell-attached than in the whole-cell recordings. The currents were twice as large as those recorded in cells treated with normal serum. To rule out an effect of IDDM serum on T-type Ca^{2+}-channels in RINm5F cells, the influence of L-type Ca^{2+}-channels was reduced by 20 µM Cd^{2+}. IDDM serum was without effect on the remaining currents. The stimulatory effect of IDDM serum on the whole-cell Ca^{2+}-current amplitude and Ca^{2+}-channel activity was most likely attributed to an increase in the open probability, due to reduction of the slow closed times. Cells treated with IDDM serum displayed greater transients in cytoplasmic free Ca^{2+}, when depolarized with 30 mM K^+, than cells treated with control serum. Sera from three different IDDM subjects demonstrated similar results. Whereas IDDM serum subjected to ammonium sulphate precipitation remained active, serum concentrated upon passing a protein A column lost its effect.IDDM serum deprived of IgM was without stimulatory effect. It is suggested that proteins in the IgM fraction of IDDM serum activate a soluble cytosolic constituent interacting with voltage-activated L-type Ca^{2+}-channels in insulin-producing cells, resulting in increased cytoplasmic free Ca^{2+}.

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A NOVEL LOW-CONDUCTANCE K^+-CHANNEL PRODUCING MEMBRANE POTENTIAL OSCILLATIONS IN MOUSE PANCREATIC β-CELLS.

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We have used the patch-clamp technique to investigate the pharmacological and biophysical properties of an oscillating Ca^{2+}-activated K^+-current induced by GTPγS in mouse pancreatic β-cells. These K^+-conductance changes are evoked by periodic increases in the cytoplasmic Ca^{2+}-concentration and transiently repolarize the β-cell thus giving rise to a bursting pattern. The K^+-conductance oscillations were reversible inhibited by carbamylcholine (300 µM). By contrast, α_2-adrenergic stimulation did not alter oscillatory behavior but evoked a small sustained outward current. At 0 mV K^+-currents evoked by GTPγS were highly sensitive to TEA (85% block by 1 mM). The TEA-resistant component which carried 80% of the current at -40 mV, was neither affected by apamin (1-5 µM) nor tolbutamide (0.5 mM). The current was highly selective for K^+ as evidenced by a 51 mV change of the reversal potential for a seven-fold change in external K^+. Fluctuation analysis yielded a single channel conductance of 0.4 pS in symmetric KCl-solutions (140 mM) corresponding to approximately 0.1 pS in physiological ionic gradients. These results indicate that there exists a novel Ca^{2+}-gated K^+-conductance in pancreatic β-cells which may contribute to the oscillatory electrical pattern seen at intermediate glucose concentrations.

OP 37
Diabetes and the Heart

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CARDIOVASCULAR RISK FACTORS IN RELATION TO GLUCOSE TOLERANCE IN A CAUCASIAN POPULATION

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Aim of the study was to evaluate the relationship between cardiovascular risk factors and the degree of glucose intolerance in a caucasian population. Randomly selected subjects (N=2488) underwent an Oral Glucose Tolerance Tests (OGTT). After classification based on WHO-criteria, a second OGTT was performed within 3-5 weeks in all subjects with IGT and diabetes and randomly (1:3) in those with normal glucose tolerance. Subjects were classified into Normal/Normal, Normal/IGT, IGT/IGT, IGT/DM and DM/DM glucose tolerance categories, on the basis of two tests. Mean fasting and 2h-postload glucose across the Normal/Normal, Normal/IGT, IGT/IGT, IGT/DM and DM/DM-categories were 5.4, 5.7, 6.0, 6.6, 7.5 mmol/l and 5.1, 7.7, 9.1, 10.7, 14.2 mmol/l, respectively. The Body-Mass-Index (BMI) were 26.1, 27.3, 28.1, 28.7 and 28.8. Significant trends, adjusted for age, gender and BMI, were found across the various categories for Waist-Hip-Ratio (0.89, 0.90, 0.94, 0.95, 0.96, $p<0.001$), triglyceride (1.5, 1.8, 2.1, 2.1, 2.2, $p<0.001$), HDL-cholesterol (1.4, 1.3, 1.2, 1.2, 1.1, $p<0.001$), systolic blood pressure (131, 141, 145, 145, 146, $p<0.001$) and diastolic blood pressure (80.1, 83.7, 84.5, 85.2 and 85.0, $p<0.001$) whereas no such differences were found for total and LDL-cholesterol. In conclusion, this analyses clearly demonstrates that the different glucose tolerance categories based on repeated OGTT are related to various known cardiovascular risk factors.

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PROTECTION FACTORS AGAINST SEVERE CARDIOVASCULAR SEQUELS IN LONG-STANDING TYPE II DIABETES MELLITUS.

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In 1976 a longitudinal study of the incidence of cardiovascular (cv) complications and the underlying risk factors (rf) was started. 456 unselected type II diabetic outpatients (D) were included. At follow-up in 1990, only 17.2% of D had not suffered from any cv complications (cv death, stroke, myocardial infarction, gangrene). The age at baseline of this group was 53.3± 10.0 years. Diabetic subjects with cv disease differed from the group without in baseline systolic blood pressure (SBP) ($p=0.0001$), age ($p=0.0001$), body mass index ($p=0.008$), triglycerides ($p=0.006$) and HbA_I ($p=0.001$). In multiple logistic regression analysis SBP ($p=0.0001$), HbA_I ($p=0.003$) and triglycerides ($p=0.04$) were significant independent predictors for the incidence of cv disease (overall prediction was 88.8%). The age adjusted relative risk for cv complications in the upper quartile of HbA_I was 1.5 (95%CI 1.14-1.87) in comparison to the lowest quartile. The results of this longitudinal study demonstrate that a variety of risk factors are operative in the development of severe cv complications. Furthermore, it was shown for the first time in prospective manner that near normal metabolic control measured as HbA_I values was significantly protective against development of severe cv disease in long-standing type II diabetes.

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RENAL DISEASE AND CARDIOVASCULAR RISK FACTORS IN INSULIN DEPENDENT (TYPE 1) DIABETES IN EUROPE:

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The frequency of diabetic renal disease and the distribution of cardiovascular risk factors was examined in 3296 insulin-dependent (type 1) diabetic patients from 31 diabetes centres in 16 countries throughout Europe. The age of the patients was 32.3 ± 10.3 years (mean ± sd) and duration 14.2 ± 9.5 years. Two percent of men and one percent of women were receiving renal replacement therapy. Seventy-one percent of patients had normal urinary albumin excretion rate (UAE <20 ug/min), 21% had "microalbuminuria" (UAE 20-200 ug/min) and 8% had "macroalbuminuria" (UAE >200 ug/min). The proportion of patients with normal UAE decreased steadily with duration, from 83% in those with diabetes for less than 5 years to 57% after more than 30 years of diabetes. The prevalence of macroalbuminuria increased with duration to reach a plateau of 18% after 20-30 years of diabetes, and declined thereafter to 12%. The unadjusted prevalence of cardiovascular disease was 7.6, 10.9 and 20.1% in patients with normal, micro and macroalbuminuria respectively ($p<0.0001$). Unadjusted mean systolic blood pressure was 118, 125 and 135mmHg in the three groups and diastolic pressure was 74, 77 and 84mmHg ($p<0.0001$). Other cardiovascular risk factors, including plasma cholesterol, triglycerides increased significantly with level of UAE. This study will allow closer investigation of the relationship between cardiovascular risk factors and urinary albumin excretion in insulin dependent diabetes.

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GENETIC VARIATION IN PATIENTS WITH DIABETIC NEPHROPATHY: ASSOCIATION WITH A MARKER FOR CORONARY HEART DISEASE.

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Diabetic nephropathy is associated with excessive coronary heart disease (CHD) and alterations in serum lipid and clotting factor concentrations occur early in the natural history of this complication. To determine if genetic factors are responsible for these changes, markers in the form of restriction fragment length polymorphisms (RFLP) of apolipoproteins B and E and the fibrinogen gene were compared in 45 type 1 (insulin dependent) diabetics requiring renal replacement therapy or with proteinuria, 51 type 1 (insulin dependent) diabetics >10 years duration and normo-albuminuric and 55 non diabetic subjects. No differences in allele frequencies were observed between groups for the fibrinogen RFLP identified using the restriction enzyme Bcl 1, the Apo E RFLP identified using Cfo 1 and the Apo B RFLP identified by Eco R1. However, the Apo B RFLP identified by Xba 1 demonstrated an over-representation of the minor allele (presence of cutting site) in the diabetic nephropathy group, vs non diabetic controls $X^2 = 7.26$ ($P<0.01$) and vs the normo-albuminuric diabetic group $X^2 = 5.22$ ($P<0.02$). The difference was particularly pronounced in nephropaths requiring renal replacement therapy before the age of forty vs non diabetic controls $X^2 = 5.92$ ($P<0.05$). The results suggest a genetic predisposition to CHD in diabetic renal disease and may explain high frequency of CHD in this susceptible group.

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Cardiovascular disease risk factors as predictors of Type 2 (non-insulin-dependent) diabetes in elderly subjects

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Little is known about predictors of diabetes in the elderly. Therefore risk factors measured in a cross-sectional study in subjects aged 65-74 years living in East Finland were correlated with the risk of developing diabetes 3.5 years later. Sixty-nine of 892 initially nondiabetic subjects developed diabetes during the follow-up. Converters to diabetes had higher body mass index (BMI) and waist-hip ratio as well as higher levels of 2 hour glucose and insulin and higher prevalence of family history of diabetes (FHD) than nonconverters. Furthermore, levels of diastolic blood pressure and total triglycerides (TG) were higher and HDL cholesterol lower among converters than among nonconverters. The highest risk for developing diabetes was associated with impaired glucose tolerance (WHO criteria) (odds ratio = 9.8, 95% confidence intervals = 6.1-15.8). The risk of diabetes was 3.7 (3.2-6.1) among subjects in the highest quartile of 2 hour insulin distribution (>86.3 mU/l), 3.5 (2.0-6.1) in those with total triglycerides >2.5 mmol/l, 2.7 (1.5-4.6) in those with waist-hip ratio >1.0, 2.5 (1.5-4.4) in those with HDL cholesterol <1.0 mmol/l, 2.1 (1.2-3.6) in those with BMI >30 kg/m^2, 1.8 (1.0-3.1) in those having hypertension, and 1.7 (1.0-2.9) in those with FHD. Thus, cardiovascular risk factors related to insulin resistance are predictors of diabetes also in the elderly.

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LIFE STYLE CHANGES DECREASE RATES OF GLUCOSE INTOLERANCE AND CARDIOVASCULAR (CVD) RISK FACTORS: A SIX YEAR INTERVENTION STUDY IN A HIGH RISK HINDU INDIAN SUBCOMMUNITY

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Primary prevention of NIDDM and CVD are major goals of modern health care. We therefore studied a Hindu 'Indian' subcommunity in Dar es Salaam with known high rates of impaired glucose tolerance (IGT), NIDDM and other CVD risk factors, to determine if health education over a period of six years could result in significant improvement. 218 (80.7%) (108 male; 110 female) of 270 (131 male, 139 female) members of the subcommunity over 14 years age were studied in 1986. Repeat examination in 1992 was performed in 170 (78.0%) subjects. In the first survey, 26.5% had IGT, and 11.8% NIDDM. In the repeat survey of the IGT subjects 9 (20.0%) persisted with IGT but only 1 (2.2%) progressed to diabetes. Of the 20 NIDDM subjects 40.0% continued to show diabetic tolerance, 15.0% IGT and 45.0% had reverted to normal. Of the NGT subjects 4.8% progressed to IGT and 4.8% developed diabetes. Thus the NIDDM prevalence had fallen from 11.8% to 8.2% and IGT from 26.5% to 10%. Small but significant reductions were observed in mean fasting blood glucose, 2hr blood glucose, serum cholesterol and triglycerides, blood pressure, and weight. The level of physical activity increased significantly ($p<0.001$). Thus community based life style modifactions can result in significant reduction in the prevalence of IGT, NIDDM and other CVD risk factors over a prolonged period.

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Nephropathy: Prospective Studies

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A 5 YEAR STUDY OF GLOMERULAR FILTRATION RATE IN NORMOTENSIVE IDDM PATIENTS WITH MICROALBUMINURIA.

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Forty normotensive (129/ 80 (SD 11/8) mmHg) insulin dependent diabetic (IDDM) patients with persistent microalbuminuria 84 (range 30-300) mg/24h with a mean age of 30 (8) year and a duration of diabetes of 17 (6) year were followed prospectively for 5 years with annually clinical examinations including measurement of glomerular filtration rate (GFR, Cr-EDTA-clearance). Mean GFR at baseline was 120 (18) ml/min/1.73m2. 14 out of 40 patients developed clinical diabetic nephropathy (albuminuria > 300 mg/24h). These patients had a significant reduction in GFR (mean -2,2 (3,8) ml/min per year, $p=0.05$) while GFR remained stable in the 26 patients with urinary albumin excretion < 300 mg/24h at follow-up (delta GFR= +0.5 (2.1) ml/min per year, NS) The difference in rate of decline of GFR was significant, mean 2.7 ml/min, $p<0.05$. The 9 patients who developed diabetic nephropathy during the first 2 years of observation had a rate of decline in GFR of -3.5 (-11-1) ml/min per year, $p<0.001$. Using multiple regression analysis of variance the rate of decline in GFR was independently correlated to onset of diabetic nephropathy ($p<0.001$) and systolic blood pressure at baseline ($p<0.05$) but not to baseline values of urinary albumin excretion, diastolic blood pressure, GFR, age, duration of diabetes or to mean haemoglobin A1c, protein or salt intake during the observation period. Conclusion: Normotensive patients with persistent microalbuminuria have a stable GFR while development of clinical overt diabetic nephropathy herald a progressive loss of kidney function.

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LONG-TERM (18 YEAR) PROGNOSIS FOR NORMO- AND MICROALBUMINURIC TYPE 1 (INSULIN-DEPENDENT) DIABETIC PATIENTS

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To study the prognostic significance of microalbuminuria in Type 1 diabetes a long-term (18 $\pm$ 2 (SD) years), second follow-up study was performed. 44 male patients with previous measurement of urinary albumin excretion rate (UAE) (RIA, $\geq$3 timed collections) were identified for reinvestigation. Retrospectively 30 patients were considered initially normoalbuminuric (UAE <15 μg/min) and 14 initially microalbuminuric ($15 \leq$ UAE < 150 μg/min) (initial diabetes duration 12 $\pm$ 3 vs 13 $\pm$ 3 years, ns, age at diagnosis 12 $\pm$ 5 vs 13 $\pm$ 4 years,ns). At follow-up UAE (three overnight collections)/proteinuria, blood pressure, s-creatinine, and kidney function ($n=16$) were measured. The normo- and microalbuminuric cohorts (no follow-up in 4/0 patients) differed with respect to all cause mortality (1 vs 5 death, $p=0.01$, Fisher's exact test), development of renal failure (0 vs 4, $p=0.008$) and of overt diabetic nephropathy (1 vs 10, $p=0.00001$). Among initially normoalbuminuric patients four showed microalbuminuria at follow-up, while 20 remained normoalbuminuric after 31 $\pm$ 5 years of diabetes. Two initially microalbuminuric patients stayed such during antihypertensive therapy, and one spontaneously became normoalbuminuric. Remeasuring of glomerular filtration rate in nine persistently normoalbuminuric patients suggested a modest (age-dependent?) decline (132 $\pm$ 5 vs 125 $\pm$ 12 ml/min/1.73m^2 ($p=0.10$), follow-up period 20 $\pm$ 2 years, age 45 $\pm$ 5 years). In conclusion the occurrence of microalbuminuria in Type 1 diabetes is a strong risk marker for overt diabetic nephropathy, renal failure and death. Conversely normoalbuminuria indicate a good long-term prognosis with well-preserved kidney function.

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THE PROGRESSION OF NEPHROPATHY IN NON-INSULIN-DEPENDENT-DIABETES IS SIMILAR TO THAT IN INSULIN DEPENDENT-DIABETES.

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There is agreement in literature concerning the evolution of glomerular filtration rate (GFR) in insulin-dependent but not in non insulin-dependent diabetic nephropathy. Our aim was to investigate the decline of GFR in 6 microalbuminuric (20-200 µg/min) (D_1) and 7 macroalbuminuric (D_2) (200-1500 µg/min) insulin-dependent diabetics, and 8 microalbuminuric (D_3) and 9 macroalbuminuric (D_4) non insulin dependent diabetics. Blood pressure levels were treated when systolic and diastolic values arose above 160/95 mmHg. Age, HbA_{1c} and creatinine were 29±3 (Mean ± SE), 7.9±0.3 % and 1.1±0.1 mg/dl respectively in D_1; 41±2, 8.2±0.3 and 1.7±0.2 in D_2; 57±4, 8.3±0.3 and 1.0±0.1 in D_3 and 64±3, 8.0±0.2 and 1.6±0.2 in D_4. GFR was measured by multiexponential analysis of plasma decay of ^{51}Cr EDTA after intravenous injection. GFR was 129±7 ml/min/1.73m^2 and 128±6 (n.s.) in D_1; 77±5 and 65±5 ($p<0.01$) in D_2; 135±8 and 132±7 (n.s.) in D_3 and 58±6 and 43±6 ($p<0.01$) in D_4 at baseline and after one year follow-up respectively. Strict antihypertensive treatment was then commenced in D_2 and D_4 to maintain systolic and diastolic values below 140/85 mmHg. The rate of decline of GFR was slackened from 12±2 to 5±2, $p<0.01$ in D_2 and from 15±3 to 6±2, $p<0.01$, in D_4 (ml·min^{-1}1,73m^{-2} per year). These results demonstrate that: 1) the rate of decline of GFR is similar in macroalbuminuric insulin-dependent and non insulin dependent diabetics and is improved by strict antihypertensive treatment and 2) GFR does not decrease in microalbuminuric insulin-dependent and non insulin-dependent diabetics during one year follow-up.

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Non-progression of microalbuminuria over 3 years in 740 Type 2 (non-insulin dependent) diabetic patients.

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After 3 months diet therapy, 740 newly-presenting Type 2 (non-insulin dependent) diabetics were allocated to continued diet therapy. Over 3 years 204 required additional therapy for symptoms or Fasting Plasma Glucose (FPG) >15 mmol/l. Urine samples were obtained yearly. Albuminuria was corrected for dilution by regression on the creatinine. In 536 patients treated with diet alone, albumin decreased from 14.7 mg/l (4.4 to 48.9) (geometric mean, SD interval) to 10.8 mg/l (4.1 to 28.8; difference $p<0.001$) during the initial 3 months' diet. Over 3 years it rose slightly to 12.1 mg/l (4.1 to 36.3 ; $p<0.05$). In 69 patients with raised excretion >= 50.0 mg/l at diagnosis, albumin fell after 3 months' diet from 115.5 mg/l (52.0 to 256.5) to 25.2 mg/l (8.1 to 78.4 ; $p<0.001$) and remained similar at 22.0 mg/l (5.4 to 89.5; n.s.) over 3 years, higher ($p<0.001$) than in other patients who also showed little change over 3 years. In patients requiring additional therapy, albumin fell from 16.1 mg/l (5.6 to 46.3) at diagnosis to 10.8 mg/l (4.2 to 27.4; $p<0.001$) after 3 months diet and 11.6 mg/l (4.5 to 30.0; n.s.) at 3 years. Albumin at diagnosis correlated with systolic ($r_S = 0.150$, $p<0.05$) and diastolic ($r_S = 0.197$, $p<0.01$) BP and FPG ($r_S = 0.201$; $p<0.001$) in this group.

In conclusion, in Type 2 diabetes microalbuminuria reduces in response to initial diet therapy, but shows little progression even in those with initially raised excretion. Progress of nephropathy is slow and microalbuminuria is unlikely to provide a sensitive indicator of response to different therapeutic regimens.

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ALBUMIN EXCRETION RATE IN NON-DIABETIC OFF-SPRING OF TYPE 2 DIABETIC PATIENTS.

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In type 1 diabetes, there is evidence that nephropathy clusters in families suggesting that genetic factors may act as determinants of glomerular permeability. The role of inherited factors is unknown in type 2 diabetes. The aim of the present study is to evaluate AER in non diabetic offspring of type 2 patients. On 200 type 2 patients with diabetes duration > 10 yr, 181 were normoalbuminuric (albumin excretion rate, AER < 20 µg/min) and 19 microalbuminuric (AER = 20-200 µg/min). We have randomly selected A) 7 offspring of microalbuminuric patients and B) 15 offspring of normoalbuminuric patients. Groups A and B were matched for age, sex, BMI and blood pressure. All offspring were normotensive, had normal creatinine clearance, normal glucose tolerance (oGTT), and sterile urine culture. We calculated the mean of AER (RIA Kit Pharmacia) on three overnight urine collections. AER was significantly higher in group A than in group B (4.42±1.54 SE vs 1.75±0.34 µg/min, $p < 0.05$). These preliminary results suggest that an increased glomerular permeability is present in offspring of non insulin-dependent patients with microalbuminuria.

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MICROALBUMINURIA IN NON-DIABETIC FIRST DEGREE RELATIVES OF TYPE 2 DIABETIC PATIENTS.

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In patients with Type 2 diabetes microalbuminuria (MA; albumin excretion rate ≥15µg/min) is associated with insulin resistance. Insulin resistance is a common finding among first degree relatives of patients with Type 2 diabetes. Is MA in these subjects also related to insulin resistance? To address this question we measured AER in 915 non-diabetic first degree relatives of patients with known Type 2 diabetes (REL; age 51.4±0.7yrs; BMI 25.8±0.2 kg/m^2). Glucose tolerance was assessed with a 2h oral glucose tolerance test (75g glucose). 54 subjects (6%) had unknown diabetes and were excluded from the study. Of the remaining subjects, 232 (27%) had impaired glucose tolerance (IGT). MA was as frequent among subjects with normal glucose tolerance (NGT;6.4%) as among subjects with IGT (9.4%). REL with MA had higher systolic blood pressure (144±3 vs 134±1mmHg; $p<0.01$), lower HDL-cholesterol values (1.11±0.04 vs 1.23±0.01mM; $p<0.05$) and higher fasting blood glucose values (5.4±0.1 vs 5.2±0.1mM; $p<0.01$) than normoalbuminuric relatives. In conclusion, in first degree relatives of patients with Type 2 diabetes, microalbuminuria is associated with hypertension, elevated blood glucose and low HDL-cholesterol, factors which have been considered components of the metabolic syndrome. The data thus suggest that microalbuminuria could be genetically linked to insulin resistance, the common denominator of the metabolic syndrome.

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Genetics I

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MUTATIONS IN GLUCOKINASE GENE CAUSE EARLY ONSET TYPE 2 DIABETES

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Tight linkage between the glucokinase gene (GCK) and early-onset-non-insulin-dependent diabetes mellitus has been recently reported. We have cloned and partially sequenced human GCK, allowing us to design pairs of primers to specifically amplify in vitro each of the 12 exons. We have scanned GCK for mutations in multigenerational families linked to GCK using single strand-polymorphism conformational analysis. We found abnormal conformers which cosegregated with diabetes in exons 4, 6, 7 and 8 in different families. In addition, conformers in exon 1a and in intron 9 that do not cosegregate with diabetes were noted. Amplification and direct sequencing of exon 7 revealed the following mutations: T228M, G261R and E279AM. All the individuals with mutations are heterozygous and express both the normal and the mutant protein. The cosegregation of the mutation with Type 2 diabetes in these families suggests that it is the cause of the glucose intolerance. The presence of the mutations may result in an overall decrease in glucokinase activity in β cells leading to an increase in the glucose concentrations necessary to stimulate insulin release.

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Type 2 (Non-insulin-dependent) Diabetes is linked to the glucokinase gene

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We studied the genetics of Type 2 (Non-insulin-dependent) diabetes by using linkage studies in three large maturity-onset-diabetes of the young (MODY) pedigrees. In all pedigrees diabetes resulted from autosomally dominant inherited β-cell dysfunction. Glucokinase is an excellent candidate gene for Type 2 (Non-insulin-dependent) diabetes as it is expressed in the β-cell and acts as the pancreatic glucose sensor. We studied a dinucleotide repeat polymorphism 10 kb 3' to the glucokinase gene using PCR. Linkage was found with a peak lod score was 5.1 at a recombination fraction (θ) of zero in a large 5 generation pedigree, BX, with 18 diabetic members diagnosed between the ages of 12 and 65. This suggests that a defective glucokinase gene contributes to the diabetes phenotype in this pedigree. In the original MODY pedigrees M and R the glucokinase locus was excluded (lod scores −7.36 & −2.1 at $\theta = 0$). The β-cell function in pedigree BX was mild and less severe than in pedigrees M and R as shown by HOMA analysis [60 (43–85): 30 (11–79) mean (SD range) % normal]. The glucokinase gene is the first candidate gene to be linked with Type 2 (non-insulin-dependent) diabetes and can result in diabetes presenting in middle and old age.

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CLINICAL CHARACTERISTICS OF TYPE 2 DIABETES LINKED TO THE GLUCOKINASE GENE

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The clinical characterisation of Type 2 diabetes linked with the glucokinase locus on chromosome 7p in a pedigree BX was established. Thirty family members were evaluated. Eighteen (11 females, 7 males) had the glucokinase allele that segregated with diabetes. History, examination and diabetic complications were assessed. Beta cell function and insulin sensitivity were measured using a Continuous infusion of Glucose with Model Assessment. Seventeen of 18 subjects with the allele segregating with diabetes were diabetic. The 18 affected subjects were similar in age 46±20 v 48±22years and BMI 26±6 v 25±3kg/m^2 to unaffected subjects. Six subjects were diagnosed on screening and 5 by glycosuria in pregnancy. Fourteen subjects were diet treated and 3 by sulphonylurea. Fasting glucose levels 4.3–12.6mmol/l increased with age (Rs =0.6,p<0.05). Beta cell function was impaired 56% normal v 129% normal in unaffected subjects (p<0.001). Affected and unaffected subjects had similar fasting insulin (8.3(4.7–14.8) v 8.5(5.8–12.6) mU/l), insulin sensitivity (53(32–85) v 56(38–83)% normal), and total cholesterol levels (4.6±1 v 4.9±1mmol/l). Microvascular and macrovascular tissue damage was uncommon. Two males (69 and 79 years) had evidence of cardiovascular disease. Biothesiometer readings were normal, but multivariate analysis showed age and carrying the allele linked to diabetes as independent determinants (t=5.2 p<0.001 and t=3.7 p<0.01). Urinary albumin:creatinine ratios were similar (0.9 (0.3–2.5) v 1.5(0.4–5.8)g/mol. Type 2 diabetes linked with the glucokinase locus is mild, however a defect in the glucokinase gene may have a role in the pathogenesis of classical Type 2 diabetes.

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LINKAGE STUDIES GIVE EVIDENCE FOR GENETIC HETEROGENEITY IN TYPE 2 DIABETES MELLITUS.

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Evidence for linkage between Adenosine Deaminase (ADA) and Glucokinase (GCK) loci and Maturity-Onset-Diabetes-of-the-Young (MODY) has been demonstrated. We have investigated by linkage analysis 33 MODY families and 20 families with late-onset type 2 (non-insulin-dependent) diabetes. Total lodscores in the MODY families versus GCK and ADA loci were respectively +13.45 and -68.1. A homogeneity test provides statistical support of genetic heterogeneity: 47 % of MODY families showed linkage to GCK (Lodscore: +29.6). In these families age of apparent onset was <12 years in recent generations. All but 3 of the 18 non-GCK-linked families have age of onset >12 . One of these 3 families gave a positive lodscore (>1) with ADA. None of the 20 late-onset type 2 families showed evidence of linkage with GCK. However, one late-onset type 2 family gave a positive lodscore (>1) with ADA. These results suggest that the GCK gene is implicated in the pathogenesis of very early onset type 2 diabetes, and that other loci are possible involved in MODY. ADA could be linked to the minority of our MODY and late-onset type 2 diabetic families. Genetic heterogeneity of type 2 diabetes is related to clinical heterogeneity.

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DIFFERENT PHENOTYPIC EXPRESSION BY THREE MUTANT ALLELES OF GLUCOKINASE GENE IN MODY.
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Close linkage between the glucokinase (GCK) locus and Maturity-Onset-Diabetes-of-the-Young (MODY) has recently been demonstrated. Three MODY multigenerational pedigrees were investigated, two of which exhibited evidence of linkage with GCK locus (Lodscores: F51=14.10, F28=2.22), and the third which did not (F160). The screening of the 12 exons of human GCK, using Single-Stranded-Conformational Polymorphisms (SSCP) analysis, revealed abnormal conformers in exon 8, which cosegregated with these MODY families. Direct sequencing of the amplified exon 8 showed 3 different missence mutations: $Glu^{300} \rightarrow Lys^{300}$ (F28), $Glu^{300} \rightarrow Gln^{300}$ (F51, confirmed by allele specific amplification of the mutated sequence in the 39 affected individuals of the kindred), $Leu^{309} \rightarrow Pro^{309}$ (F160). Subjects having the codon 300 mutation in kindred F51 were characterised by mild fasting hyperglycemia. In contrast, in family F160, all the diabetic subjects required early treatment with insulin or sulfonylurea. Most of the diabetic members of family F28 were glucose intolerant, or had well controlled diabetes. In these families, there appears to be phenotypic differences that may reflect the nature of the mutation and its possible effect on GCK activity. The mutations elicit that MODY has complete penetrance with age of onset <10 years in the younger generations.

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A POLYMORPHISM OF THE ALDOSE REDUCTASE LOCUS IS A PREDICTIVE MARKER OF DIABETIC RETINOPATHY
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Aldose reductase (ALR2) is thought to play an important role in the pathogenesis of diabetic nephropathy, retinopathy and neuropathy. This study investigated ALR2 genotypes in patients with type 1 diabetes with and without complications. Three regions of the human ALR2 gene were amplified using PCR and these products were subsequently used as probes to screen for restriction fragment length polymorphisms (RFLP). Two RFLPs were detected using the restriction endonucleases Pst-I and Bam-HI with probes to the 5' and 3' ends of the ALR2 gene. One hundred and thirty patients with type I diabetes (forty-one diabetic controls, fifty-three with diabetic nephropathy/retinopathy, thirty-six with retinopathy alone) and fifty-seven normal controls were investigated. There were no significant differences in the frequency of 5.7 and 5.3 kilobase (kb) 5'-PstI/ALR2 alleles and genotypes in any of the patient or normal control groups. Patients with retinopathy alone had a significant increase in the frequency of the 8.2 (kb) 3' Bam-HI/ALR2 allele and homozygous genotype compared to the diabetic group. The 5.7-8.2/5.3-8.2 kb 5'ALR2-3'ALR2 genotype was found in 52.9% of the diabetic patients with retinopathy but was completely absent from the diabetic control group ($p<0.0005$, $Pc=0.005$). In conclusion, we have identified an ALR2 gene polymorphism which is associated with diabetic retinopathy.

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Lipoproteins

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GENDER DIFFERENCES IN APO E PHENOTYPES OF A TYPE 2 (NON-INSULIN DEPENDENT) DIABETIC POPULATION
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Apolipoprotein E (apo E) is of particular importance in lipid metabolism as it mediates the elimination of potentially atherogenic remnant particles derived from triglyceride-rich lipoproteins. In this context, apo E polymorphism was analysed in an Italian population of type 2 (non-insulin dependent) diabetic patients (males, 211; females, 225) and in a corresponding, non-diabetic control cohort (males, 296; females, 69). There were significant differences ($p<0.05$) in allele frequencies between diabetic, male and female patients due to an under-representation of the $\varepsilon 4$ allele in the female group. No differences in allele frequencies were noted when non-diabetic male and female subjects were compared. Comparisons between diabetic and non-diabetic subjects also demonstrated significant differences ($p<0.05$) in allele frequencies between female subjects but not between male subjects. A closer examination of the female diabetic population revealed that under-representation of the E4 allele was particularly notable in older patients; those ≥60y of age had a significantly lower ($p<0.05$) $\varepsilon 4$ allele frequency than controls or male diabetic patients, whereas the younger female patients (≤59y) did not. The results are consistent with the proposal that the $\varepsilon 4$ allele may represent a particular risk factor for female diabetic patients.

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THE INFLUENCE OF HYPERINSULINEMIA IN THE PRESENCE OF EUGLYCAEMIA ON CHOLESTEROL SYNTHESIS IN HUMANS.
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The aim of this study was to evaluate the acute effects of insulin on the rate of whole body cholesterol synthesis as assessed by plasma mevalonate (MVA) concentrations in normal subjects. Ten healthy men aged 36±7 years, BMI 23.9±2.5 Kg/m^2 underwent a 3-h euglicaemic hyperinsulinemic (1mU/Kg/min) clamp study from 9 AM to 12 AM after 14-h fasting. Blood samples were drawn every 30' for insulin (IRI) and every 60' for MVA, lipids and apoproteins. In eight subjects the plasma MVA physiological variations were evaluated on a different day in the fasting state at 9, 10.30 and 12 AM. MVA concentrations were assayed by CG/CI/MS after extraction with CH_2Cl_2:PrOH (9:1). Results (mean±SD) were as follows:

CLAMP STUDIES

time	0'	60'	120'	180'
MVA (ng/ml)	2.53±0.5	2.39±0.7	1.67±0.7**	1.51±0.7**
IRI (μU/ml)	10.6±4.3	97±31**	109±29**	113±38**
Tot Chol. (mg/dl)	169±55	166±38	164±41	157±42
HDL Chol. (mg/dl)	51±11	48±11	44±10*	46±7*
Tg (mg/dl)	75±21	70±21	68±20*	69±20*
Apo A1 (mg/dl)	90±9	80±9	90±8	78±25
Apo B 100 (mg/dl)	76±24	68±25*	71±24*	68±18*

DIURNAL MVA VARIATIONS

time	9 AM	10.30 AM	12 AM
MVA (ng/ml)	2.3±0.6	2.2±0.6	2.03±0.5

* $p<0.05$ ** $p<0.001$ vs baseline

During the clamp studies a slight significant decrease in HDL-cholesterol, triglyceride (120'-180') and Apo B-100 (60'-120'-180') levels was noted. MVA levels decreased significantly by 37% and 43% at 120' and 180' respectively ($p<0.001$). A significant inverse relationship was found between IRI and MVA levels at 180'.The percent decrease from baseline in MVA levels due to diurnal variations was 11%at 10.30 AM and 17% at 12 AM. In conclusion, this study demonstrates that hyperinsulinemia in the presence of euglycaemia decreases in healthy males the circulating levels of mevalonate, the immediate product of HMG CoA Reductase and an index of whole body cholesterol synthesis.

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THE DEPENDENCE OF REVERSE CHOLESTEROL TRANSPORT ON CONTROL OF DIABETES IN TYPE-1 (INSULIN-DEPENDENT) DIABETES

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Changes in reverse cholesterol transport (RTC) from peripheral tissues and the arterial wall to the liver may be affected by control of diabetes and contribute to the higher incidence of atherosclerosis in diabetics. RTC parameters - lecithin:cholesterol acyl transferase (LCAT) activity and qualitative alterations of high density lipoproteins (HDL) - were studied in 18 Type-1 diabetics (7 men, 11 women without vascular complications of diabetes) consecutively admitted to our clinic for decompensation of diabetes for a period of 7-34 days. LCAT activity was determined isotopically using the method of autologous substrate as fractional esterification rate (FER) and molar esterification rate (MER) and the method of homologous substrate (LCATh) eliminating the effect of the patient's plasma on the rate of cholesterol esterification and reflecting the amount of enzyme. After diabetes compensation, fructosamine decreased from 2.47±0.23 to 2.11±0.32 in men, and from 2.55±0.57 to 2.22±0.37 mmol/l in women (both $p < 0.05$); this was associated with increases in FER (6.49±2.1 vs 7.85±2.5 in men, and 6.50±2.2 vs 8.09±1.8 %/h in women, both $p < 0.05$) and in MER (96.2 ±2.30 vs 130±43 in men, and 94.1±37 vs 115.7±31 umol/l/h in women, both $p<0.05$). No significant changes in LCATh, in HDL2 and HDL3 subfractions or in triglyceride content in HDL were observed, only men showed a rise in HDL cholesterol. We conclude that diabetes compensation in Type-1 diabetics may enhance the rate of cholesterol esterification, probably more affecting properties of plasma rather than changing the amount of LCAT.

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APO B CONTAINING LIPOPROTEINS SHOW MULTIPLE ABNORMALITIES IN INSULIN-DEPENDENT DIABETIC PATIENTS WITH KIDNEY DISEASE

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Microalbuminuric and proteinuric Type 1 (insulin-dependent) diabetic patients exhibit multiple lipid and lipoprotein abnormalities. In order to characterize these changes we examined 65 normoalbuminuric (AER 7(0-19) μg/min), 45 microalbuminuric (AER 50 (20-178) μg/min), 35 proteinuric (AER 422 (220-1490) μg/min) and 56 healthy controls matched for age, duration of diabetes, BMI and glycaemic control. The mass concentrations of VLDL, IDL, LDL represent the sum of triglycerides, cholesterol, protein and phospholipids in each fraction separated by sequential ultracentrifugation. In addition apo B in $VLDL_1$ (Sf 60-400), $VLDL_2$ (Sf 20-60) and IDL (Sf 12-20) was measured after separation by density gradient ultracentrifugation. LDL particle size was determined by nondenaturing gradient gel electrophoresis.

	NORMO	MICRO	PROT	CONTROL
VLDL mass (mg%)	73±6	102±10[a]	98±11[a]	96±10
IDL mass (mg%)	34±2	43±4[a]	49±4[c]	39±3[e]
LDL mass (mg%)	266±10	294±12[a]	315±16[b]	286±9[a]
$VLDL_1$apoB (mg/dl)	1.0±0.2	1.1±0.2	1.1±0.2	1.3±0.2
$VLDL_2$apoB (mg/dl)	4.4±0.4	5.6±0.5[a,d]	5.6±0.5[a]	4.2±0.4[e]
IDL apoB (mg/dl)	10.9±0.6	14.1±0.9[b]	14.9±1.1[b,d]	12.4±0.8
Apo B (mg/dl)	80.3±2.1	90.9±3.3[b]	88.2±3.7[a]	85.9±3.0
LDL particle (Å)	260±2	259±2	257±2	255±2

[a]p<0.05, [b]p<0.01 and [c]p<0.001 vs NORMO; [d]p<0.05 vs CONTROL; [e]p<0.01 vs PROT

Apo E allele and phenotype frequencies were similar in all groups. In micro- and macroalbuminuric patients apo E4 compared to apo E2 was associated with much higher increase of LDL cholesterol (+37%) than in normoalbuminuric group (12%). In conclusion, Type 1 diabetic patients with kidney disease exhibit increased transport rates of apo B containing lipoproteins and the presence of apo E4 further aggravates abnormalities of LDL metabolism.

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APOLIPOPROTEINS B AND A-I/C-III GENETIC POLIMORPHISMS AND CORONARY HEART DISEASE IN DIABETES MELLITUS.

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Plasma lipid abnormalities play a major role in the development of coronary heart disease and may be genetically conditioned,also in diabetic patients.We have evaluated restriction-fragments-lenght polimorphisms (RFLPs) of apolipoprotein B gene and of apolipoproteins A-I/C-III genetic cluster in 95 type 2 (non-insulin-dependent) diabetic patients, of whom 48 were affected by coronary heart disease (CHD)(34 males,mean age 54±6 years,known diabetes duration 10.2±3.4 years) and 47 were not (35 males,mean age 51±8,diabetes duration 8.9±4.1 years).DNA from peripheral blood was amplified by Polimerase Chain Reaction and digested by the restriction enzymes XbaI for Apo B and SacI for Apo A-I/C-III.Genotype frequency of the involved alleles (X1 and X2 for Apo B, S1 and S2 for Apo A-I/C-III) was then obtained. No association was observed among X1 and X2 alleles frequency,CHD and plasma levels of total cholesterol(C),HDL-C,LDL-C,triglycerides,Apo A-I and Apo B. On the contrary S1/S2 heterozygosis was more frequent in CHD- than in non CHD-diabetic patients (X^2= 7.7,p= 0.006) and was associated with increased plasma triglycerides (3.05±2.04 vs 1.61±0.92 mmol/l,$p<0.001$). Moreover S2 allele frequency was significantly higher in patients with CHD than in those without (X^2= 7.1,$p<0.01$).These data indicate that some RFLPs of the Apo A-I/C-III genetic cluster may be associated with hypertriglyceridaemia and CHD in type 2 (non-insulin-dependent) diabetes mellitus.

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ABNORMAL DISTRIBUTION OF VLDL SUBFRACTIONS IN TYPE 1 DIABETIC PATIENTS: COULD A REDUCTION IN HEPATIC LIPASE ACTIVITY PLAY A ROLE?

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Aim of the present study was to test whether changes in lipolytic enzyme activities could explain the abnormal VLDL composition found in type 1 diabetic patients. Therefore, fasting lipid composition (free and esterified cholesterol, triglycerides, phospholipids) of four VLDL subfractions of decreasing size (A,B,C,D), isolated by density gradient ultracentrifugation, and plasma post-heparin lipolytic enzyme activities (lipoprotein lipase=LPL, hepatic lipase=HL) were evaluated in 13 male normolipidemic type 1 diabetic patients in optimal blood glucose control (HbAlc 6.9±0.4%) (M±SEM) and in 9 control subjects matched for sex, age (29±2 vs 29±2 yrs), BMI (24.7±0.6 vs 24.5±0.7 kg/m²), plasma cholesterol (4.22±0.15 vs 4.29± 0.15 mmol/l) and triglycerides (0.85±0.07 vs 0.76± 0.09 mmol/l). A significant increase in smaller VLDL (D) (50.5± 2.7 vs 37.4±3.1%, $p<0.005$) and a decrease in the intermediate ones (C) (20.6±1.7 vs 27.9±1.5%, $p<0.005$) was observed in diabetic patients. Furthermore they had significantly reduced HL values (233±28 vs 332±42 mU/ml, $p<0.05$); LPL was similar in the two groups. HL activity was inversely correlated to the amount of VLDL D ($r=-0.72$, $p<0.01$) and directly to that of VLDL C ($r=0.61$, $p<0.05$). In conclusion, smaller (more atherogenic) VLDL are increased in type 1 diabetic patients; since HL activity is directed mainly toward the smallest VLDL, both its reduction and the inverse correlation with VLDL D in diabetic patients suggest a specific role of this enzyme in the abnormal VLDL metabolism of type 1 diabetic patients.

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Hormone Action

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EFFECTS OF C-PEPTIDE ON MICROVASCULAR FUNCTION IN EXERCISING FOREARM MUSCLE IN TYPE I DIABETES

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Microvascular dysfunction is frequently found in patients with type I diabetes. Recently C-peptide has been shown to reduce glomerular hyperfiltration and stimulate glucose utilization in type I diabetes. This study was undertaken to examine the influence of C-peptide on blood flow (BF), capillary diffusion capacity (CDC) and substrate exchange of exercising forearm muscle in type I diabetics. Patients (n=17) and healthy controls (n=14) performed rhythmic forearm exercise using a handergometer before and during 60 min i.v. infusion of C-peptide (6 nmol/kg/min) or NaCl. BF and CDC were lower in diabetics compared to controls (BF: 29±2 vs 40±3 ml/min/100 ml, CDC: 5.4±0.4 vs 7.8±0.6 ml/min/100 ml, p<0.01-0.001). CDC and BF both increased during C-peptide infusion (BF +27±4%, CDC +52±9%) in the diabetics, no significant changes were seen in either group during NaCl. Forearm uptake of oxygen and glucose in diabetics increased during C-peptide infusion but not during NaCl. It is concluded that infusion of C-peptide in young diabetics restoring levels results in a normalization of both blood flow and CDC during exercise as well as augmented uptake of oxygen and glucose by exercising muscle. The findings indicate that C-peptide may be of importance for microvascular function in exercising muscle in type I diabetes.

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OVEREXPRESSION OF INSULIN RECEPTORS INHIBITS IGF1 EFFECT ON C- FOS TRANSCRIPTION IN CHO CELLS.

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Insulin (IR) and IGF1 (IGF1-R) receptors share a high degree of homology and show overlapping biological actions. Both hormones induce transcription of "progression factors" and cellular growth. Aims of the present work are to investigate : 1) whether insulin increases c-fos mRNA cytoplasmic content, in CHO-K1 cells (which express a very low number of IR), via the IGF1-R and 2) the effect of overexpression of IR in CHO (CHO-IR) on insulin and IGF1 capacity to induce c-fos expression. CHO-K1 have been transfected with the plasmid pCMV5-HIR, by Ca co-precipitation, and a clone (CHO-IR) overexpressing IR (15 fold) has been selected. CHO-K1 and CHO-IR have been serum starved for 16 h before experiments. Cells have been incubated with insulin (50 ng/ml) or IGF1 (50 ng/ml) for 30' at 37°C, solubilized and total RNA has been isolated and transferred by Northern blot and hybridized with a 5 Kb c-fos probe. Our results show that, in CHO-IR, insulin increases c-fos mRNA cytoplasmic accumulation 2 fold higher than the effect obtained in CHO-K1 cells; instead overexpression of functional IR (CHO-IR) inhibit by 50% the effect of IGF1. Conclusions: insulin and IGF1 activate transcription of c-fos via a common saturable pathway; overexpression of IR inhibits IGF1 activation of c-fos.

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COMPARISON OF THE METABOLIC EFFECTS OF rh-INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) AND OF INSULIN IN MAN.

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To compare the effects of elevated plasma concentrations of IGF-1 and insulin on C-peptide, glucose, amino acid and free fatty acid (FFA) metabolism, groups of 8 overnight-fasted male healthy volunteers received during 8 h euglycaemic clamping either "low" or "high" doses of IGF-1 and insulin (5 or 30µg/kg/h IGF-1, 4.8 or 31.2 mU/kg/h insulin). These doses of IGF-1 and insulin resulted in identical increases in glucose uptake (~1.6mg/kg/min at "low" doses, ~7.2mg/kg/min at "high" doses). Plasma C-peptide levels decreased by 57±4% and 36±6% after "high" doses of IGF-1 and insulin, respectively (p=0.013). Plasma FFA decreased by 44±5% and 55±5% after "high" doses of IGF-1 and insulin, respectively (ns). Plasma leucine flux (1-^{13}C-leucine infusion technique) decreased by 43±4% and 39±3% after "high" doses of IGF-1 and insulin, respectively (n.s.). Leucine oxidation decreased by 56±4% after "high" dose IGF-1 but only by 38±6% after insulin (p=0.02). Therefore, acute increases in plasma IGF-1 inhibit lipolysis (plasma FFA) and whole body protein breakdown (leucine flux) similarly to insulin, whereas IGF-1 suppresses insulin secretion (C-peptide) and irreversible leucine catabolism (leucine oxidation) more than insulin; this suggests that insulin-like effects of IGF-1 are in part mediated via insulin receptors and in part via IGF-1 receptors.

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Insulin demand in type 1 diabetes mellitus is reduced during administration of insulin-like growth factor-I.

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Recombinant human insulin-like growth factor (rhIGF)-I administration lowered insulin, C-peptide, and triglyceride (TG) levels in healthy and type 2 diabetics subjects, decreased glucose levels in diabetic subjects, and appeared to improve insulin sensitivity in both groups. We investigated whether rhIGF-I has comparable effects in 2 subjects (age 29 and 30 years, BMI 24 and 28 kg/m^2) with type 1 diabetes mellitus (duration 27 and 17 years) during a study of 16 days. They received a diet (30 kcal/kg) and insulin by sc pump infusion (day 1-16). 10 µg rhIGF-I/kg·h were given sc on days 9-16 with a second pump. Fasting and postprandial glucose, insulin, IGF-I, growth hormone (GH), and TG levels were determined with routine methods. Pre- and postprandial glucose levels were below 6.5 and 10 mmol/l, respectively, and didn't differ between the two study periods. Total and free IGF-I levels increased during rhIGF-I infusion 4.5 and 2-fold above control levels. The basal insulin infusion rate (29.6 and 23.6 IU/24 h; day 1-8) decreased (13.0 and 11.8 IU/24 h; day 9-16) as did the insulin bolus injections (from a total of 32 and 49 IE/24 h [day 1-8] to 24 and 20 IE/24 h [day 9-16]). Hence, fasting and postprandial insulin serum levels decreased during rhIGF-I administration which also lowered GH and TG levels. In conclusion, rhIGF-I administration reduces insulin requirements of type 1 diabetics probably by direct effects on glucose metabolism via IGF type 1 receptors.

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DECREASE OF INSULIN SENSITIVITY AND CLEARANCE AFTER CHRONIC GROWTH HORMONE TREATMENT IN TURNER'S SYNDROME.

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Biosynthetic GH therapy (GHT) is a well-known approach in the treatment of short statural girls with Turner's syndrome but its metabolic effects are still under investigation. Aim of the study was to evaluate the effects of GHT on glucose turnover, insulin clearance and lipids in six patients (age: 8-13 yrs) with normal glucose tolerance and basal GH secretion. An euglycaemic hyperinsulinaemic (25 mU/kg/h) clamp was performed before and after 157±15 days of GHT (0.1 U/kg/day s.c. for 6 days/week). GHT did not increase fasting GH levels (2.8+0.5 µU/ml) while an increase in growth velocity was seen in 5 out of 6 patients. Fasting RD was 4.1±0.4 mg/kg/min before treatment without changes after GHT (3.5±0.2 mg/kg/min) despite a 143% increase in insulin levels (5.0±1.0 vs 12.2±2.2 µU/ml; $p<0.02$). Triglyceride levels significantly increased after GHT (33.9±3.6 vs 55.7±8.0 mg/dl; $p<0.05$). During the clamp GHT decreased Rd/I levels (0.30±0.02 vs 0.20±0.03 mg/kg.min/µU/ml; $p<0.05$) and a lower inhibition of HGO was demonstrated (78.1±4.4 vs 58.8±6.5 %, $p<0.05$). In addition insulin clearance was normalized after GHT with a decrease of 39.0±6.6% (30.95±3.5 vs 18.05±1.34 L/kg.min,$p<0.05$). In conclusion GHT induces peripheral and hepatic insulin resistance, associated with a decrease in insulin clearance.

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GROWTH HORMONE UPREGULATES HEPATIC GLUCOSE TRANSPORTER (GLUT2) GENE EXPRESSION IN THE DIABETIC RAT.

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The effects of diabetes on hepatic GLUT2 gene expression are controversial and the hormonal regulation of expression remains unclear. To investigate these phenomena we rendered 12 male Sprague Dawley rats diabetic by i.p. injection of streptozotocin (40mg/Kg) and randomised them to 4 groups plus 4 control (**C**) animals. Diabetics (**D**)(blood glucose 36 ± 0.5 mmol/l) received no treatment. Treatment groups received daily injections of: (**A**) 2.5 IU insulin s.c. (blood glucose 19.9 ± 2.3 mmol/l), (**B**) 5.0 IU insulin s.c. (blood glucose 3.3 ± 0.1), (**G**) 0.8 IU recombinant growth hormone s.c. (blood glucose 38.7 ± 2.4 mmol/l) for 16 days. Animals were killed, livers snap frozen, total RNA extracted and northern blot hybridization analyses performed using a cDNA probe for GLUT2 (gift from G I Bell). Autoradiographs were quantitated by laser densitometry and reprobed with an 18S control probe (gift from D T Denhart). GLUT2 mRNA was reduced by 45% in (**D**) verses (**C**)($p<0.02$). Insulin treatment at both doses (**A**)(**B**) did not increase GLUT2 expression compared with (**D**). Growth hormone caused a two fold increase in GLUT2 expression (**G**) compared with (**D**)($p<0.03$), restoring levels to control values. We conclude that in the absence of circulating insulin hepatic glucose uptake may be regulated in part by growth hormone.

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Insulin Synthesis

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GLUCOSE-SENSITIVE NUCLEAR COMPLEX BINDING TO RAT I AND HUMAN INSULIN GENES DETECTED IN ISOLATED RAT ISLETS

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In cultured islet cells, glucose stimulation of the rat I insulin gene (RI) acts through specific DNA sequences in the 5'-flanking region. Our aim is to identify nuclear proteins that transmit this glucose effect. The binding of rat islet nuclear extracts to the glucose response element (GluRE) of RI (-247 to -196) was tested using electrophoretic mobility shift assays. Several islet-specific DNA-protein complexes were detected. Competition experiments using wild-type GluRE and mutated fragments showed that 22 nucleotides (-228 to - 207) are sufficient to retain all binding activities. To determine whether glucose had effects at the level of enhancer binding proteins, islets were incubated (1-3 h) at 2 and 20 mM glucose, nuclear extracts isolated, and their binding capacity tested. A single DNA-protein complex was augmented by glucose, the other RI-nuclear protein complexes remaining unchanged. Homologous sequences of the human insulin gene (-228 to -207) demonstrated one single complex with rat islet extracts, which corresponded to the glucose-sensitive complex of RI. Glucose stimulation of islets enhanced the binding also to the human probe. **In conclusion**, this is the first demonstration of a glucose-sensitive islet nuclear protein, with similar binding characteristics to the RI and human insulin genes, suggesting a possible role in the glucose regulated expression of both genes.

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ß CELL-SPECIFIC INSULIN GENE EXPRESSION: UBIQUITOUS AND TISSUE-SPECIFIC REGULATORS.

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In transient transfection assays a relatively short promoter fragment (< 500 base pairs) of the human insulin gene directs efficient transcription only in ß type cells, thus the tissue-specificity of insulin gene expression is at least partly due to diffusible trans-acting factors. We have investigated the regulatory sequences and factors involved in this specificity. Deletion analysis of the 5' region reveals the presence of both positively and negatively acting regulatory elements. A very powerful positive element at -345 (with respect to the transcription start site) contains a binding site for the ubiquitous factor Sp1. A second very powerful positive element at -240 contains a binding site for a helix-loop-helix factor which is widely distributed, and not identical to the ß cell-specific factor IEF-1. These factors do not activate the insulin promoter in non-ß cells, nor is this selectivity due to the promoter, which can be activated by a promiscuous enhancer in non ß cells. Active transcription may require the factor IUF-1, which is present only in ß type cells, and binds to three sites in the 5' region of the human insulin gene.

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PROTEOLYTIC MATURATION OF THE PROINSULIN-CONVERTING CARBOXYPEPTIDASE H N THE ISLETS OF LANGERHANS

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Proinsulin conversion in the insulin secretory granule requires endopeptidase and carboxypeptidase H (CPH) activities. To determine if CPH is itself processed during segregation to granules, its biosynthesis was investigated and compared with that of insulin in isolated rat islets. Islets were pulse-chase-radiolabelled and the newly-synthesized proteins immunoprecipitated and analyzed by electrophoresis and fluorography. CPH appeared initially as a 57kDa precursor which was converted to a 54kDa protein with a half- time of 30 min. This was significantly faster than proinsulin conversion (half-time of 45 min). N-glycanase treatment showed that CPH conversion was due to proteolysis and not oligosaccharide modification. The proteins were secreted in parallel in response to glucose stimulation indicating that they were sorted to granules at approximately the same rate following synthesis. Transport to granules was efficiently reduced in islets indubated at 20°C as shown by significant inhibition of proinsulin conversion at this temperature. Conversion of CPH was not affected at 20°C indicating that its processing occurs before sorting to granules. This is consistent with the finding that only the mature form of CPH was secreted. These results indicate that proteolytic conversion of CPH might be required for its activation or segregation to the site of proinsulin conversion in the secretory granules.

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POST-TRANSLATIONAL PROCESSING OF THE PROPROTEIN-CONVERTING ENZYME, PC2, IN THE ISLETS OF LANGERHANS

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Insulin secretory granules contain two endopeptidase activities which cleave proinsulin on the C-terminal side of $Arg^{31}Arg^{32}$ and $Lys^{64}Arg^{65}$ to produce insulin. The type 2 endopeptidase was identified recently as the mammalian subtilisin-related protease, PC2. To investigate the post-translational processing of PC2 compared with that of insulin, isolated rat islets were pulse-chase-radiolabelled and the newly-synthesized proteins immunoprecipitated and analyzed by electrophoresis and fluorography. PC2 was synthesized initially as a 75kDa precursor which was processed to a 65kDa protein via three intermediates with a half-time of 2 hours. This was significantly slower than proinsulin conversion (half-time of 45 min). N-terminal sequence analysis of native PC2 revealed that it was cleaved after $Arg^{105}Lys^{106}Lys^{107}Arg^{108}$ and $Gly^{109}Tyr^{110}Arg^{111}$. Newly-synthesized proinsulin-related peptides (proinsulin, intermediates and insulin) were secreted within 60 min in response to glucose stimulation. In contrast, PC2 secretion required at least 2 hours indicating that it was sorted to granules more slowly than insulin. The finding that only the low molecular weight form of PC2 was secreted suggested that cleavage of the 75kDa precursor occurs in the Golgi complex prior to its segregation into secretory granules. These results indicate that proteolytic processing of PC2 might be required for its activation or segregation to secretory granules.

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BIOSYNTHESIS AND PROTEOLYTIC PROCESSING OF PC1 AND PC2 ENDOPEPTIDASES IN PANCREATIC ß-CELLS.

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Proinsulin conversion occurs in the ß-granule compartment of pancreatic ß-cell. It follows that the enzymes that cleave proinsulin must be co-ordinately delivered to the ß-granule in an active form. In this study we examined the biosynthesis and proteolytic processing of the putative processing endopeptidases PC1 and PC2 in parallel to proinsulin. Using specific peptide antisera directed against the C-terminals of PC1 and PC2, immunoblot analysis showed that these proteins were present in isolated rat islets and insulinoma secretory granules. Two PC1 (92kD and 66kD) and two PC2 (75kD and 67kD) molecular forms were identified. Isolated rat islets were incubated (20min) in a media containing increasing glucose concentrations (2.8mM-16.7mM) in the presence of [^{35}S]methionine. PC1, PC2 and (pro)insulin were then immunoprecipitated from islet lysates and analysed by electrophoresis. PC1 and PC2 biosynthesis were specifically stimulated by glucose in parallel to proinsulin. It was also noticed that after 20min labeling incubation PC1 and PC2 processing had begun, whereas no proinsulin processing was observed. In conclusion, these results show that PC1 and PC2 biosynthesis are regulated by glucose, and also suggest that the processing of these proteins occurs earlier in the ß-cell secretory pathway than that of proinsulin.

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COORDINATE REGULATION OF THE TWO *XENOPUS* NONALLELIC INSULIN GENES IN ADULT PANCREAS

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The two insulin genes in *Xenopus Laevis* are expressed differentially in prepancreatic embryos. We now examine adult pancreatic regulation in response to alterations in temperature, glucose administration, somatostatin treatment, and aging. Insulin mRNA levels were quantitated by slot blot hybridization with specific probes, and were expressed as x 10^8molecules/5ugRNA±SEM. Compared to frogs at 20°C, frogs at 29°C showed a coordinate decrease in insulin I and II mRNA levels (Insulin I, 3.41±0.34 vs. 2.39±0.17; Insulin II, 2.59 ±0.36 vs. 1.67±0.09; $p<0.05$); there were no significant changes at 12°C. Both insulin I and II mRNA levels decreased slightly in frogs given prolonged glucose and in those fed *ad libitum*; there were no changes after a single dose of glucose or in frogs given somatostatin. Insulin I and II mRNA levels were higher in older frogs (36 months; Insulin I 3.68±0.43, Insulin II 3.26±0.38 vs. 6 months; 2.14±0.15 and 1.21±0.06; $p<0.05$), and there was a modest reduction in the percentage of insulin I mRNA with aging (6 months 63.6±3.1 vs. 36 months 53.9±2.7; $p<0.05$). We conclude that the two nonallelic insulin genes are regulated coordinately in adult pancreas, and suggest that the mechanisms regulating insulin gene expression in prepancreatic embryos are distinct from those of the adult pancreas.

OP 43
Insulin Resistance and Macrovascular Disease

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THE BOTNIA STUDY: CONCOMITANTS OF ABDOMINAL OBESITY IN PERSONS PREDISPOSED TO TYPE 2 DIABETES.

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Epidemiological studies have shown that abdominal obesity (AOB) is associated with an increased risk for diabetes, hyperlipidemia and hypertension as well as cardiovascular disease. The impact of abdominal obesity defined as waist/hip ratio (WHR) ≥1.0 in men and ≥0.8 in women among 898 (422 males/476 females) glucose-tolerant first-degree relatives of Type 2 diabetic patients was studied. 29% of males had AOB, which was associated with higher diastolic BP (82±1 vs 79±1 mmHg; p<0.05), fasting insulin (9±1 vs 8±1 μU/ml; p<0.01), triglycerides (1.7±.1 vs 1.5±.1 mmol/l; p<0.05), BMI (27.3±.3 vs 25.0±.2 kg/m^2; p<0.001) and fat percentage (23.6±.4 vs 21.0±.2 %; p<0.001) vs those with WHR <1.0. 73% of females had WHR ≥0.8, which was associated with higher diastolic BP (79±1 vs 77±1 mmHg; p<0.05), fasting insulin (8±1 vs 7±1 μU/ml; p<0.05), cholesterol (6.1±.1 vs 5.7±.1 mmol/l; p<0.05), triglycerides (1.3±.1 vs 1.0±.1 mmol/l; p<0.001) and BMI (25.7±.2 vs 24.1±.3 kg/m^2; p<0.001), but similar fat percentage (30.8±.5 vs 30.2±.4 %) vs. those with WHR <0.8. In males and females AOB correlated with BMI (r=0.438; p<0.001 and r=.260; p<0.001) butwith fat percentage .among men only (r=0.260; p<0.001).
We conclude that abdominal obesity in first-degree relatives of Type 2 diabetic patients is associated with hyperlipidemia, increased blood pressure and hyperinsulinemia.

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INSULIN RESISTANCE AND HYPERTRIGLYCERIDEMIA IN CHILDREN OF NON-INSULIN DEPENDENT DIABETICS

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Type 2 diabetes appears to be an inherited condition. Our aim was to identify early metabolic abnormalities in non-diabetic offspring of one, type 2, diabetic parent with strongly positive (n=58, age 27.8±7.0 yrs) or negative family history (n=38, age 27.4±6.7 yrs), compared with 31 matched offspring of nondiabetic parents (NDP). After an overnight fast, blood was taken for glucose, insulin, C-peptide, lipids and insulin receptors, while all the subjects had a 75g oral glucose tolerance test. The number of insulin receptors was estimated by incubating freshly separated red blood cells with Mono ^{125}I-(TyrA14)-human insulin. The positive family history group had significantly higher fasting levels of triglyceride (1.09±0.2 vs NDP: 0.93±0.1 mmol/l, p<0.001), insulin (102.8±46.4 vs NDP: 77.5±32.4 pmol/l, p<0.01) and C-peptide (0.69±0.2 vs NDP: 0.61±0.1 nmol/l, p<0.05) and lower number of insulin receptors (9.1x10^3 vs NDP: 11.2x10^3, p<0.01). After glucose challenge (120 min) the increases in both insulin and C-peptide concentrations were significantly greater in the first group offspring (289.2±241.1 pmol/l, 2.2±1.4 nmol/l respectively) than in NDP (192.4±170.3), p<0.05, (1.5±0.9), p<0.01. No significant differences were found in fasting and postchallenge glucose levels. The negative family history group had only significantly lower number of insulin receptors (9.4x10^3) compared with NDP (p<0.05). The results indicate the genetic origin of these metabolic changes.

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INSULIN-RESISTANCE AND ISCHEMIC HEART DISEASE IN TYPE 2 DIABETES MELLITUS

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To assess if, in type 2 diabetes mellitus, patients with ischemic heart disease (IHD) are more insulin-resistant compared to patients without IHD, we performed an insulin tolerance test (ITT), along with the evaluation of other cardiovascular risk factors, in two groups of male patients, 36 with and 36 without IHD, with comparable age, length of diabetes, body mass index (BMI) and metabolic control. Patients with IHD showed a higher insulin-resistance (K_{ITT} index 2.45±0.18 vs 3.12±0.13, p = 0.0036), total (235±8 vs 208±6, p = 0.011) and LDL-cholesterol (149±7 vs 128±5, p = 0.013), prevalence of hypertension (83 vs 44%, p < 0.001) and microalbuminuria (34 vs 18%, p = 0.009). No differences were found between HDL-cholesterol, triglycerides, insulin and C-peptide plasma levels and waist to hip ratio. Multiple logistic regression analysis revealed only hypertension (odds ratio 2.32, conf. int. 95% 1.29-4.18, p = 0.005), insulin-resistance (odds ratio 1.9,conf. int. 95% 1.04-3.46, p = 0.036) and total cholesterol (odds ratio 1.01, conf. int. 95% 1.001-1.028, p = 0.038) independently related to IHD. After correction for age, K_{ITT} was the only metabolic feature related to the age of clinical onset of IHD, including in the analysis as independent variables even BMI, total and HDL-cholesterol, triglycerides and length of diabetes. In conclusion: 1) type 2 diabetic patients with IHD are more insulin-resistant compared to patients without IHD; 2) hypertension, insulin-resistance and total cholesterol are the only features independently related to IHD; 3) the higher is the insulin-resistance level, the earlier is the age of clinical onset of IHD.

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INSULIN RESISTANCE IN FAMILIAL AND NONFAMILIAL HYPERCHOLESTEROLEMIA

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Insulin resistance has been associated with high very-low density lipoprotein triglyceride and low high-density lipoprotein cholesterol levels. Whether or not hypercholesterolemia is an insulin resistant state has not been investigated. Therefore, we studied 8 patients with familial hypercholesterolemia and 13 corresponding controls with normal total cholesterol level (<5.3 mmol/l) (age 31±1 vs. 27±2 yrs, mean±SEM, p=NS; body mass index 23±1 vs. 23±1 kg/m^2, p=NS; total cholesterol 9.4±0.4 vs. 4.5±0.2 mmol/l, p<0.001)(Study I). Furthermore, we studied 25 patients with nonfamilial hypercholesterolemia and 18 corresponding controls (total cholesterol <5.3 mmol/l) (age 52±1 vs. 55±1 yrs, p=NS; body mass index 26±1 vs. 27±1 kg/m^2, p=NS; total cholesterol 7.4±0.1 vs. 4.5±0.1 mmol/l, p<0.001)(Study II). All subjects had normal glucose tolerance and did not have hypertension or any drug treatment which could influence glucose metabolism. Insulin responses in an oral glucose tolerance test did not differ between patients and controls in Studies I and II. In 3-h euglycemic hyperinsulinemic (insulin infusion rate 80 mU/m^2/min) clamp studies the rates of whole body glucose uptake were similar in patients and controls in Study I (71.5±6.3 vs. 69.4±3.4 umol/kg/min, p=NS) and Study II (60.5±3.2 vs. 58.0±2.8 umol/kg/min, p=NS). Similarly, rates of glucose oxidation (Study I: 20.1±1.8 vs. 19.4±0.9 umol/kg/min, p=NS and in Study II: 20.0±0.6 vs. 20.3±0.7 umol/kg/min, p=NS) and glucose nonoxidation (Study I: 51.4±5.3 vs. 50.0±3.1 umol/kg/min, p=NS and in Study II: 40.5±2.8 vs. 37.7±2.7 umol/kg/min, p=NS) did not differ between patients and controls. We conclude that impaired insulin mediated glucose uptake is not a characteristic finding in patients with familial and nonfamilial hypercholesterolemia.

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INSULIN-LIKE GROWTH FACTOR-1 REFLECTS BLOOD GLUCOSE LEVEL AND INTRAABDOMINAL FAT MASS IN OBESITY

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Abdominal obesity is associated with Type 2 (non-insulin dependent) diabetes mellitus. However, the effect of obesity and fat distribution on Insulin-like growth factor-1 (IGF-1) concentrations is not clear. The aim was to characterize the association between visceral adipose tissue and IGF-1 in obese patients before and after a moderate energy restriction (1200 kcal/day). In 60 obese patients (body mass index (BMI), 27-39 kg/m^2) IGF-1, growth hormone, insulin and blood glucose concentrations were tested for correlation with anthropometric measures. After an overnight fast, blood samples were drawn at the pre-trial visit, and again after 8 weeks and 16 weeks of treatment. Visceral adipose tissue was estimated from CT-calibrated equations. Linear regression analyses, and ANOVA-tests were performed. We found a significant association between IGF-I and visceral adipose tissue (r= -0.41, p= 0.006, females only (n= 51)). No correlation was found between IGF-1 and BMI or body weight. During weight loss (8.1± 5.8 kg, mean±SD), blood glucose levels decreased significantly (p= 0.003). A significant inverse correlation between IGF-1 and blood glucose levels were present before as well as after 8 and 16 weeks of treatment (r= -0.37, p= 0.007). **Conclusion:** IGF-1 reflects the intraabdominal fat mass (visceral adipose tissue) rather than obesity *per se*. IGF-1 and blood glucose levels are inversely correlated in obesity.

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OVERWEIGHT, HYPERTENSION AND HYPERLIPIDEMIA IN CHILDREN AND ADOLESCENTS WITH TYPE I DIABETES.

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Cardiovascular risk factors contribute to reduced life expectancy in diabetes mellitus. However, little is known on the prevalence of overweight, hypertension and hyperlipidemia in pediatric patients with insulin-dependent diabetes. A database encomprising 310 patients of German descent (age 2 - 23 years) followed longitudinally at one institution for up to 20 years (5053 visits) was evaluated. z-scores (SDS) for height, weight and blood pressure were calculated. During the first 5 years of diabetes, z-score was + 0.26 ± 0.03 (mean ± SE) for height and + 0.66 ± 0.02 for weight. Subsequently, progressive overweight developed: If diabetes lasted for > 10 years, weight-SDS increased to + 0.85 ± 0.04, while height-SDS decreased to - 0.27 ± 0.06 (p < 0.0001, Wilcoxon). Systolic blood pressure was elevated, even during the early course of diabetes (SDS: + 0.87 ± 0.02, p < 0.0001) and increased further with the duration of diabetes (r = + 0.1, p < 0.001). Z-score for diastolic blood pressure was + 0.29 ± 0.01, with no significant change during the course of diabetes. At the beginning of diabetes, cholesterol averaged 4.50 ± 0.05 mmol/l and triglycerides 1.08 ± 0.04 mmol/l. While cholesterol remained constant in boys, a positive correlation with duration of diabetes was present in girls (r = 0.23; p < 0.0001). Conclusion: Overweight, hypercholesterolemia and hypertension are common in pediatric and adolescent patients with type I diabetes and contribute to future cardiovascular risk. Studies based on large populations are necessary to detect gradual changes during the early course of diabetes. This progression should be preventable by improved therapeutic regimen.

OP 44
Nephropathy in Children

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PREVALENCE OF EARLY MICROVASCULAR COMPLICATIONS IN YOUNG TYPE I DIABETIC PATIENTS: ROLE OF PUBERTY, DURATION OF DIABETES, SEX AND AGE AT ONSET OF DIABETES.

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The prevalence and correlates of the early signs of renal, retinal and nervous microvascular complications have been evaluated simultaneously in 317 young type I diabetic patients. Microalbuminuria has been detected on 11% of patients and appeared to be strongly and positively related to HbA1c (p=0.006) and less significantly to age at onset (p=0.066) and duration of diabetes (p=0.031). Glomerular hyperfiltration has been identified on 20.7% of patients and a weakly positive association has been found with HbA1c (p=0.068) and male gender (p=0.048) and a negative association with age at onset (p=0.045). Retinopathy has been detected on 22.7% of patients and it was associated with duration of diabetes (p=0.001) with age at onset (p=0.030). Peripheral somatic nervous dysfunctions have been detected on 18.5% of patients, a strong association has been identified with duration of diabetes (p=0.005) and HbA1c (p=0.001). Autonomic nervous dysfunctions have been detected on 5.1% of patients. Microalbuminuria has not been detected in prepubertal patients while a similar frequency of retinopathy, glomerular hyperfiltration and nervous dysfunctions have been observed in prepubertal and postpubertal patients. These results suggest that diabetes *per se* (namely short-term metabolic control and duration of diabetes) plays the major role on the onset of nervous complications. Both puberty and diabetes control are involved in the appearance of microalbuminuria. Glomerular hyperfiltration does not show any strong relationship with the variables examined. The appearance of retinopathy was strongly related to duration of diabetes and weakly to increase of age at onset of diabetes.

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THE INCIDENCE OF NEPHROPATHY IN JUVENILE TYPE 1 DIABETES HAS DECREASED.

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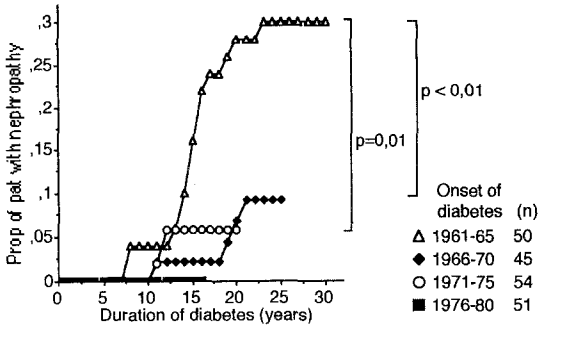
To find out whether the incidence of nephropathy in juvenile type 1 diabetes has changed during the last 30 years have we studied all 213 juvenile type 1 diabetic patients with onset <15 years diagnosed 1961-1980 in a district of southeast Sweden. All patients could be traced and 92% of the patients was followed from onset to 1991 or death. Nephropathy was defined as urinary albumin excretion >200µg/min or consistent albuminuria, albustix ≥1. The time of onset of nephropathy was obtained from medical records. Survival analysis and log rank test were done. The patients were grouped according to year of onset with 5 years intervals. The incidence of nephropathy decreased significant from 1961-65 to 1966-70 and 1971-75. None of thous diagnosed 1976-80 has developed nephropathy.



Conclusion: The incidence of nephropathy has decreased considerably,with few new cases during the last five years.

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ALBUMIN EXCRETION RATE IN THE SECOND HALF OF NORMAL RANGE PREDICTS PERSISTENT MICROALBUMINURIA IN DIABETIC CHILDREN

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Urinary albumin excretion rate (AER) greater than values obtained in normal subjects is considered a risk factor for the development of kidney disease and failure in type 1 diabetes. Little is known about minimal elevation of AER during childhood as a predictor of later development of persistent microalbuminuria and incipient diabetic nephropathy in youth.

In 1982 we evaluated AER in a cohort of type 1 diabetic children and adolescents, aged 2 to 17 years, with duration of diabetes longer than 1 year. Among these patients 8 had persistent microalbuminuria (AER > 30 $\mu g/min/1.73 m^2$ in at least 3 out of 5 consecutive timed overnight collections). Twenty children showed AER persistingly in the second half of normal range. Ten years after, 14 out of these patients developed persistent microalbuminuria, while only 1 out of 39 children with AER in the first half of normal range developed persistent microalbuminuria. The positive predictive value for persistent microalbuminuria of an AER in the second half of normal range was 67%; the negative predictive value for persistent microalbuminuria of an AER in the first half of normal range was 95%.

Diabetic children with AER in the second half of normal range have an increased risk to develop persistent microalbuminuria and, consequently, clinical diabetic nephropathy.

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EARLY ONSET OF DIABETES MELLITUS IS AN EXTRA RISK FACTOR FOR DIABETIC NEPHROPATHY

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Aims: 1. Is early onset of diabetes a risk factor for diabetic nephropathy(DN) as is assumed in diabetic retinopathy(DR). 2. How do these patients compare to diabetic controls without DN matched for age at onset, sex and duration according to glycaemic control, DR, lipids and smoking.

Data of all IDDM patients diagnosed before the age of 25 and with duration of disease > 8yrs (n=171) were analysed (age at onset, duration, DR, HbA1c, lipids and smoking). DN was defined as an urinary albumin/creatinin ratio > 3 mg/mmol. Patients were divided according to age at diagnosis: I: 0-4yrs, n=19, mean duration 24yrs (12-38); II: 5-9yrs, n=35, 24 (10-39); III: 10-14yrs, n=48, 21 (8-57); IV: 15-19yrs, n=35, 19 (8-53); V: 20-24yrs, n=34, 20 (8-50).

Results: 35 patients (21%) have DN: 4(21%)in I; II: 10(29%), III: 12(25%), IV: 6(17%), V: 3(9%). Even when matched for duration, DN is found significantly more often in patients with onset before the age of 15: 21/83 in I-III (25%, mean duration 18yrs, 8-33) vs. 7/63 in IV-V (11%, mean duration 17yrs, 8-33), $p<0.05$, whereas HbA1c is not different (7.9±1.8 vs. 7.6±1.5%).

DN patients have higher HbA1c (8.4±1.9 vs. 7.4±1.3%, $p<0.01$) and more DR (76 vs. 45%, $p<0.01$) compared to matched controls; lipids and smoking were not different.

Conclusions:1. Age at onset < 15yrs is an extra risk factor for DN. 2. Glycaemic control is worse and DR more frequent in patients with DN.

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RELATIONSHIP BETWEEN BLOOD PRESSURE AND URINARY ALBUMIN EXCRETION RATE IN CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES. COMPARISON OF RESULTS TO NON-DIABETIC CHILDREN.

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During six months a nation-wide screening for blood pressure and urinary albumin excretion rate (AER) was carried out in 502 males and 440 females with Type 1 diabetes (approximately 80% of total) treated at 22 paediatric departments. Mean age (range) for the patients was 13.5 years (2-18) with a mean diabetes duration of 64 months (0-211). In addition 663 children (334 males, 329 females), mean age 13.9 (range 6-18) years served as a control group with respect to blood pressure. For all diabetic children blood pressure were recorded once using an automated apparatus. In normal control children blood pressure were recorded by a single investigator with the same random-zero-sphygmomanometer. Microalbuminuria was defined as AER >20-150 $\mu g/min$ in at least 2 out of three timed overnight urine collections and diagnosed in 43 children. Seven had overt proteinuria (>150 $\mu g/min$). For both sexes blood pressure (systolic and diastolic) increased with age in the diabetic patients and in the control group. No statistical significant difference was observed between diabetic children with AER <20 $\mu g/min$ and normal control children. In contrast 29 out of 43 adolescents with microalbuminuria had diastolic blood pressure in the upper quartile. By multivariate logistic analysis the only predictors of microalbuminuria were age and diastolic blood pressure. These findings suggest that elevated arterial blood pressure may be related to the increased prevalence of microalbuminuria observed in adolescents with Type 1 diabetes. Elevated blood pressure in childhood should lead to examination of urinary albumin excretion rate.

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DECREASE OF GLOMERULAR HYPERFILTRATION IN SHORT-TERM DIABETIC ADOLESCENTS WITHOUT MICROALBUMINURIA.

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Objective: the study has been designed to follow prospectively glomerular filtration rate (GFR) and urinary albumin excretion (UAE) of young patients with short-term insulin dependent diabetes mellitus and normal UAE.

Research design and methods: The study population consisted of 19 patients with glomerular hyperfiltration (146.8±11.1 ml/min/1.73m2)and 21 patients with normal GFR (114.0±17.5 ml/min/1.73m2), matched for duration of diabetes (8.3±2.7 vs 9.3±3.7 yrs)and age (13.9±4.0 vs 15.4±3.2 yrs). GFR has been assessed by radiosotopic tracer and UAE by radioimmunoassay at the initial of the study and after 30.5±10 months of follow-up.

Results: GFR decreased in the two groups but Δ GFR of patients with glomerular hyperfiltration was greater than Δ GFR of patients with normal GFR (0.83±0.55 vs 0.27±0.56 ml/min/month $p<0.005$). UAE (log10: 0.83±0.42 vs0.86±0.39 $\mu g/min$), blood pressure and prevalence of microalbuminuria (16% vs 19%) were comparable between the two groups at follow-up. Rate of fall of GFR was positively correlated with initial GFR ($p<0.001$) but not with initial UAE, blood pressure or with changes in HbA1c, UAE and blood pressure or pubertal development during follow-up.

Conclusion: glomerular hyperfiltration over 3 years of follow-up is associated with higher decline in GFR without greater appearance of microalbuminuria or increase of UAE, than in patients with normal GFR.

OP 45
Genetics II

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A NEW MARKER FOR TYPE I DIABETES IN THE MHC CLASS I REGION CLOSE TO HLA-X

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Type-I diabetes is associated with the Major Histocompatibility Complex (MHC), in particular the class-II DR and DQ loci. It has been suggested that the MHC class-I region may confer disease susceptibility independent of the class-II region. We have employed molecular probes and Southern blots to investigate the class-I region of 73 patients with type-I diabetes and 48 normal controls. The P3 probe with the restriction endonuclease Pst-I recognises 2 loci - P3A is located 40 kilobase (kb) centromeric to the HLA-B locus whilst P3B resides 650 kb telomeric of HLA-B close to HLA-X. Allelic fragments of 4.0 and 3.8 kb correspond to P3A and 1.8 and 1.5 kb to P3B. A highly significant increase in the frequency of the 1.5 kb fragment was found in the patient group compared to normal controls (80.6% vs. 37.5% p <0.0005). The 1.5 kb P3B fragment was present on 100% of HLA-A1,B8,DR3 haplotypes in both patient and control groups. The HLA-A1/1.5 kb P3B combination was present in 36% of patients and only 18.4% of controls. No association was found with P3A. These results suggest that this MHC region (HLA-X) contains a susceptibility gene for type-I diabetes.

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Pooled, Multiplex, Single Nucleotide Primer Extension (SNuPE): A Novel and Powerful Tool for Genetic Studies in Diabetes.

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The detection of mutations in candidate genes in patients with Type 2 diabetes is only the first step in proving a causative link between the mutation and the disease. Studies of the prevalence of the mutation in large populations of diabetic and control subjects play an important role in determination of its pathological relevance. We have adapted the technique of SNuPE to detect mutant sequences at a number of different loci simultaneously and with sufficient sensitivity for use on pooled samples of genomic DNA. Using multiplex SNuPE, three mutations previously detected in patients with Type 2 diabetes were sought for in a further 42 highly insulin-resistant Type 2 diabetic patients selected from the UK Prospective Diabetes Study to represent those >98th percentile for fasting insulin. Insulin receptor (IR) Val-Met^{985} was found in 2/42, IR Lys-Glu^{1068} was not detected, and glucose transporter (GLUT4) Val-Ile^{383} was found in 2/42. IR Met^{985} had previously been described at a similar frequency in a non-diabetic population. GLUT4 Ile^{383} had not been found in any normal subject in two previous studies. Pooled SNuPE was used to rapidly search for this mutation in a population of 240 British Caucasian controls (age 53-60) with no personal history of diabetes. Four of 240 controls had the Ile^{383} variant. We conclude that pooled, multiplex SNuPE is a valuable tool for genetic epidemiological research in diabetes and that the relevance of the Ile^{383} variant of GLUT4 to Type 2 diabetes requires further study.

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MHC CLASS II GENES IN TYPE 1 DIABETES MELLITUS ASSOCIATED WITH POLYENDOCRINE AUTOIMMUNE DISEASES

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Some patients with type 1 insulin-dependent diabetes mellitus present with polyendocrine autoimmune diseases (type 1b). This syndrome occurs often later in life, with a slower course to insulin dependency, than does type 1 diabetes mellitus free of these associations (type 1a). Are these two syndromes genetically distinct ? We analysed HLA DRB1, DQB1, DQA1 and DPB1 allelic polymorphisms after gene amplification by means of the polymerase chain reaction (PCR) using allele-specific oligonucleotidic probes (PCR-ASO) or restriction fragment length polymorphism (PCR-RFLP) in 215 control, 62 type 1a and 63 type 1b Caucasian subjects. Both diabetic groups presented significant enrichment in several risk alleles :

- DR3 (63% in 1a, 48% in 1b, vs 20% in controls; Pc<0.001);
- DR4 (45% in 1a, 43% in 1b, vs 20% in controls; Pc<0.001);
- DQB1* 0302 (36% in 1a, 33% in 1b, vs 12% in controls; Pc<0.001);
- DQB1* 0201 (71% in 1a, 64% in 1b, vs 34% in controls; Pc<0.001);
- DQA1* 0301 (44% in 1a, 46% in 1b, vs 22% in controls; Pc<0.001);
- DQA1* 0501 (73% in 1a, 61% in 1b, vs 41% in controls; Pc<0.001).

However, there were major differences in some gene profiles among the two patient groups. Type 1a group differed from type 1b and control groups by significant enrichment in DR4 subtype 0402 (13% in 1a, vs 0.5% in controls and 5% in 1b; Pc<0.02) and DPB1 *0301 allele (31% in 1a, vs 13% in controls and 15% in 1b; Pc<0.04). The expected linkage desequilibria (DR3 DQB1* 0201 DQA1* 0501) and (DR4 DQB1* 0302 DQA1* 0301) were present with significant increased frequency in both diabetic groups. However, allele DPB1* 0301 presented independently from these linkages as a supplementary risk marker in type 1a patients. Association of DPB1* 0301 to DR4 was found in 16% of type 1a patients (vs 1.5% in control and 6% in type 1b; Pc<0.01) and association of DPB1* 0301 to DR3 in 13% of the 1a group (vs 0.5% in control and 3% of 1b group; Pc<0.01). These DRB1* 0402 and DPB1* 0301 genes may contribute to confer some specificities to HLA genetic profile and could be responsible for speed and severity of insulitis in type 1a patients.

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MANGANESE SUPEROXIDE DISMUTASE (MnSOD) GENE POLYMORPHISMS IN IDDM PATIENTS AND CONTROLS.

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Beta cell cytotoxity may be due to IL-1 induced generation of reactive oxygen species. Genetic variation in the enzyme MnSOD locus could reflect differences in radical scavenger potential. We studied possible restriction fragment length polymorphisms (RFLPs) of this locus in IDDM patients (n=154) and controls (n=178). Only the restriction enzyme TaqI revealed a polymorphic pattern consisting of 7 fragments, including two diallelic polymorphisms comprising fragments of 2.3 kb/2.0 kb (RFLP A) and 1.5 kb/1.2 kb (RFLP B). No IDDM specific fragment pattern was observed. Only 6 of 9 possible genotypes were found. Deletion of the TaqI-site identifying RFLP B was always accompanied by deletion of the TaqI-site identifying RFLP A. This suggests that nucleotide changes may occur simultaneously in different regions of the gene. Deletion of the RFLP A TaqI-site A was not necessarily associated with deletion of the RFLP B TaqI-site. A trend for difference in overall genotype frequency between patients and controls was observed (p=0.11). This was due to significant differences in frequencies of the homozygous genotypes (p<0.03), demonstrating an association of simultaneous homozygosity of fragment 1 and 5 with IDDM. We hypothesize that RFLPs reflects differences in gene expression level, protein level and/or specific activity of MnSOD of importance for interindividual differences in beta cell susceptibility/resistance to IL-1 cytotoxicity.

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ANALYSIS OF GENES ENCODING 3 KEY PROTEINS IN INSULIN RESISTANT GLUCOSE UTILIZATION OF SKELETAL MUSCLE FROM TYPE 2 DIABETICS

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Using single stranded conformation polymorphism (SSCP) and direct nucleotide sequencing we have analyzed for mutations in 3 major candidate genes: Glut 4, glycogen synthase (GS) and the catalytic subunit of the GS-related protein phosphatase 1 (PP1β). Total RNA was isolated from muscle of Type 2 diabetics and controls, and cDNA was synthesized using reverse transcriptase. Promoter and coding regions were amplified applying PCR. Glut 4: A single base variation (sbv) was detected at codon 130: AAC (Asn) and AAT (Asn). The allele frequency (af) in Type 2 diabetics (n=52) and controls (n=42) was similar. Glut 4 promoter sequence was kindly given by J. Buse and the first base in codon 1 was assigned + 1. Two sbv's were found in the promoter at ÷ 162: A and G and at ÷ 773: C and T. The af was comparable between groups. GS: No mutations were detected in the coding region. PP1β: A sbv was found at codon 67: CAA (Gln) and CAG (Gln). Again, af was similar in the two groups. Conclusion: Analysis of the entire coding regions of Glut 4, GS and PP1β and the promoter of Glut 4 revealed no mutations with predictable functional implications but 4 polymorphisms which are frequent in the population.

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SIB-PAIR ANALYSIS OF THE GLUT1 GLUCOSE TRANSPORTER GENE IN TYPE 2 (NON-INSULIN DEPENDENT) DIABETES MELLITUS.

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Population association analysis of genetic factors in type 2 diabetes has so far yielded inconclusive results. An alternative approach is represented by linkage analysis in affected sib-pairs, where the observed concordance for a particular marker of a candidate gene is compared to that expected under the assumption of no linkage between marker and disease. With this method it is possible to study linkage in the absence of multigenerational families, extremely rare in type 2 diabetes. In the present study we have studied the glucose transporter gene GLUT1, a candidate gene for diabetes. The GLUT1 gene was analyzed in 55 sib-sets from Italy and Britain, for a total number of 78 sib-pairs, with two markers of this locus, the Xba-1 RFLP (2 alleles) in the GLUT1 gene and an Msp-1 RFLP (3 alleles), at 0.2 estimated recombination frequency. The results did not show departure from independent segregation between marker and disease at all the function of the allele frequency (F(p)) considered, except for F(p)=1/p for the Xba-1 RFLP, which resulted significant (p<0.01). However, F(p)=1/p gives a very strong weight to the allele frequency, probably over estimating its significance. We conclude that the GLUT1 gene is unlikely to play a major role in the aetiology of type 2 diabetes, although an accessory role for this gene can not be excluded.

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Exercise

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EFFECTS OF TRAINING ON INTERACTION OF INSULIN AND CONTRACTIONS IN MAN.

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To study if muscle contractions in addition to maximal insulin stimulation can increase glucose uptake in muscle (GUR), and if physical training will affect the contraction induced increase in GUR six young (23±2 years, mean±SEM), untrained men performed one-legged ergometer exercise at 70% of VO2max of the leg 30 min per day, 6 days per week for 10 weeks. Then femoral veins and the radial artery were cannulated and a euglycaemic clamp was performed. Blood flow was determined by thermodilution. After 120 min infusion of insulin of 480 mU/min/sqm subjects began 30 min of ergometer exercise with both legs at 70% of VO2max. During the 90-120 min of insulin infusion plasma insulin concentration was 16.3±0.6 nmol/l (2323±80 μU/ml). Glucose uptake in the leg (GURL) was higher (P<0.05) in trained (T) leg compared with untrained (UT) leg (T:127±13 μmol/min/kg (22.8±2.3 mg/min/kg), UT: 104±12 μmol/min/kg (18.7±2.2 mg/min/kg)). During contractions P-insulin was 17.8±0.8 nmol/l (2537±118 μU/ml) and GURL was increased dramatically (UT: 231±29 μmol/min/kg (41.6±5.3 mg/min/kg) and T: 283±33 μmol/min/kg (50.9±6.0 mg/min/kg)). During contractions the GURL and the contraction induced increase in GURL was significantly higher in T-legs than in UT-legs (P<0.05). Conclusions: physical training increases insulin responsiveness in muscle; contractions are also in presence of maximal insulin concentration a powerfull stimulus for glucose uptake; training increases the contraction induced enhancement of glucose uptake in muscle.

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COMPARATIVE BIODISPONIBILITY OF A NATURALLY LABELLED [13C]FRUCTOSE AND A [13C]GLUCOSE ORAL LOAD DURING MODERATE EXERCISE IN HEALTHY SUBJECTS.

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In a previous qualitative study, we demonstrated that 50g of naturally labelled [13C]fructose (13C-F) were used as a fuel to the same extent as than 50g of glucose (13C-G) during moderate exercise in normal subjects. To quantify sugar oxidation, 5 healthy male volunteers walked during 90 min on a treadmill at their 45% VO2 max. They randomly received 50g of 13C-F or 13C-G in 150 ml of water or 150 ml water alone (in 1 dose, after 15 min adaptation to exercise). Exogenous sugar oxidation was calculated from 13CO2 in expired air and total carbohydrate, lipid and protein oxidation was evaluated by simultaneous indirect calorimetry. Under the 3 conditions ie 13C-G, 13C-F, water alone, respectively : energy production rate was similar : 2803±274, 2863±485, 2784±294, KJ/90 min, m±SEM, ANOVA NS; protein oxidation rate: 7.3±1.0, 6.9±0.7, 8.1±0.9, g/90min, ANOVA NS. Total carbohydrate oxidation was equivalent : 80.5±10.7, 76.9±9.4, 70.0±11.3 (g/90min, ANOVA, NS). From the 50g of exogenous sugars : 18.4±3.2 of 13C-G and 17.8±1.3 of 13C-F were oxidized (g/90min, NS). There was no significant differences in lipid oxidation : 34.2±3.3, 39.2±7.2, 43.1±5.9 (g/90min, ANOVA, NS). In conclusion : glucose and fructose ingested by healthy subjects, in moderate amount, during moderate exercise, present the same biodisponibility as energetic fuels. These results confirm our previous qualitative experiment.

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EVALUATION OF 24 HOURS ENERGY EXPENDITURE IN TYPE I DIABETES BY RESPIRATION CHAMBER.

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No data are available on 24h metabolism monitoring in type I subjects; we evaluated the differences in energy balance and main substrate fluxes between ten type I patients (BMI 23+1; fat free mass(ffm) 63.4+4.6 kg) and eight normal healthy volunteers (BMI 22+1; ffm 61.2+6.5 kg). Patients were treated with intensive insulin therapy (0.6UI/Kg).and were in good metabolic control (HbA1c=5.5+0.7%) During the 30hrs spent in the chamber VO2, VCO2, RQ, 24h energy intake (EI), energy expenditure (EE), day-time EE, night-time EE, basal EE, EE during the exercise (40%of maximal effort), main substrate oxidation (lipids, carbohydrates and proteins) and overall diet-induced termogenesis (DIT) were calculated. The results were corrected for the urinary N2 loss. DIT was found significantly lower in diabetics vs controls (6.7+1.3% vs 11.8+4.7%, $p<0.05$). A regression analysis of DIT on 24h glycaemia showed for diabetic patients an inverse correlation ($r=0.7$, $p<0.01$). EE during exercise was 5.7+0.8kcal/kg ffm/min in diabetics vs 6.90+0.48kcal/kg ffm/min in controls ($p<0.01$).Since DIT is highly related to the theoretical cost of glucose storage and no difference was found in carbohydrates oxidation.these data indicate that in diabetics glucose storage is reduced when hyperglycaemia occurs. EE during exercise was found significantly lower in type I patients although neither hypoxia nor ketonemia occured; this probably indicates an adaptative response of insulin-dependent subjects to increased physical activity.

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MECHANISMS OF ENHANCED INSULIN SENSITIVITY IN WELL TRAINED IDDM PATIENTS

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The aim was to examine the mechanisms of improved insulin sensitivity in well trained male type 1 diabetic patients (n=7, age 31±3 yrs, body mass index 24±1 kg/m^2, duration of diabetes 14±4 yrs, insulin dose 43±3 U/day, HbA_{1c} 8.2±0.4%, $V0_2max$ 50±3 ml/kg/min) as compared to untrained (n=6, $V0_2max$ 42±4 ml/kg/min) but otherwise matched patients. Total body glucose disposal rate was 27% greater in the trained than untrained patients (54.7±2.4 vs 42.9±1.8 µmol/kg/min, $p<0.02$) as determined during a 4 hour euglycemic (5.1±0.1 vs 5.0±0.1 mM) insulin clamp with similar hyperinsulinemia (624±56 vs 648±85 pM) in the two groups, respectively. The difference was due to a 38% greater nonoxidative glucose disposal in the trained patients (38.3±2.6 vs 27.8±1.8 µmol/kg/min, $p<0.01$). Glucose oxidation rate (indirect calorimetry) was similar in the two groups. The arterialized blood – deep venous blood glucose concentration difference (A–V difference) was 75% greater (1.4±0.1 vs 0.8±0.2 mmol/l, $p<0.02$) and forearm glucose uptake 81% greater (38.1±4.2 µmol/l forearm/min, $p<0.02$) in the trained than untrained patients, respectively. In the whole group, the A–V difference correlated with total body ($r=0.79$, $p<0.002$) and nonoxidative glucose disposal ($r=0.69$, $p<0.01$). Forearm blood flow (pletysmography) was in the basal state similar in the two groups and remained unchanged during insulin infusion. In conclusion: 1) Improved insulin sensitivity in trained IDDM patients is due to increased nonoxidative glucose disposal. 2) The greater glucose disposal is accounted for by a higher glucose fractional uptake rather than increased blood flow.

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LATE POSTEXERCISE HYPERGLYCAEMIA IN WELL-CONTROLLED TYPE I DIABETIC PATIENTS: FACT OR FICTION ?

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We have tried to experimentally confirm reports of exercise induced late postexercise hyperglycaemia in 8 type I diabetic patients (5 women, 5 men; age 27 (3) years (mean (SD); HbA_{1c} 7.3 (1.2) %, VO_2max 32.7 (8.9) ml/kg body weight; BMI 23.8 (2.6) kg/m^2). Patients were subjected to moderate prolonged exercise at a heart rate of 120 bpm for one hour (MP), stepwise incremental exercise beginning at a work load of 80 watts increased by 40 watts every 3 min until exhaustion (E), a combination of both (MP+E) or rest (R). Experiments were performed in random order on 4 individual days, beginning at 5 pm and ending at 6 am the following morning. Insulin dose and carbohydrate intake were constant on all days. On no occassion were blood glucose levels significantly higher on the morning after exercise than at baseline (baseline vs. 12 h post-exercise): MP 5.4 (2.2) vs. 4.7 (1.5); E 6.4 (2.9) vs. 5.6 (2.8), MP+E 6.3 (3.8) vs. 5.8 (2.3)l, R 6.4 (3.0) vs.5.5 (3.5) mmol/l. All changes in hormone and substrate levels measured (serum growth hormone, glucagon, insulin, lactate, ketone bodies) were as to be expected from commonly accepted concepts. Heart rate, blood pressure and lactate rose significantly upon moderate prolonged exercise, more so with exhaustive exercise. Blood glucose fell signifcantly upon prolonged moderate but not upon exhaustive exercise. We did see several hypoglycaemic episodes in the early morning hours. However, contrary to other reports we did not observe late postexercise hyperglycaemia in well controlled patients with type I diabetes.

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Work capacity and oxygen uptake in insulin dependent diabetic patients with micro- and macroalbuminuria.

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Recent data indicate that microalbuminuria (micro) and macroalbuminuria (macro) are associated with reduced oxygen uptake during maximal exercise. The aim of this study is to further investigate the association of degree of nephropathy to work capacity and maximal oxygen uptake in IDDM. 35 IDDM patients classified as normoalbuminuric (normo), micro (urine albumin excretion (UAE) 20-200 ug/min) and macro (UAE>200 ug/min) and 14 healthy non-diabetic subjects (ctr) participated. Diabetes duration and glycaemic control were similar in the three groups. Age and sex distribution were not different between the four groups. Exercise test was performed on a ergometer bicycle until exhaustion (50 W/3 min). Oxygen uptake was calculated from the formula: Oxygen uptake (ml/kg/min) = work capacity (W) x 13 + (3.5 ml/min x body weight (kg) / body weight (kg). No symptoms or signs of ischaemic heart disease occurred. Results:

	No	RHR	MHR	WC	OU
ctr	14	74(11)	173(10)	208(42)	44(5)
normo	12	84(7)	175(10)	205(38)	40(7)
micro	12	84(17)	170(14)	194(69)	38(11)
macro	11	86(14)	152(19)	141(56)	31(9)

No:number of persons, RHR:resting heart rate(min -1), MHR:maximal heart rate(min-1), WC:work capacity, OU:oxygen uptake, mean(+/-1SD), s:p<o.o5.

Patients with macro had significantly lower work capacity and oxygen uptake than ctr and normo, but a trend towards progressive decreased oxygen uptake and work capacity from normo to macro is seen.

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Clinical Pancreatic Transplantation

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THE ULTRASTRUCTURE OF ISOLATED HUMAN PANCREATA IMPROVES ATFER A COLD STORAGE OF AT LEAST 24 h
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Transplantation of human pancreas is becoming an effective treatment for insulin dependent (Type 1) diabetes mellitus. Final success of pancreas transplantation depends also on viability and structural integrity of the organ, which can be influenced by storage time of the pancreas prior to transplantation. We investigated the ultrastructural morphology of endocrine and exocrine pancreas in 4 organs stored for transplantation. Specimens were taken from the tail of the pancreas of a donor in situ and after a three hour perfusion with cold Belzer UW solution (ViaSpan). In three other organs, specimens were taken from the tail after the same perfusion and cold storage in fresh ViaSpan for a total of 19, 24,and 48 hours. Ultrastructural examination of the exocrine pancreas showed that the overall morphology was better in 24 hour specimens in comparison with in situ, 48, 3, and 19 hour samples, respectively. In particular, this applied to endoplasmic reticulum and secretory granules. In the endocrine pancreas, islet cell examination showed that in 19 hour specimen, B cells were almost degranulated and their cytoplasm was disorganized; granules of A cells were collapsed. The general islet morphology looked increasingly better at 3, 24, and 48 hours. These preliminary results suggest that a cold storage of at least 24 hours before procedures for transplantation gives better structural conditions of the pancreas. Interestingly, large-scale retrospective studies showed that, up to 30 hours, the pancreas graft functional survival rates progressively improved with increasing storage times.

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IMPAIRED INSULIN SECRETION AND PERIPHERAL GLUCOSE UPTAKE IN HUMAN PANCREAS TRANSPLANT RECIPIENTS.
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Metabolic responses to 75 g oral glucose were studied in nine combined pancreas-kidney transplant recipients with systemic insulin secretion. Four recipients had impaired glucose tolerance (IPx), and five had normal glucose tolerance (Px). Eight non-diabetic kidney transplant recipients (Kx) and eight normal subjects (Ns) served as controls. Identical immunosuppression was given to the transplant recipients. ß-cell function was assessed from insulin secretion rates using the combined "minimal model". Glucose kinetics were assessed with dual-isotop technique (3-^{3}H-glucose infusion, 1-^{14}C-glucose oral administration) and indirect calorimetry. Total incremental insulin secretion was 2-fold increased in Kx, 1.6-fold increased in Px, but not significantly different in IPx compared to Ns ($p<0.05$ in Kx vs IPx and Px vs IPx). Maximal insulin secretion rate was in Kx: 0.14±0.02; Px:0.09±0.02; IPx:0.05±0.01; Ns:0.07±0.01 nmol•l^{-1}•min^{-1} ($p<0.05$ in Kx vs IPx and Px vs IPx). Rate of exogenous glucose appearance and hepatic glucose production were similar in all groups. Glucose uptake (0-120 min) was significantly reduced in IPx:385±17 vs Ns:556±39; Kx:523±66; Px:622±53 mg•kg^{-1} ($p<0.05$). Postglucose rate (0-120min) of oxidative and non-oxidative glucose metabolism was reduced by 45% in IPx compared to the other groups ($p<0.05$). Conclusion: Reduced glucose disposal in pancreas recipients with impaired glucose tolerance is most likely secondary to impaired ß-cell function.

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THE EFFECT OF INSULIN RESISTANCE ON GLUCOSE TOLERANCE AFTER PANCREAS TRANSPLANTATION
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Insulin resistance is present in recipients of pancreatic graft receiving immunosuppressive therapy. To investigate quantitavely its impact on glucose tolerance, hyperglycemic insulin clamp (12 mmol/l for 120 min.) was performed in 10 normoglycemic pancreas and kidney recipients (PKR), 11 nondiabetic kidney recipients on the same immunosuppression (KR), 10 Type 2 diabetics satisfactorily treated with diet (DM2), and 17 matched healthy controls (HC). In PKR, KR and DM2, insulin resistance was proved using the hyperinsulinemic isoglycemic clamp technique at insulin levels of ~100 and ~2000 mU/l as 39 and 29%, 42 and 24%, and 38 and 18% decreases of metabolic clearance rates of glucose in comparison with HC, respectively ($p<0.01$). During the last 20 min of the hyperglycemic clamp, glucose utilisation rates in PKR, KR and DM2 were 8.2±1.7, 9.0±1.5 and 4.6±1.3 g/kg/min and were all significantly lower than 17.3±1.3 g/kg/min (mean ±SE) in HC ($p<0.01$). Basal insulinemia was increased in PKR, KR and DM2 ($p<0.05$). Both the early and the late insulin responses were normal in PKR and KR, but were impaired in DM2. We conclude, that the main cause of decreased glucose tolerance in recipients with well functioning pancreatic graft is not insufficient insulin secretion, but insulin resistance, which is roughly of the same degree as in nondiabetic kidney recipients and Type 2 diabetics on diet.

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EFFECT OF SOLITARY PANCREAS TRANSPLANTATION ON THE PROGRESSION OF DIABETIC NEPHROPATHY.
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Pancreas transplantation (PTx) halts development of early diabetic nephropathy (DN) in renal allografts. We evaluated effects of solitary PTx on progression of DN in Type I (insulin-dependent) diabetic patients (D) with native kidneys. 12 D, 32±7 yrs old (duration of D: 21±5 yrs), had renal function studies and biopsy before and 5 yrs after PTx. 6 D, 29±10 yrs old (duration of D: 18±7 yrs), did not receive PTx and were studied at similar times (C). HbA_1, decreasing from 10.3±1.3 to 6.5±0.7% ($p<0.001$) following PTx, was unchanged in C (11.9±2 and 11.3±2.6, NS). Creatinine clearance went from 100±22 to 67±24 ml/min/1.73 m^2 in PTx ($p<0.001$) and from 94±19 to 86±28 in C (NS); albumin excretion went from 497±871 to 486±926 mg/24 hrs in PTx (NS) and from 24±26 to 260±526 in C (NS). Mesangial fractional volume increased from 0.33±0.08 to 0.38±0.11 in PTx ($p=0.007$) and from 0.29±0.06 to 0.37±0.12 in C ($p=0.054$). Glomerular basement membrane width was 594±139 and 559±113 nm in PTx (NS), and 613±165 and 591±174 in C (NS), at baseline and 5 yrs, respectively. Following PTx % sclerosed glomeruli increased from 13±16 to 37±16 ($p<0.001$) and interstitial fractional volume from 0.26±0.05 to 0.34±0.08 ($p=0.004$). The outcome was similar when PTx and C were matched for baseline renal structure. Thus, solitary PTx, despite euglycemia, cannot halt progression of established DN.

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FOLLOW-UP OF AUTONOMIC NEUROPATHY AFTER SUCCESSFUL PANCREAS TRANSPLANTATION

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One important aim of pancreas transplantation is stabilisation or regression of diabetic complications. Therefore a prospective controlled study of autonomic neuropathy was performed in 25 type 1-diabetics after simultaneous pancreas/kidney transplantation over a period of 53 (30-74) months. Group 1: n=15 both organs functioning. Group 2: n=10 pancreatic graft loss 2 (1-11) months after transplantation with kidney functioning.
Cardiac autonomic neuropathy (cANP) was measured by R-R-variability during deep breathing [E/I-ratio, mean R-R-distance, variability of heart frequency (VHF)], Ewing-test [30/15-ratio] and Valsalva maneuver [Max/Min-ratio]. Orthostatic dysfunction was diagnosed by measuring blood pressure during lying and standing. Neuropathic symptoms were analyzed using a graded questionnaire. The results 2 (1-5) respectively 53 (30-74) months after transplantation:
Group 1: E/I: 1,1 ± 0,19 → 1,09 ± 0,9 (n.s.); R-R-dist.: 689 ± 125 → 768 ± 81 (p≤0,01); VHF: 6,75 ± 10,1 → 5,8 ± 5,31 (n.s.); 30/15: 1,03 ± 0,01 → 1,07 ± 0,05 (p≤0,01); Max/Min: 1,23 ± 0,18 → 1,23 ± 0,21 (n.s.).
Group 2: E/I: 1,04 ± 0,02 → 1,05 ± 0,03 (n.s.); R-R-dist.: 619 ± 83 → 759 ± 140 (p≤ 0,01); VHF: 3,34 ± 1,6 → 3,86 ± 2,85 (n.s.); 30/15: 1,00 ± 0,02 → 1,04 ± 0,04 (p≤0,01); Max/Min: 1,1 ± 0,04 → 1,26 ± 0,24 (n.s.).
Orthostatic dysfunction was measured in 7 out of 15 (47%) in group1 and 6 out of 10 (60%) in group 2. It improved in both groups (to 27% respectively to 40%), but it didn't reach significance.
Neuropathic symptoms like gastroparesis [7 of 25 (group 1+2)], erectile impotence [3 of 25], nocturnal sweating [13 of 25] or gustatory sweating [6 of 25] didn't change significantly.
In contrast to sensory-motor neuropathy normalisation of blood glucose doesn't lead to a marked regression of autonomic neuropathy even more than 4 years posttransplant.

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QUALITY OF LIFE ASSESSMENT IN TYPE 1 DIABETIC PATIENTS AFTER ORGAN TRANSPLANTATION. COMPARISON OF TWO METHODS.

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Quality of life assessment is important especially for expensive and incisive therapeutic interventions like organ transplantation, however the tools to measure quality of life are still under debate. We therefore compared results obtained by a home-based questionnaire (217 questions) with those of a structured evaluation (77 questions) judged by the responsible physician. The patient sample consisted of 48 type 1 diabetics: Group 1 (n=22): patients after successful transplantation of pancreas and kidney. Group 2 (n=19): patients with functioning kidney graft but insulin requirement. Group 3 (n=7): patients after rejection of both organs. The age of the patients (36±1y), the duration of diabetes (24±1y) and the degree of secondary complications were very similar in all groups. In the 10 items scored from 1 to 5 consisting of anxiety (a), indifference (b), annoyance (c), pessimism (d), depression (e), vocational (f) and financial (g) situation, partner relationship (h), leisure time activity (k) and overall quality of life (l) there were much higher scores in group 1 and 2 compared to group 3. However, there was no significant difference between group 1 and 2. Quality of life was markedly ($p<0.05$) lower in 7 (d-l) items in comparison to group 1 and 2 when judged by the physician, but only in 3 (f, g, k) out of the 10 parameters for the patient-based questionnaire. Only in 2 parameters (i.e. anxiety and overall quality of life) a significant difference ($p<0.05$) existed between both methods indicating a rather good agreement on many aspects of life using opposite approaches of quality of life assessment. The physician tends to score more positively in those patients with functioning transplants and worse in the patients after rejection. In conclusion both methods are valid instruments in assessing quality of life, which has to be tested in a large number of patients and not only in a cross-sectional but also in a prospective study.

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Hormone Receptors

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INSULIN RECEPTOR ISOTYPE EXPRESSION CORRELATES WITH RISK OF NON-INSULIN-DEPENDENT DIABETES

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The human insulin receptor exists in two structurally distinct isoforms, HIR-A and HIR-B, which are generated by alternative splicing. Altered expression of these receptor isoforms has been detected in skeletal muscle from patients with non-insulin-dependent diabetes. To assess whether this phenomenon precedes the clinical onset of type 2 diabetes, we examined receptor expression in skeletal muscle biopsies from non-diabetic first degree relatives of non-insulin-dependent diabetic patients with varying levels of insulin resistance (and increased risk of developing type 2 diabetes) in comparison with skeletal muscle from insulin-sensitive controls. Polymerase Chain Reaction analysis of mRNA from muscle biopsies detected the exclusive expression of HIR-B. These data suggest that insulin resistance - the hallmark of manifest non-insulin dependent diabetes and perhaps a primary defect leading to type 2 diabetes - is associated with altered expression of insulin receptor isotypes.

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Insulin receptor isoform expression in skeletal muscle membranes of NIDDM patients

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The human insulin receptor exists in two isoforms (HIR-A α-subunit 719 aa and HIR-B α-subunit 731 aa) with distinct patterns of tissue specific expression. We have recently shown that skeletal muscle of non-diabetic individuals contain only mRNA encoding HIR-A while in skeletal muscle of NIDDM (non-insulin dependent diabetes mellitus) patients also mRNA encoding HIR-B is found. In this study we investigated the human insulin receptor isoform expression on the protein level with a polyclonal antibody which discriminates between HIR-A and HIR-B. The antibody showed clearly distinct displacement of insulin binding in skeletal muscle membranes of non-diabetics and NIDDM patients (displacement of specific ^{125}I-insulin binding: 13 non-diabetic patients 70.0% ±14.34, 12 NIDDM patients 32.6% ±17.45). In additon, different displacement of ^{125}I-insulin in plasma membranes from patients with NIDDM (81.2% displacement of specific insulin binding) and non diabetic patients (39.9%) could be obtained with an anti-serum (PA 12) to the 12 amino acids (aa 717 - 728) of the B-receptor form. These data suggest that the altered expression of receptor isotype mRNA in the skeletal muscle of NIDDM patients leads to an altered receptor isoform pattern in the plasma membrane.

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ALTERNATIVELY SPLICED VARIANTS OF THE INSULIN RECEPTOR AND ITS FUNCTIONAL CORRELATES IN MUSCLE FROM PATIENTS WITH TYPE 2 DIABETES AND NORMAL SUBJECTS.

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Two insulin receptor (IR) mRNA transcripts, resulting from alternative splicing of exon number 11 of the receptor gene, are regulated in a tissue-specific manner. To examine the significance of this observation, the relative expression of IR splice variants, insulin binding to solubilized, WGA-purified receptors, and IR kinase phosphorylation of poly (Glu-Tyr (4:1)) were measured in vastus lateralis muscle of 9 patients with insulin resistant Type 2 diabetes and 16 healthy subjects. Using an RNA-based fluorescens-labelled PCR assay which was quantitative over a range of known ratios of the two mRNA transcripts we found no difference in the relative expression of IR transcripts: 67 $\pm$ 7% IR-exon + 11 in diabetics versus 70 $\pm$ 6% IR-exon + 11 in controls. The recovery of IR was comparable: 96 $\pm$ 13 and 109 $\pm$ 13 fmol/100 mg muscle (ns) in diabetics and controls, respectively, and no significant differences were found in IR affinity or in basal and insulin stimulated IR kinase activity. We conclude: 1) About 70% of IR mRNA is the exon+11 splice variant in muscle from both Type 2 diabetic patients and normal subjects and 2) insulin binding and IR kinase activity are unrelated to the relative expression of alternatively spliced isoforms of IR in human muscle.

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A GLYCINE-1008 TO VALINE MUTATION IN THE INSULIN RECEPTOR IN A WOMAN WITH TYPE A INSULIN RESISTANCE

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We examined the insulin receptor gene in a Japanese woman with type A insulin resistance. At the age of 20, she was referred to hospital for evaluation of acanthosis nigricans and polycystic ovary. 75 g oral glucose tolerance test showed a diabetic pattern and fasting hyperinsulinemia (130 μu/ml) was noted. Insulin binding was normal, but autophosphorylation and tyrosine kinase activity were reduced in partially purified insulin receptors from EB-virus transformed lymphocytes. We determined the nucleotide sequence for all 22 exons of the insulin receptor gene by direct sequencing of genomic DNA amplified with polymerase chain reaction. Substitution of valine for glycine at codon 1008 in the tyrosine kinase domain was identified in her one allele, which was the same mutation reported previously (Science, 245, 66, 1989). Her father had the same mutation and impaired glucose tolerance with mild hyperinsulinemia, but her mother and two brothers were normal. From the previous reports as well as our case of deletion mutation (ibid, 63, 1989), it is concluded that a single mutant allele in the tyrosine kinase domain develops insulin resistance dominantly.

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PRESENCE OF GLUCAGON AND GLP-1(7-36)AMIDE SPECIFIC RECEPTORS IN HUMAN FAT MEMBRANES.

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GLP-1(7-36)amide (tGLP-1), insulinotropic intestinal peptide, is lipolytic in isolated rat adipocytes, and specific receptors in rat fat membranes were found. Now we have explored the presence of glucagon and tGLP-1 specific receptors in human fat. Previous informed consent given, fat was obtained from the abdominal wall of patients undergone cholecystectomy. The fat membranes (30,000 g pellet) were solubilized with 1% Triton X-100 in 50 mM Hepes, 10 Mm $MgSO_4$, pH 7.6. [^{125}I]glucagon or [^{125}I]tGLP-1 (3 fmol each) were incubated, for 15 min at 25°C, with solubilized membranes (3-10μg) in 100 μl of 50 mM Hepes, pH 7.6, containing 10 mM $MgSO_4$, 0,1% bacitracin, 500 Uic/ml trasylol and 2% BSA, in the absence or presence of increasing concentration of either unlabelled peptides. Both [^{125}I] peptides displayed specific binding, linear to the range of the membrane protein-content and displaceable only by the equal unlabelled peptide. The maximal binding (10 μg membrane protein) represented 2.3±0.2% for [^{125}I]glucagon, and 10.4±1.0% for [^{125}I]tGLP-1. The binding capacities for tGLP-1 were at least 5 times higher than those for glucagon at 10^{-10}, $5x10^{-10}$ and 10^{-9}M. The 50% inhibition of the maximal binding occurred, in both cases, at $<1x10^{-9}$M unlabelled peptide. This work documents the presence of specific receptors for glucagon and tGLP-1 in human fat tissue, being the tGLP-1 receptors in greater abundance than those of glucagon.

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PANCREASTATIN BINDING SITES AND ACTION IN INTACT AND PERMEABILIZED RINm5F CELLS

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To gain insight into mechanism(s) of action of pancreastatin, we have studied binding of 125-I-pancreastatin to intact clonal insulin producing RINm5F cells, and intracellular effects of pancreastatin in electropermeabilized cells. Binding of 125-I-pancreastatin (in 2.5 mM Tris-HCl, 15 mM acetate, pH 7.4, 40 min, 20°C) was displaced by 1-300 nM pancreastatin and not by 1 μM somatostatin, GLP-1(7-36) amide, or GIP. Binding kinetics and Scatchard analysis reveal approximately 30 000 receptors per cell with K_d about 14 nM, and indicate internalization of the ligand. Cells were electropermeabilized (2.5 kV/cm) and incubated in 140 mM K-glutamate, pH 7.0, with ATP and ATP-regenerating system, 20 min, 37°C. An increase in the ambient Ca^{2+} concentration from 10^{-8} to 10^{-4} M resulted in a 3.2-fold stimulation of insulin release. This Ca^{2+}-induced insulin release was inhibited (-33%,$P<0.001$) by 10 nM pancreastatin, and potentiated (+37%,$P<0.001$) by protein kinase C (PKC) activator, 10nM phorbol ester TPA. Down-regulation of PKC by 200 nM TPA for 24 h markedly reduced the acute effects of both 10 nM pancreastatin and 10 nM TPA. Pretreatment with 100 ng/ml pertussis toxin abolished the effect of pancreastatin. Hence, pancreastatin may inhibit the insulin secretory process interacting both with specific receptors in plasma membrane and with a PKC- and G-protein-regulated intracellular site directly involved in exocytosis.

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GENETICS OF TYPE-I-DIABETES IN OUR BB RAT SUBLINE

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Crossing studies have shown that inheritance of Type-I-diabetes in BB rats can be explained by two or three recessive genes. These different findings prompted us to determine the inheritance of diabetes in our BB rat subline by crossing one diabetic BB male (F19) with females of the nondiabetes-prone congenic rat strains LEW.1BB and BB.1A characterized by the MHC or the genetic background of our BB rats and females of the diabetes-resistant DA rat strain which differs in both MHC and genetic background including three coat colour genes. The F1 females were backcrossed onto the same diabetic BB male used for the F1 production. All backcrossed hybrids (BC) were observed for diabetes development up to an age of 200 days at which time the pancreatic insulin content was determined in all nondiabetic BCs. The coat colour genotypes of albino DA-BCs were determined by appropriate crosses. From the diabetes incidence in BCs (23.9 % LEW.1BB-BC, 28.2 % BB.1A-BC, 9.8 % DA-BC) and the results of the coat colour gene analysis in BCs of DA rats we assume that i) diabetes in our BB rat subline is determined by the MHC, lymphopenia and a third recessive non-MHC gene of major importance and ii) near the coat colour genes A and C there must be interacting genes which prevent diabetes development and positively influence the pancreatic insulin content.

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CONNECTION BETWEEN HIGH JUVENILE BODY-WEIGHT AND DEVELOPMENT OF DIABETES IN BB-RATS

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The aim was to study why among BB-rats some animals become diabetic and some do not, and to investigate whether it is possibly to select those rats destined for diabetes. Whilst the genetic background as well as the environmental factors affecting BB-rat littermates are very similar, the body-weight reflects some of the existing variance. The study included 151 BB-rats, and the body-weight of each animal was measured daily from the day of birth. A standard three-way analysis of variance was applied to these weight data. Thirty-four animals became diabetic before 100 days of age, and we found their body-weight to be increased about 10% compared to the non-diabetic animals. This was significant for each day of life from the 1st to the 45th, with p-values from 0.05 to 0.0001. The weight increase for the individuals destined to become diabetic was seen for both sexes but was most pronounced among male BB-rats. Furthermore, when investigating whether juvenile body-weight has any predictive value we found that the incidence of diabetes at 100 days of age could be doubled from 22.5% to 46.7% ($p<0.01$) by selecting the heaviest animal in each litter. We conclude that, for BB-rats, a connection exists between high juvenile body-weight and the risk of diabetes development in early adult life.

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COMPARISON OF EFFECT OF TREATMENT WITH DAILY SUBCUTANEOUS INJECTIONS OF INSULIN AND SUSTAINED RELEASE INSULIN IMPLANTS ON METABOLIC CONTROL AND FEEDING PATTERNS IN BB AND STREPTOZOTOCIN DIABETIC RATS.

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Accurate methods for assessing and maintaining various levels of metabolic control are mandatory for studies of diabetic microangiopathy in diabetic animals. We compared the effect of conventional insulin therapy (CIT) (U40 Bovine Ultralente insulin, NOVO) and sustained release insulin implants (SRII) (Møllegaard, Denmark) on metabolic control and feeding patterns. Food intake and plasma glucose concentration (PG) were measured 2-hourly for 24 hours and HbA1 (Corning) determined in (1) non-diabetic (n=17) (2) established diabetic (n=12 STZ, 13 BB/E) rats 7 days before substituting CIT with a SRII and again 27 days post-implant. Mean ± SEM (range) PG was 5.8 ± 0.1 (5.1-6.3), 7.8 ± 1.3 (2.4-17.8) and 7.3 ± 0.4 (5.5-10.0) mmol/l respectively. Corresponding mean ± SEM HbA1 values were 4.4 ± 0.3, 4.9 ± 0.4 and 4.9 ± 0.2%. Mean ± SEM food consumption of non-diabetic rats was significantly higher ($p< 0.01$) during the 12 hour dark cycle (1.41 ± 0.20g) than the light (0.47 ± 0.08g) cycle. In all diabetic animals, irrespective of type and treatment of diabetes, this diurnal variation was abolished and overall intake of food higher ($p< 0.01$) than in non-diabetics (1.60 ± 0.13 vs 0.94 ± 0.17g). We conclude (1) PG is very unstable in diabetic rats maintained on CIT and random PG and HbA1 are misleading in assessing overall metabolic control; (2) in contrast, SRIIs achieve a relatively stable PG which is adequately reflected by these parameters; (3) diabetic rats feed continuously and are hyperphagic.

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FUNCTIONAL AND METABOLIC PERTURBATIONS IN ISOLATED PANCREATIC ISLETS FROM THE GK RAT, A GENETIC MODEL OF NON-INSULIN-DEPENDENT DIABETES.

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Spontaneously diabetic non-obese GK rats exibit high basal plasma glucose and insulin levels and a poor insulin secretory response to glucose. We studied 1) insulin biosynthesis and release 2) glucose metabolism, in islets freshly isolated from GK rats and normal Wistar rats, the latter being used as control animals. In GK rats, islet insulin content was decreased when expressed per islet but normal when related to DNA content. The basal rate (2.8 mM glucose) of both (pro)insulin and total protein biosynthesis was doubled in islets from GK rats. As judged from the (pro)insulin/total protein synthesis ratio, (pro)insulin biosynthesis was normally stimulated by 16.7 mM glucose. Both basal and glucose-stimulated insulin release were dramatically decreased in islets from diabetic rats. A reduced secretory response to leucine (10mM) or leucine+glutamine (10 mM each) and a lack of response to monomethylsuccinate (10mM) were also observed. By contrast the insulinotropic capacity of non-nutrient secretagogues such as gliclazide (0.062mM) or the combination Ba^{2+}(2mM)-theophylline(1.4 mM) remained normal. Glucose oxidation (estimated as the production of $^{14}CO_2$ from D-[6-^{14}C]glucose) was severely impaired while no major alteration of glycolytic flux (as judged from the conversion of D-[5-^{3}H]glucose to $^{3}H_2O$) could be detected. Accordingly, the D-[6-^{14}C]glucose oxidation/D-[5-^{3}H]glucose utilization ratio was less markedly increased in response to a rise in glucose concentration in islets from GK rats than in islets from control rats. Thus, in islets from diabetic GK rats, glucose-induced insulin release but not insulin biosynthesis was impaired. This defect is associated with, and probably due to, a deficient islet mitochondrial function.

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PREFERENTIAL ALTERATION OF OXIDATIVE RELATIVE TO TOTAL GLYCOLYSIS IN ISLETS OF RATS WITH INHERITED OR ACQUIRED NON-INSULIN-DEPENDENT DIABETES

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A current hypothesis ascribes to an alteration of D-glucose transport into the B-cell the impairment of glucose-induced insulin release in experimental models of non-insulin-dependent diabetes. In the present study, the rate of glycolysis, as judged from the conversion of D-[5-^{3}H]glucose to ^{3}HOH and when expressed relative to the islet protein content, was not affected in islets from either adult rats injected with streptozotocin during the neonatal period (STZ rats) or spontaneously diabetic rats obtained by repeated selective breeding (GK rats). In both STZ and GK rats, however, the paired ratio between D-[3,4-^{14}C]glucose oxidation and D-[5-^{3}H]glucose utilization was 25% lower than in control animals. Such a decrease was observed at both low and high hexose concentration and persisted in the absence of extracellular Ca^{2+}. In contrast, the ratio between either D-[2-^{14}C]glucose or D-[6-^{14}C]glucose oxidation, which informs on the generation of $^{14}CO_2$ from the C_1 and C_2 of glucose-derived acetyl residues, and D-[3,4-^{14}C]glucose oxidation was not impaired in the diabetic rats. These findings suggest that a preferential alteration of oxidative relative to total glycolysis, rather than an impaired transport or phosphorylation of D-glucose, contributes to the secretory defect not solely in a cytotoxic but also in an inherited model of non-insulin-dependent diabetes.

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THE NUMBER OF LIVER INSULIN RECEPTOR IS DECREASED IN THE INSULIN RESISTANT GK RAT.

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The spontaneously diabetic non obese GK rat (as compared to control Wistar rat) has been found to exhibit higher basal plasma glucose and insulin levels and a very poor insulin secretory response to glucose. Insulin action was assessed in adult GK females at basal and submaximal (euglycemic clamp) insulin levels. Liver insulin receptor properties and hepatic glucose production (GP) were determined under these two conditions. GP in the GK rats was significantly higher ($p<0.001$) in the basal state. At submaximal hyperinsulinemia (and euglycemia), it was less effectively suppressed ($p<0.001$) than in the controls, thus demonstrating liver insulin resistance. At both basal state and clamp condition, binding of ^{125}I-A14-insulin to liver membranes of GK rats was significantly decreased ($p<0.005$) by 20-30%. Tracer binding was inhibited by 50% at the same insulin concentrations. Solubilized and wheat germ agglutinin purified receptors of GK and control rat liver exhibited similar affinity for insulin and kinase activity (for both autophosphorylation and phosphorylation of the artificial substrate poly(Glu-Tyr)4:1. This indicates that liver insulin resistance in the GK rat is at least partly accounted for by a decrease in receptor number.

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PHOSPHOLIPID FATTY ACID COMPOSITION AND GLUCOSE TOLERANCE IN THE ISRAELI SAND RAT.

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Recent dietary studies in rats have demonstrated an association between increasing tissue long chain n-3 fatty acid phospholipids and improved insulin sensitivity of glucose metabolism. However, this relationship has not been examined in animal models that spontaneously develop non-insulin dependent diabetes mellitus. The Israeli Sand rat demonstrates a range of glucose and insulin responses when maintained on standard laboratory chow, providing a suitable animal model. The aim of our study was to examine the relationship between glucose tolerance and tissue phospholipid fatty acid composition in Israeli Sand rats. At 24 weeks of age we classified our rats into 4 groups according to blood glucose and insulin levels. Group A were normoglycemic, normoinsulinemic; B were normoglycemic, hyperinsulinemic; C were hyperglycemic, hyperinsulinemic; and D were hyperglycemic, hypoinsulinemic. In the fed state rats were sacrificed and tissues removed for subsequent analysis. The % fatty acids as 20:5n-3 in liver phospholipids were 6.2 $\pm$ 1.5, 6.7 $\pm$ 1.4, 4.6 $\pm$ 0.6 and 3.6 $\pm$ 0.5; the 20:4n-6 levels were 7.1 $\pm$ 0.6, 6.8 $\pm$ 0.3, 7.0 $\pm$ 0.7 and 9.4 $\pm$ 0.9, which resulted in a ratio of 20:4n-6/20:5n-3 of 1.3 $\pm$ 0.3, 1.1 $\pm$ 0.3, 1.6 $\pm$ 0.3 and 2.7 $\pm$ 0.2 for groups A,B,C and D respectively. Although cross-sectional, our results are consistent with the suggestion that an increase in tissue 20:4n-6/20:5n-3 ratio is associated with a deterioration in glucose tolerance.

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ISLET MONOAMINE OXIDASE ACTIVITY IN OBESE HYPERGLYCEMIC MICE AND THEIR LEAN LITTER-MATES

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ß-Cell monoamines, located in secretory granules, are inactivated by monoamine oxidase (MAO), a H_2O_2-generating enzyme, which may influence the redox state of the ß-cell and thereby insulin secretion. We studied the relation between islet MAO-activity, plasma insulin and glucose in obese (ob/ob) mice. MAO activity was assayed radiochemically with serotonin, dopamine and ß-phenylethylamine (PEA) as substrates. An overnight fast in lean mice increased the MAO-activity by 35-70% towards all three substrates. In obese mice the MAO-activity towards serotonin decreased after fasting (-25%), whereas no effect was recorded with dopamine or PEA. Thus islet MAO towards dopamine and PEA was higher (30-50%) in fasted lean *vs* fasted obese mice, whereas it was lower (-40%) towards serotonin. A correlation analysis in obese mice showed a negative correlation between plasma glucose and islet MAO-activity towards PEA ($r = -0.61$; $p < 0.05$) and dopamine ($r = -0.65$; $p < 0.02$) respectively. The data suggest that the plasma glucose levels modulate islet MAO-activity and thereby influence the monoamine content and redox state of the ß-cell which in turn may affect insulin secretion. In the obese mouse this regulatory influence is disordered, and this may contribute to the hyperinsulinemic state of the obesity syndrom.

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METHYLGUANIDINE INHIBITS NITRIC OXIDE PRODUCTION AND PREVENTS DIABETIC VASCULAR DYSFUNCTION IN RATS.

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We assessed the ability of methylguanidine to inhibit nitric oxide (NO) production and prevent diabetic vascular dysfunction. Methylguanidine inhibited endotoxin-induced NO synthase isolated from RAW 264.7 macrophages (~1/40 as potent as N^G-mono methyl-L-arginine (NMMA) and aminoguanidine) and constitutive NO synthase isolated from rat brain (~1/50 as potent as NMMA but equipotent to aminoguanidine). Diabetes was induced (45 mg/kg body weight streptozotocin i.v.) in four groups of male Sprague-Dawley rats: controls (n=10), methylguanidine-treated controls (n=8), diabetics (n=10), and methylguanidine-treated diabetics (n=9). Methylguanidine was administered orally (2 mg/ml in drinking water) and by single daily s.c. injection (25 mg/kg body weight). Plasma glucose (7.2±0.8 [SD] vs 23.3±6.7 mM for controls vs diabetics), water consumption (46±6 vs 108±68 ml/day), and urine volume (14±6 vs 111±68 ml/kidney/day) were unaffected by methylguanidine in controls and diabetics. ^{125}I-albumin permeation (µg plasma/min/g wet weight) was increased by diabetes ($p < 0.001$) in retina (47±12 vs 116±30), sciatic nerve (47±13 vs 121±22), aorta (62±20 vs 155±37), and kidney (727±239 vs 1,011±265), and was normalized by methylguanidine in diabetic rats (retina, 55±18; sciatic nerve, 50±10; aorta, 85±41; kidney, 738±169) without affecting albumin permeation in controls. These experiments establish methylguanidine as an inhibitor of NO synthase and suggest a role for increased NO production in the pathogenesis of diabetic vascular complications.

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Neuropeptide Y augments the vasoconstrictor action of noradrenaline in experimental diabetes.

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Abnormal vascular responses may influence the development of diabetic microvascular complications. However, previous studies have not shown a consistent effect of diabetes on vascular reactivity. We compared the vasoconstrictor response to noradrenaline (NA) in isolated mesenteric arterioles (diameter 293±8 (mean±SE) µm) between non-diabetic and streptozotocin-induced diabetic (STZ-D; 4 weeks' duration) Wistar rats. We also examined the effects of neuropeptide Y (NPY), a powerful vasoconstrictor co-released with NA from sympathetic nerve endings, at a sub-vasoconstrictor dose on NA-induced vasoconstriction. Arteries were dissected and mounted in a myograph and dose-response curves constructed for NA ($5x10^{-7}$ to 10^{-4} M) with and without NPY (10^{-7}M). Control arteries (n=16) showed no difference in ED50 (dose causing 50% vasoconstriction) between NA-only and NPY-augmented NA responses (ED50 $1.6±0.4x10^{-6}$ M vs $2.5±0.5x10^{-6}$ M respectively; p=0.2). By contrast, diabetic vessels (n=20) showed enhanced constriction with NPY-augmentation (ED50 $0.9±0.2x10^{-6}$ M vs $2.1±0.3x10^{-6}$ M; p<0.0001). In diabetic rats plasma NPY levels were higher (1056±207 vs 433±113 pmol/l; p=0.03). In conclusion, STZ-D Wistar rats show an NPY-augmented response to NA contraction and higher plasma NPY levels, indicating dysregulated sympathetic vascular control, which may be relevant to the development of diabetic microvascular complications.

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EVIDENCES FOR INCREASED SYMPATHETIC ACTIVITY IN TYPE-II DIABETIC RATS.

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It was previously postulated that an overactive sympathetic nervous system is implicated in type-II diabetes. To investigate this hypothesis, we evaluated some parameters of sympathetic transmission in a rat model of type-II diabetes, induced in neonatal animals by the treatment with streptozotocin (90 mg/kg ip). Adult diabetic rats, which respond to glucose load with pronounced hyperglycemia and reduced insulin secretion, have two- to three-fold higher levels of noradrenaline in the plasma, pancreas or brain, when compared to normal controls. In addition diabetic rats have a significantly higher blood pressure which seems to be sympathetically mediated because following ganglionic blockade by chlorisondamine (0.6 mg/kg ia) residual blood pressure was not significantly different from controls. Stress-induced hyperglycemia in diabetic rats is antagonized by the α_2-adrenoceptor antagonist SL 84.0418 (at 3 mg/kg ip) and attenuated by chlorisondamine. In addition, SL 84.0418, which does not affect basal insulin secretion in normal rats at this dose, potently stimulates basal- or glucose-evoked insulin secretion in diabetic rats, and these effects are further attenuated by chlorisondamine. It is suggested that type-II diabetic rats have an overactive sympathetic nervous system, which results in hyperglycemia and inhibition of insulin secretion, and that α_2-adrenoceptor blockade by SL 84.0418 normalizes hyperglycemia by disinhibiting insulin secretion.

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A NEW EXPERIMENTAL MODEL FOR PARTIAL REDUCTION OF PANCREATIC ß-CELL MASS IN THE RAT

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The aim of this study was to develop a technique to make a subtotal reduction of pancreatic B-cell mass without surgical removal of pancreatic parenchyma. The superior mesenteric artery, which supplies 1/3 of the pancreas with blood, was located in anesthetized rats and clamped. Then 0.2 ml of either streptozotocin (SZ; 40 mg/kg bodyweight) or saline was injected intravenously. After 10 min the arterial clamp was removed and the animals were allowed to recover from anesthesia. All animals injected with saline (n = 6) had a normal intraperitoneal glucose tolerance test (GTT) 7 days later. A total of 8 out of 9 of the SZ-treated rats were normoglycemic and had a normal GTT 7 and 14 days after surgery, whilst 1 was diabetic. The number of islets (>25 µm) in the part of the pancreas to which SZ had free access, was very low, whilst the islet number in the part supplied by the superior mesenteric artery was similar to that of the control animals (≈2 islets/mg pancreas). It is concluded that the present model can be used to selectively destroy the ß-cells in approximately 2/3 of the islets. In this animal model the remaining B-cells are presumably exposed to an increased functional demand in an otherwise normal pancreatic gland.

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IDENTIFICATION OF RESIDUAL PANCREATIC ß-CELLS IN STREPTOZOTOCIN TREATED RATS BY INSULIN IN SITU HYBRIDIZATION.

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Beta-cells surviving the toxic effect of streptozotocin may be difficult to identify by immunocytochemistry for insulin because of depletion of the cellular insulin stores, due to sustained hyperglycemia. We therefore performed in situ hybridization of rat preproinsulin mRNA (a cocktail of three oligonucleotides was used) in pancreata of streptozotocin diabetic rats (60 mg per kg body weight, n=5) and compared the staining results with those after immunocytochemistry. Quantitative analysis of 10 islets in each pancreas, followed throughout 20 serial sections alternatively subjected to in situ hybridization and immunocytochemistry, revealed that immunocytochemistry detected 20-25% less Beta-cells in the streptozotocin diabetic rat pancreas than in situ hybridization. The highest number of in situ hybridization positive and immunocytochemistry negative Beta-cells were found in small islets where only 20% in situ hybridization positive Beta-cells were also detected by immunocytochemistry. These results suggest that in situ hybridization for preproinsulin mRNA is the method of choice for the identification of residual or regenerating Beta-cells with very low insulin content.

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EFFECT OF ELEVATED BLOOD GLUCOSE ON INSULIN-STIMULATED GLUCOSE TRANSPORT IN RAT MUSCLE.

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Type II diabetes mellitus is characterized by skeletal muscle insulin resistance. To investigate the effect of chronically elevated blood glucose on insulin-stimulated glucose transport, muscles from F_1 Hybrids (H) of spontaneously type II diabetic (non-ketotic and non-obese) GK-Wistar rats and control Wistar rats (C) were studied at 100, 200, and 500 g of body weight. Serum cholestorol and triglycerides did not differ between groups. The glucose induced insulin response was impaired by 87% ($p<0.01$) in perfused pancreases of H rats. An intraperitoneal glucose injection (2 g/kg) increased blood glucose after 60 minutes significantly greater in H than C (100 g: 8.9 ± 0.3 vs 5.7 ± 0.4 mmol/L; 200g: 13.6 ± 0.6 vs 6.8 ± 0.4 mmol/L; 500 g: 19.5 ± 0.7 vs 7.8 ± 0.6 mmol/L; $p<0.001$ for all groups). Epitrochlearis and soleus muscles were incubated in vitro for 1 hr in increasing concentrations of insulin. The 100, 200, and 500 g C and H insulin dose response curves for 3-0-methylglucose transport did not differ. However, the basal 3-0-methylglucose transport rate in soleus muscles from 500 g H rats was decreased ($p<0.05$) compared to C muscles. In conclusion, these results provide evidence that elevated glucose per se is not responsible for the development of peripheral insulin resistance in rats.

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EFFECT OF PROLONGED HYPERGLYCEMIA ON IN VIVO INSULIN SECRETION IN RATS: ROLE OF THE AUTONOMIC NERVOUS SYSTEM.

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We have previously shown that prolonged hyperglycemia induced in non-diabetic rats (HG), durably potentiated the ß-cell responsiveness to glucose. The fact that in vitro glucose-induced insulin secretion was, on the contrary, markedly impaired, suggested that the potentiation of insulin secretion was related to extrapancreatic factors, especially those involving central nervous control of insulin secretion. Rats were rendered hyperglycaemic by a 48h-glucose infusion (20 mM). To evaluate the role of parasympathetic system (PS), HG rats were submitted to bilateral subdiaphragmatic vagotomy, 6h after the end of infusion. Insulin secretion was investigated by a glucose tolerance test. The $\Delta I/\Delta G$ was markedly increased in intact HG rats (3.86+0.86) compared to controls (1.02 ± 0.30).Vagotomy reduced the difference between groups: $\Delta I/\Delta G$ became similar in vagotomized HG rats (1.69 ± 0.25) and in intact controls. This suggests that increased cholinergic activity was involved in the high insulin secretion in HG rats. The role of the sympathetic system (SS) was investigated by studying the effect of an intraperitoneal injection of various doses of the alpha2 adrenergic blocker oxymetazolin (OM). The OM concentration producing half maximal decrease of $\Delta I/\Delta G$ was doubled in HG rats compared to controls. This strongly suggests a decreased sensitivity of insulin secretion to the inhibiting effect of catecholamines in HG rats. Therefore both PS and SS could be involved in the potentiating effect of prolonged hyperglycemia on the in vivo insulinresponse to glucose.

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TAMM-HORSFALL GLYCOPROTEIN IN EXPERIMENTAL DIABETES.

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Tamm-Horsfall protein (TH) is a glycosylated polypeptide secreted only by kidneys. The aim of these studies was to estimate the significance of TH in diabetes by studying TH urinary excretion, cellular location of TH mRNA by *in situ* hybridisation, and cellular immunostaining of TH in the distal straight tubule (DST) on tissue sections from age matched controls (C) and 10 (D10) and 50 (D50) days moderately diabetic rats.

Urine was collected from each group for several 4 hour periods. One kidney was snap frozen for *in situ* hybridization and the other was perfusion fixed for immunocytochemical staining. Densitometry was used to quantitate tissue staining.

Urinary excretion of TH increased significantly from 0.5 µg/day in controls to 1.2 in D10 and 1.78 in D50. The mRNA staining expressed in relation to control measurements of 100 in C was 50 in D10 and 29 in D50. The immunocytochemical staining decreased significantly from 100 in C to 41 in D10 and 30 in D50. The decrease was most pronounced in the cortex.

In conclusion, the TH protein formation and excretion in the DST is disturbed in diabetic rats, indicating a dysfunction in the DST. It is possible that this is an early manifestation of diabetic kidney disease.

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HYPERSECRETION OF AMYLIN FROM THE PERFUSED PANCREAS OF GENETICALLY OBESE RATS

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To clarify alterations in amylin secretion during the development of obesity, we studied the secretion of amylin from the perfused pancreata of lean (Fa/?) and genetically obese (fa/fa) Zucker rats at 9, 18, and 54 weeks of age. Amylin concentration in the effluent was determined by radioimmunoassay (Peninsula). Secretion of amylin induced by 16.7 mM glucose in obese rats was significantly greater than in lean rats at 9 and 18, but not at 54 weeks, whereas the secretion of amylin induced by 10 mM arginine in obese rats was significantly greater than in lean rats at all ages. Maximum secretion of insulin and amylin induced by arginine occurred at 18 weeks in obese rats and at 54 weeks in lean rats. The secreted amylin-to-insulin molar ratio induced by glucose in obese rats at 18 weeks was significantly greater than in lean rats (obese : 1.23±0.05%; lean : 0.99±0.04%, $p<0.01$). At 54 weeks, the molar ratios induced by glucose and arginine in obese rats were significantly greater than in lean rats, although insulin secretion did not differ significantly. These findings suggest that hypersecretion of amylin from the pancreata of genetically obese rats may be linked to the development of obesity.

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Human islet amyloid polypeptide accumulates in β-cell lysosomes and perivascular spaces in transgenic mice.

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The species specific amino acid structure of islet amyloid polypeptide (IAPP) is an important factor in formation of islet amyloid in type 2 (non-insulin-dependent) diabetes mellitus. We used a transgenic mouse model to investigate whether accumulation of IAPP in β-cells is a result of the amino acid sequence. Pancreatic tissue was obtained from transgenic mice (>5 months), expressing genes for human IAPP (hIAPP)(7 breeding lines) or rat IAPP (rIAPP)(2 lines) and from control mice. IAPP was immunolocalised on ultrathin sections for electron microscopy using antisera to rIAPP (which cross reacts with hIAPP and mouse IAPP) and to hIAPP C-terminal flanking peptide (hC-IAPP). The investigators were blind to the genetic status of the animals. Immunoreactivity (IR) for IAPP was located in insulin granules of all animals. High density of IAPP-IR was only found in B-cell lysosomal lipofuscin bodies of animals expressing the hIAPP gene. Amorphous perivascular IAPP-IR deposits were detected in two hIAPP mice. hC-IAPP-IR was localised to insulin granules in hIAPP transgenic animals but was not found in lysosomes of any mice studied. We suggest that hIAPP in transgenic mice, as in man and monkeys, is processed via a B-cell lysosomal pathway and can accumulate in perivascular spaces and lipofuscin bodies.

PS 2
Hormone Receptors

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AN INSULIN RECEPTOR GENE DELETION IN A KINDRED WITH A FAMILIAL FORM OF INSULIN RESISTANCE.

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Mutations in the insulin receptor gene have mainly been identified in patients with extreme insulin resistance. We have investigated a family in which both parents and all five daughters have moderately elevated levels of fasting insulin ranging between 160-220 pmol/L except for a 6-year old girl with levels of 85 pmol/L. One daughter is obese and has fasting hyperglycemia. [^{125}I] insulin binding to EBV-transformed lymphoblasts was either low or at the lower limit of the normal range. Using denaturing gradient gel electrophoresis we did not find a mutation in the insulin receptor gene. However, in exon 3 we could not detect the father's DNA sequence in any of the daughters. Southern blot analysis revealed a deletion in the father's genomic DNA. When cDNA was synthesized from the paternal genomic DNA using reverse transcriptase and amplified by polymerase chain reaction, two cDNA species were identified: one with a normal sequence; in the other species exon 3 was deleted. The deleted allele was inherited by all the daughters. In conclusion, the deletion mutation appeared to cause a moderate degree of insulin resistance in this kindred. These observations suggest that the prevalence of insulin receptor mutations may be higher than is generally believed.

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INSULIN INTERACTION WITH ITS OWN RECEPTORS IS BLOCKED BY RECEPTOR AUTOANTIBODIES: I-123 INSULIN IMAGING IN A PATIENT

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Insulin receptor antibodies (IRA) of a patient with autoimmune hypoglycemia were *in vitro* characterized and their effect on *in vivo* biodistribution of I-123 insulin was evaluated. IRA inhibited insulin binding on HIR3T3 fibroblasts, IM9 lymphocytes and human placental membranes; induced aggregation of the receptors on the cell membrane; immunoprecipitated the proreceptor, alfa and beta subunits from cell lysates. A biodistribution study in the patient after i.v. injection of 1 mCi I-123 Tyr A14 insulin showed lack of tracer uptake by the liver (9% of the injected dose, n.v. 21.1±1.7%). Time activity curves generated on the heart and on the liver were parallel suggesting that activity in the liver represents only the tracer in the hepatic blood compartment. Clearance of insulin from the blood was indeed slower than in controls with a T1/2 of 11.5 min (n.v. 3.2±0.1 min) and mainly exerted by the kidneys. Analysis of plasma radioactivity showed that insulin degradation did not occur at the same rate as in controls.

In conclusion, IRA detected *in vitro* by different techniques significantly alter *in vivo* biodistribution of I-123 insulin by preventing its interaction with receptors and its subsequent degradation.

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INCREASED DISSOCIATION RATE OF INSULIN FROM ITS RECEPTOR IN A PATIENT WITH STEINERT'S ATROPHIC MYOTONIA. B. Casanova, F.J. Arrieta, A. Casla, A. Suárez, N. Pulido and A. Rovira. Fundación Jiménez Díaz. UAM, Madrid. Spain.

Steinert disease is inherited through a dominant autosomic gene and may be associated with insulin resistance. The insulin binding, dissociation kinetics, insulin internalization and degradation were studied in lymphocytes transformed with Epstein-Barr virus from a 14 yr-old patient with Steinert disease. Insulin sensitivity index, obtained by the modified minimal model, was $0.6x10^{-4}$ $min^{-1}/(\mu U/ml)$ (normal: $3.5\pm0.4x10^{-4}$, mean±SE, n=14). Either maximal specific ^{125}I-insulin binding (26.2% /10^7 cells, n=2), maximal insulin binding capacity (0.48 pmol/10^7 cells) and affinity (ID_{50}: 0.75 nM) were within the normal range (31.8±4.7, 0.8±0.2 and 0.7±0.1, respectively). ^{125}I-insulin dissociation rate at pH 6.0 or in the presence of 500 ng/ml insulin was 23% and 70% higher than in controls respectively. Insulin internalization was decreased (8.2 vs 18.4±2.8% of total bound at t=0), and a smaller fraction of internalized ^{125}I-insulin remained bound to its receptor (PEG-precipitable: 32.4 vs 51.7±6.8% of total intracellular). ^{125}I-insulin was more rapidly degraded (TCA-soluble radioactivity in the medium: 100 % higher than in controls after 60 min incubation at 37°C). We conclude that the higher dissociation rate of insulin from its receptor at the cellular surface and at the intracellular compartment, together with a decrease in the internalization of the insulin-receptor complex may explain the insulin resistance present in this patient with Steinert disease.

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INFLUENCE OF INSULIN RECEPTOR α-SUBUNIT PROTEOLYSIS ON HORMONE-RECEPTOR INTERACTIONS

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Insulin binding to rat liver plasma membranes (LPM) promotes proteolysis of the 135 kDa α-subunit of the insulin receptor (αIR) to a fragment of 120 kDa, a process suggested to play a role in the hepatic IR down-regulation onset. In the present study, transient-state and steady-state [^{125}I]-insulin binding assays in combination with chemical cross-linking procedures and SDS-PAGE, were used to characterize the influence of αIR proteolysis on insulin-receptor interactions in LPM. Degradation of αIR occurred even in the absence of hormone (basal degradation) with insulin binding augmenting proteolysis above basal level. By the time maximal binding was achieved, insulin-stimulated α-subunit proteolysis had contributed in half to maximal degradation attained (≈30%). The effect of insulin on αIR proteolysis was more pronunced at low (<12%) than at high receptor occupancy, suggesting that proteolytic activity responsible for αIR degradation is saturable. Proteolytic degradation of α-subunit increases by 3-fold the apparent affinity of the IR as determined by displacement assays. A slower rate of insulin dissociation from 120 kDa complexes is responsible, at least in part, of the higher affinity showed by the degraded receptors. Our results suggest that αIR proteolysis may have a regulatory effect on cellular sensitivity to insulin by altering IR affinity.

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BIOCHEMICAL AND MORPHOLOGICAL EVIDENCE THAT INSULIN CAN UP-REGULATE ITS RECEPTORS IN THE PLASMA MEMBRANE

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The aim was to further characterize the acute effects of insulin on its cell surface receptors. Isolated rat adipocytes were preincubated at 37°C for 20 min with or without insulin (1000 μU/ml). Receptor cycling was stopped with 2 mM KCN and insulin dissociated. ^{125}I-insulin was bound to the cell surface and cross-linked to the receptors with 0.5 mM disuccinimidyl suberate. Insulin pretreatment increased binding ~4-fold ($p<0.001$) through an enhanced number of binding sites. This effect was recovered in plasma membranes (despite receptor internalization) when prepared after the cross-linking procedure ($p<0.01$). SDS-PAGE and autoradiography confirmed a 3-5-fold increase of ^{125}I-insulin bound to the 135 kDa α-subunit of the insulin receptor. This effect was markedly attenuated in insulin-resistant cells (cAMP-treatment). Furthermore, immuno-gold labelling (using a monoclonal antibody directed against the C-terminus of the receptor β-subunit) of the cytoplasmic face of plasma membranes, assessed with electron microscopy, was enhanced ~3-fold following insulin pretreatment. Trypsin (1 mg/ml) treatment of cells removed > 90% of the ^{125}I-insulin binding capacity of subsequently prepared plasma membranes, suggesting that no "hidden", trypsin-resistant receptor pool exists.

Conclusion: Insulin rapidly increases the number of functioning insulin binding sites in the rat adipocyte plasma membrane possibly through a conformational change.

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TYROSINE KINASE ACTIVITY IN INSULIN RECEPTORS FROM HYPOMAGNESEMIC RATS

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Decreased insulin sensitivity has been referred in diabetic patients with hypomagnesemia. The aim was to study the effect of hypomagnesemia on insulin binding and tyrosine kinase activity of the insulin receptor from liver, muscle and fat. Rats (180 g) were fed with normal (n=9) or low Mg^{+2}-containing diet (n=15), reaching a serum Mg^{+2} level of 1.90±0.09 mg/dl (mean±SE) and 1.10±0.04, respectively ($p<0.001$). Solubilized receptors from liver, skeletal muscle and epididymal fat were partially purified by Wheat Germ Agglutinin affinity chromatography. Specific ^{125}I-insulin binding (10^{-10}M) and tyrosine kinase activity towards an exogenous substrate (Poly Glu^{80}-Tyr^{20}) were measured. No differences in the insulin binding to liver, muscle or fat insulin receptors were found between hypomagnesemic and control rats. Hypomagnesemic rats presented a decrease in basal and in insulin-stimulated tyrosine kinase activity of insulin receptors from liver (basal: 2.4±0.3 vs 3.8±0.6 pmol $^{32}P/\mu g$ receptor, $p<0.05$; 10^{-7}M insulin: 4.3±0.3 vs 9.5±1.8, $p<0.05$), skeletal muscle (basal: 3.7±0.2 vs 5.3±0.4, $p<0.01$; 10^{-7}M insulin: 22.1±0.9 vs 26.5±1.6, $p<0.05$) and fat (basal: 3.7±0.7 vs 7.6±1.5, $p<0.05$; 10^{-7}M insulin: 6.4±1.2 vs 20.7±2.6, $p<0.001$); the insulin effect on the kinase activity was also significantly decreased in the three tissues. In conclusion, the insulin resistance referred in hypomagnesemia is due in part to an impairment of the tyrosine kinase activity of insulin receptors from the three major insulin-target tissues.

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EVIDENCE FOR INCREASED GLP-1(7-36)AMIDE SPECIFIC RECEPTORS IN FAT MEMBRANES FROM NONINSULIN-DEPENDENT DIABETICS. M.L. Villanueva-Peñacarrillo, E. Mérida, E. Delgado, F. Arrieta, A. Rovira and I. Valverde. Fundación Jiménez Díaz, Madrid, Spain.

We have found GLP-1(7-36)amide (tGLP-1) specific receptors in rat and human fat membranes and a lipolytic effect of tGLP-1 in rat adipocytes. Higher tGLP-1 secretion after oral glucose in noninsulin-dependent diabetics (NIDDM) has been reported. We have studied the [^{125}I]tGLP-1 binding to solubilized fat membranes from NIDDM and weight matched controls. The fat tissue was obtained from the gluteal region after patients informed consent given. Fat membranes (30,000 g pellet) were solubilized with 1% Triton X-100 in 50 mM Hepes, 10 mM $MgSO_4$, pH 7.6. Solubilized membranes (5 μg) and [^{125}I]tGLP-1 (3 fmoles) were incubated for 15 min at 25°C, in 100 μl of 50 mM Hepes, pH 7.6, containing 10 mM $MgSO_4$, 0.1% bacitracin, 500 Uic/ml trasylol and 2% BSA, in the presence of unlabelled peptide ($0-10^{-6}$M). The maximal specific binding was 8.5 ± 0.9%, n=5, and 5.3 ± 0.3%, n=4, $p < 0.02$, in NIDDM and controls, respectively. The Scatchard plot revealed the presence of high- and low-binding sites, with respective Kds of $1.4x10^{-10}$M and 0.8 x 10^{-8}M, in NIDDM, and $1.6x10^{-10}$M and $0.8x10^{-8}$M, in controls. The total binding capacity was 6.4 and 3.0 fmol/μg in NIDDM and in controls, respectively. In conclusion, NIDDM seem to have higher tGLP-1 receptor's number accompanied by no alterated affinities when compared to matched weight control subjects. These results indicate a possible implication of tGLP-1 in the physiopathology of noninsulin-dependent diabetes mellitus, at the level of lipid mobilization.

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A COMPARATIVE STUDY OF IN VITRO AND IN VIVO INSULIN METABOLISM IN NORMAL CONTROLS

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This study compares intermediates obtained from A14-125I-insulin (A14-I) processing by human monocytes with those arising after iv injection of insulin. Monocytes from 10 normal controls were incubated with A14-I (8.33 nM) for 30 min at 37°C, acid washed and reincubated in A14-I-free buffer. Cells released 50% of internalized radioactivity within 30 min. The reversed-phase HPLC (RP-HPLC) characterization of medium radioactivity at this time, showed 8 labeled products: peak 1 (70% of medium radioactivity) coeluting with 125I-iodotyrosine, was identified as a final degradation product and peak 7 (12%) coeluting with A14-I, as intact hormone. 6 other peaks (25%), eluted at intermediate positions (peaks 2-6) or after A14-I peak (peak 8). In vivo A14-I (140-150 µCi) was injected in 5 normal controls. Serum radioactivity, extracted by Sep-Pack C18 column, was resolved by RP-HPLC into 8 peaks which appeared rapidly in plasma, reached a maximum in 15 min, and then decreased slowly. The comparison of in vivo and in vitro RP-HPLC patterns, showed that medium from monocytes contains identical products to those in blood. The immunochemical characterization of degradation products obtained in vitro and in vivo, showed that immunoprecipitability with anti-insulin antibody increased from peak 4 (50%) to peak 8 (90%, superimposable to that of A14-I). The presence of immunoprecipitable derivatives in plasma implies that the decay curve of insulin is faster and the metabolic clearance rate higher, when determined by RP-HPLC as compared with immunoprecipitation (589±29 ml/min/m^2 vs 399±23 ml/min/m^2, $p<0.05$). On Sephadex G50, peaks 4-8 eluted with A14-I and after sulfitolysis, they contained in their structure intact A-chain. Rebinding values to monocytes (% of A14-I binding) were about 25% for peaks 5 and 6 and 40% for peak 8. This material was internalized at a rate (45%) similar to that of intact insulin (51.2%). We conclude: 1) In vivo and in vitro insulin metabolism produces intermediates, with characteristics very similar to those of insulin and almost in part, rebindable to insulin receptors. 2) intermediates produced in vivo and in vitro are identical thus supporting the possibility that insulin is metabolized in various cells by a common pathway.

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EVALUATION OF COMPLEX BINDING BEHAVIOR IN THE INSULIN RECEPTOR USING A BIOSENSOR.

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Classical experiments using ^{125}I-labeled insulin show that insulin does not bind to its receptor according to a simple bimolecular interaction scheme. Rather, equilibrium and kinetic binding measurements suggest the presence of negative cooperativity, which is most clearly demonstrated by the acceleration of dissociation of ^{125}I-insulin when receptor binding sites are fully occupied by insulin. We have extended these studies using a newly-developed technology (BIAcore™, Pharmacia Biosensor AB) which monitors in real time the association and dissociation of ligand to purified substrates coupled to a biosensor. Experiments were carried out to measure association and dissociation kinetics of insulin binding to a purified cloned insulin receptor extracellular domain (IRED) bound to the biosensor. Computer programs were developed to to analyze the shapes of the kinetic binding curves along with extrapolation to equilibrium bound values. The association phase could be interpreted in terms of a simple bimolecular binding reaction with a lower affinity than seen in solution. However, the dissociation phase was non-first-order, confirming the presence of complex binding behavior in this system. We describe the detailed analysis required to distinguish among various classes of binding models incorporating negative cooperativity, multiple binding sites, or isomerization transitions during the binding process.

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RECEPTOR BINDING OF THE THREE ISOMERS OF ^{125}I-LABELED INSULIN-LIKE GROWTH FACTOR I

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The binding of insulin-like growth factor I (IGF1) to the IGF1-receptor has been characterized for receptors from a variety of sources, but in contrast to studies of the insulin receptor where Tyr^{A14}(^{125}I)-labeled human insulin is available as an ideal tracer, studies of the IGF1 receptor have been performed with various less well characterized tracers. Since the purity, homogeneity, and affinity of the tracer are all crucial for the data obtained from receptor binding assays, we have isolated and characterized the three isomers obtained upon ^{125}I-labelling of IGF1. The isomers were separated by RP-HPLC and identified by peptide sequencing. The relative receptor affinities were determined by binding to partially purified IGF1-receptors from transfected cells. The affinities of the isomers were Tyr^{24} > Tyr^{31} >> Tyr^{60}. In another experiment the curves for binding of increasing amounts of Tyr^{31}(^{125}I)-IGF1 to the IGF1-receptor and displacement of Tyr^{31}(^{125}I)-IGF1 with unlabelled IGF1 were superimposable, indicating that the affinity of Tyr^{31}(^{125}I)-IGF1 is comparable to that of unlabelled IGF1.

In conclusion, we have purified, identified, and characterized the three iodination isomers of IGF1, and we find Tyr^{31}(^{125}I)-IGF1 to be the tracer of choice for all studies involving IGF1-receptors.

PS 3
Hormone Action

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METABOLIC EFFECTS OF A GROWTH HORMONE BOLUS IN TYPE II DIABETES

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Growth hormone (GH) and insulin-like growth factors (IGF's) have been implicated in the pathophysiology of diabetic complications. We studied the response of 9 well controlled, male, diet treated diabetics (D)(mean (± SEM) duration 2.1 ± 0.8 years;mean HbA1 7.8 ± 0.4%) and 9 age, sex and weight matched controls (C) to a bolus of GH (0.012U/kg/15min) in the postprandial state. Plasma glucose decreased significantly from baseline in diabetics (7.61 ± 0.4 mmol/l) and controls (5.4 ± 0.1 mmol/l) at 20 and 25 minutes ($p<0.05$ GLM/ANOVA). Glucose in controls returned to baseline at 120 minutes. Baseline plasma insulin and C-peptide were not different between groups. Insulin decreased in controls at 20 minutes (5.8 ± 1.4 mU/l,$p<0.05$) till 50 minutes associated with significant suppression of C-peptide (baseline 0.43 ± 0.07 nmol/l to nadir (75 min) 0.23 ± 0.1,$p<0.05$). Insulin and C-peptide did not change in diabetics. Baseline insulin-like growth factor binding protein-1 was significantly higher in normals (D 0.71 ± 0.17;C 1.64 ± 0.35 ug/l,$p<0.05$) but did not change;it increased in diabetics at 75 and 90 minutes (1.4 ± 0.29, 1.3 ± 0.21 ug/l,$p<0.05$). This altered response to a GH bolus in type II diabetics may affect distribution and action of IGF's.

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ROUTE OF INSULIN ADMINISTRATION AND GROWTH HORMONE BOTH INFLUENCE HEPATIC GENE EXPRESSION OF IGF-I.

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Diabetes reduces hepatic IGF-I gene expression, but the controlling factors and the role of the portal vascular link between the pancreas and liver remain poorly understood. We therefore investigated the effects of growth hormone (GH) and the route of insulin administration (s.c. or i.p. [to mimic the hepatic portal delivery]) on hepatic IGF-I mRNA levels *in-vivo*. Fifteen streptozotocin diabetic rats were randomly assigned to 5 groups: **[D]** untreated diabetics (blood glucose (b.g.) 36.3 ± 0.5 mmol/l), **[D+Isc]** diabetic + 5.0IU/24h insulin s.c. (b.g. 3.3 ± 0.1 mmol/l), **[D+Iip]** diabetic + 5.0IU/24h insulin i.p. (b.g. 4.7 ± 0.3mmol/l), **[D+GH]** diabetic + 0.8IU/24h GH (b.g. 38.7 ± 2.4 mmol/l), **[D+GH+I]** diabetic + GH + insulin s.c. (b.g. 13.9 ± 4.3 mmol/l) plus 4 controls **[C]**(b.g. 7.4 ± 0.4 mmol/l). Hepatic IGF-I mRNA was quantified by northen blot analysis. Compared with **[C]** IGF-I mRNA levels were reduced to 21 ± 6% in **[D]**($p<0.01$), partly restored in **[D+GH]**(76 ± 4%, $p<0.05$) and not different in **[D+GH+I]**(102 ± 10%). Intraperitoneal insulin was more effective ($p<0.05$) in restoring hepatic IGF-I mRNA (71 ± 17% of **[C]**, $p<0.02$) than s.c. insulin (44 ± 4% of **[C]**, $p<0.01$). We conclude that insulin, the route of insulin delivery via the portal route to the liver and GH play an important role in hepatic IGF-I gene expression.

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METABOLIC POTENCY OF RECOMBINANT HUMAN INSULIN-LIKE GROWTH FACTOR II IN ADULT RATS

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Insulin-like growth factor II (IGF-II) is structurally related to proinsulin and insulin-like growth factor I (IGF-I). Insulin-like acute metabolic actions have been reported for both, IGF-I and IGF-II in vivo and in vitro. **Aim** of the present investigation was to further characterize the acute metabolic action of IGF-II in anaesthetized adult rats by obtaining dose response curves for hypoglycaemic action and for stimulation of glucose metabolism during euglycaemic clamping. **Results:** Maximal responses of insulin and IGF-II were identical. However, about 50 times higher doses of IGF-II were required to result in identical in vivo responses, with half-maximally effective serum concentrations for the stimulation of glucose disposal during clamp studies of about 0.8 and 50 pmol/ml respectively. A similar difference in potency was observed for the dose dependent stimulatory actions on glucose metabolism in individual target tissues, e.g. for 2-deoxyglucose uptake, glycogen formation in skeletal muscle and for lipogenesis in epididymal fat requiring half-maximally effective serum concentrations in the range of 0.8 to 3.0 pmol/ml for insulin and of 40 to 70 pmol/ml for IGF-II.

These data suggest that in vivo acute metabolic actions of insulin-like growth factor II on carbohydrate metabolism occured through insulin receptors.

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THE RESPONSE OF GLUCOSE AND FAT METABOLISM TO IGF-I, INSULIN AND COMBINED INSULIN+IGF-I IN DOGS.

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IGF-I has insulin like effects on glucose and fat metabolism with a hypoglycaemic potency 8-11% of insulin (INS) but it is unclear whether these effects are mediated via IGF-I receptors. The short term metabolic effects of 34 or 103 pmol/kg/min IGF-I, 3.4 pmol/kg/min INS, combined 3.4 INS + 34 IGF-I or combined 3.4 INS + 103 IGF-I pmol/kg/min infusions were investigated in anaesthetised dogs (n=5). D-3 ^{3}H glucose was infused for 510 min and the hormones from 180-360 min. Euglycaemia was maintained by D-3 ^{3}H glucose spiked glucose infusion. The percentage decrease in basal glucose production (Ra) with both combined infusions (59% and 54%) was not different from 3.4 pmol INS alone (51%). The percentage increase in glucose utilisation (Rd) from basal was greater with both combined infusions (444%, 498%) than with INS (331%) or 34 or 103 pmol IGF-I (317%, 331%), ($p<0.01$). The decrease in free fatty acid (NEFA) concentration with both combined infusions was not different from INS. The differential effects of INS and IGF-I on glucose Rd, glucose Ra and NEFA concentration are consistent with the relative abundance of IGF-I receptors in muscle but not in hepatocytes or adipocytes and suggest the metabolic effects of IGF-I are mediated through IGF-I receptors.

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NEGATIVE FEEDBACK REGULATION OF AMYLIN GENE EXPRESSION BY AMYLIN IN ßTC1 CELLS

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Islet amyloid polypeptide (IAPP) is known to be secreted by the ß-cell and to accumulate as amyloid deposits in Type 2 diabetes. Our aim was to study the short-term regulation (30 min) of IAPP gene expression in the glucose-responding ß-cell line ßTC1. Based on our recent observation that IAPP (2.5 µmol/l) can interact with CGRP receptors to stimulate a 4 fold increase of insulin secretion in ßTC1 cells , we investigated whether IAPP was able to regulate its own gene expression through the CGRP-receptors. IAPP gene expression in exponentially growing cells was studied by Northern blot analysis and quantified by densitometric analysis. Glucose (1-25 mmol/l) increased IAPP gene expression in a dose-dependent way, maximal stimulation was observed at 25 mmol/l and represented a 1.6 fold increase as compared to that of 1mmol/l glucose. At the optimal stimulatory concentration of glucose (25 mmol/l), IAPP (1µmol/l) inhibited its own gene expression (40% of control), without apparently modifying insulin mRNA levels. CGRP (1µmol/l) gave the same degree of inhibition of IAPP gene expression. Cycloheximide, a protein synthesis inhibitor, did not prevent the inhibitory effect of IAPP. It is concluded that IAPP can down-regulate its own gene product, probably through the activation of CGRP receptors.

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IDENTIFICATION OF A CHOLECYSTOKININ RECEPTOR SUBTYPE IN THE ENDOCRINE PANCREAS

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Cholecystokinin (CCK) is a gut hormone that regulates pancreatic endocrine functions via CCK-A receptors. CCK_4 (Trp-Met-Asp-Phe-NH_2) is 1000 fold less potent than CCK_8. The in vitro potencies and selectivity of newly synthesized CCK_4 analogues were investigated. Exchanging various amino acids, e.g. Met by Pro (compounds UCII118A, UCIV38B) or Nle (compounds QIII10, QIII36, UCV46A) and modifying Phe and/or Trp shifts the dose-response curves for glucose-induced insulin release two to three fold to the left ($p<0.001$), i.e. results in a sensitizing effect of isolated islets to glucose that is comparable to the insulinotropic effect of CCK_8. Especially above mentioned compounds which possess electron-drawing groups were effective. Other synthesized compounds with electron-donating groups had no effect. In contrast to CCK_8 the insulinotropic CCK_4 compounds were selective for the endocrine pancreas: they had no agonistic or antagonistic effect on either the contraction of the guinea pig ileum or on the feeding behaviour of mice being supplied with either compound by an implantable Alzet® pump for five days. It is concluded that by these highly selective compounds (peripheral) CCK-A receptor subtypes can be discriminated for the first time; the ß-cell CCK-A receptors are different from those in smooth muscle and those for regulating appetite.

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SYNERGISTIC EFFECTS OF GIP AND GLIBENCLAMIDE ON INSULIN AND SOMATOSTATIN SECRETION

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It is well established that glucose-dependent insulinotropic peptide (GIP) stimulates adenylate cyclase. The aim of this study was to explore further the mechanisms by which GIP stimulates secretion of pancreatic hormones. Using isolated perfused rat pancreas we have studied interactions of GIP with secretagogues, which differ in their effects on intracellular cyclic AMP-levels. 10 nM GIP potentiated both early (1-5 min) and late (6-40 min) 16.7 mM glucose-induced insulin and somatostatin release. Total 40 min insulin: 1867 ± 120 ng (control), 5011 ± 440 ng (+GIP); $n=5$, $p<0.001$. Somatostatin: 1.91 ± 0.34 ng (control), 5.31 ± 1.13 ng (+GIP); $n=5$, $p<0.01$. The early (1-5 min) and late (6-20 min) 2 µM glibenclamide-induced insulin and somatostatin responses were potentiated by 10 nM GIP. Total 20 min insulin: 147 ± 13 ng (control), 708 ± 92 ng (+GIP); $n=6$, $p<0.001$. Somatostatin: 1.22 ± 0.13 ng (control), 2.81 ± 0.37 ng (+GIP); $n=6$, $p<0.01$. Similar but less pronounced results were obtained with 1 nM GIP. In contrast, 1 or 10 nM GIP had no effect on 20 mM arginine-induced secretion of insulin, somatostatin or glucagon. Hence, GIP shows synergistic activity with the cyclic AMP-increasing secretagogues glucose and glibenclamide, and not with arginine, a secretagogue which depolarizes the ß-cell and increases the intracellular Ca^{2+} concentration but does not increase cyclic AMP.

PS 4
Insulin Action

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INSULIN DYNAMICS FOLLOWING GLUCOSE INJECTION ARE BLUNTED IN INTERSTITIAL FLUID COMPARED TO PLASMA: IMPLICATIONS FOR INSULIN ACTION?

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Insulin action in vivo is determined by both transcapillary insulin transport (TCIT) and subsequent insulin binding and activation of glucose transport. We discovered that during hyperinsulinemic euglycemic clamps, TCIT is rate-limiting for insulin action. To examine insulin (INS) and (GLU) dynamics under physiologic conditions, we performed intravenous glucose tolerance tests (IVGTTs; 0.3 g/kg; n=7) on 6 conscious dogs, and sampled mixed venous plasma (P) and thoracic duct lymph (L). At basal, INS_P was 2.1±0.2 fold greater than INS_L ($P<0.001$); no gradient existed for GLU. Upon injection (t=0), GLU_P reached peak of 340±28 mg/dl at 2 min. GLU appearance in lymph was delayed (10±2 min; $P<0.01$), and attained peak level 93±28 mg/dl below plasma ($P<0.004$). In contrast, while INS_P attained peak of 138±25 μU/ml within 2 min, appearance of INS in lymph was slower (4±1 min; $P<0.01$), reaching only 49% of INS_P (68±9 μU/ml; $P<0.01$). There was no relation between GLU disappearance rate (K_G) and INS_L (peak value, integrated area, time to initial appearance; $P>0.05$ for all). CONCLUSION: During the IVGTT, while glucose dynamics between plasma and lymph are not very different and can largely be explained by sampling delay, insulin dynamics in lymph are delayed and attenuated relative to plasma concentrations, consistent with energy-dependent transendothelial insulin transport.

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THE EFFECT OF A PURE ANTIANDROGEN ON INSULIN RESISTANCE IN POLYCYSTIC OVARY SYNDROME

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The polycystic ovary syndrome (P.C.O.) is associated with hyperinsulinemia and hyperandrogenemia but it remains unclear which is the primary event. The main aim of this study is to assess the long-term effect of a pure nonsteroidal androgen receptor blocker, Flutamide, on insulin resistance in P.C.O. Eleven anovulatory obese women with P.C.O. (BMI 29.6 ± 1.28), aged 18-28 years, received Flutamide, 500 mg daily, for 4-6 weeks. The following parameters were determined before and after treatment: 1) Insulin and glucose responses to oral glucose tolerance test (OGTT 75g) 2) Insulin tissue sensitivity index (SI) with euglycemic clamp technique 3) Androgen levels: free Testosterone (fT), Δ_4A) Androstenedione (Δ_4A) and Dehydroepiandrosterone sulfate (DHEAS). Results: 1) Glucose and insulin levels decreased throughout the OGTT: glucose at 30' (144.1 ± 10.0 vs 108.7 ± 7.0 mg% $p<0.01$) and 60' (156.7 ± 14.0 vs 119.8 ± 9.0 mg% $p<0.05$) and insulin at 60' (90.2 ± 5.0 vs 72.6 ± 5.0 mIU/ml, $p<0.03$) and 90' (85.0 ± 7.0 vs 66.0 ± 4.0 mIU/ml, $p<0.05$) 2) SI (Glucose infusion rate/immunoreactive insulin) remained unchanged (6.30 ± 0.68 vs 6.70 ± 1.0, p NS). 3) Δ_4A levels decreased (7.30 ± 0.39 vs 4.18 ± 0.30 ng/ml, $p<0.004$) but not fT (15.80 ± 1.70 vs 13.98 ± 1.8 pmol/l) and DHEAS (4008 ± 666 vs 2883 ± 601 ng/ml). In conclusion this study suggests that the long-term effect of the antiandrogen flutamide, at the present dosage, seems to alter glucose tolerance during OGTT, but this may not be due to improvement of insulin resistance, which remains unchanged during the euglycemic clamp technique.

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SPIRONOLACTONE TREATMENT INCREASES INSULIN SENSITIVITY IN HYPERANDROGENIC WOMEN.

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To evaluate whether correction of hyperandrogenism may increase in vivo insulin sensitivity, a two step (20-80 mU/m²·min) euglycemic insulin clamp, associated to 6-[3H]-glucose infusion, was performed in 11 young nonobese hirsute women (BMI < 25 kg/m², Ferriman-Gallwey score > 12) with normal glucose tolerance, as well as in 6 matched healthy controls. In hirsute patients the study was carried out before and after 3 months of antiandrogen treatment with spironolactone (100 mg/day). Fasting plasma glucose, insulin and potassium were similar before and after spironolactone (4.8±0.1 vs 4.7±0.1 mmol/l, 41±6 vs 48±4 pmol/l, and 4.3±0.1 vs 4.1±0.1 meq/l respectively, mean±SEM). Glucose disposal was significantly lower in hirsute women as compared to normal controls, and increased after treatment from 25.0±1.7 to 27.8±2.2, and from 66.7±2.2 to 71.7±2.2 μmol/min·kg fat-free mass, $p< 0.025$, respectively in the 2 steps of clamp studies. However, also after treatment it remained lower than in controls. Hepatic glucose production was similar in the 2 groups either at basal or during hyperinsulinemia and, in hirsute women, it did not change after treatment. In conclusion, antiandrogen treatment with spironolactone improved peripheral insulin sensitivity of hyperandrogenic women, supporting the hypothesis that androgen excess per se contribute to determine insulin resistance.

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IN 19 INSULINOMAS HYPERINSULINEMIA LEADS TO INSULIN RESISTANCE WHICH DISAPPEARS AFTER SURGERY

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The effect of chronic hyperinsulinemia on insulin sensitivity was evaluated in 19 patients (11 obese and 8 non-obese) with insulinoma before and in 8 patients 4 months after surgical removal of the tumor. None had family history of diabetes mellitus. One patient had mild hypertension. Plasma cholesterol and triglycerides did not show significant changes. Euglycemic hyperinsulinemic (1.0 mU/kg.min of Human Insulin) clamp was performed in each patient after 7-12 hours of fasting state, depending on the severity of hypoglycemia. The metabolized glucose index was corrected for the steady-state insulinemia. Results: metabolized glucose was highly reduced in insulinomas than in normals (23.0±2.28 and 40.33±0.83 μmol/kg.min respectively, $p<0.006$). No statistically significant difference was found between obese and non-obese insulinomas (19.33±2.28 vs 27.94±4.33 μmol/kg.min, respectively) although a strong tendency of obese insulinomas to be non-insulin resistant was observed. The metabolized glucose index was negatively correlated ($r=-0.754$) to fasting insulinemia only in non-obese insulinomas. After surgery, the metabolized glucose index was normalized (38.73±2.17 μmol/kg.min). In conclusion: 1) chronic hyperinsulinemia determines insulin resistance in insulinomas; 2) the degree of insulin resistance is inversely related to the plasma insulin concentrations; 3) surgical removal of the tumor leads to a normalization of insulin sensitivity.

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THE EFFICACY OF PHARMACOLOGICAL TREATMENT OF INSULINOMA PATIENTS EXAMINED BY EUGLYCAEMIC CLAMPS

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Complete recovery of glucose metabolism after surgical operation need not be achieved in all patients with insulinoma. A conservative treatment with diazoxide may be used as an alternative. However, two types of insulinoma patients classified as "responders" and "non-responders" to diazoxide therapy were previously observed. We evaluated euglycaemic clamps to decide the efficacy of conservative treatment with diazoxide. Nine patients with later histologically confirmed insulinoma were preoperatively examined by euglycaemic clamps on Biostator (mode 7:1) before and after 3 days of diazoxide administration (3mg/kg daily).Euglycaemic clamps were performed after an overnight fast using an insulin infusion at a rate 0.5 mU/kg/min during 90 min (Clamp I) and 1.0 mU/kg/min in the following 90 min (Clamp II). Fasting plasma glucose concentration was improved after diazoxide treatment in 5 patients ("responders", 3.6+0.6 vs 5.5+0.5 mmol/l,$p<0.01$) whereas it was unchanged in 4 patients ("non-responders", 2.6+0.2 vs 2.2+0.3 mmol/l, NS). Significantly higher plasma insulin concentration was found during Clamp I in responders and non-responders to diazoxide as compared to 5 healthy persons (75+9 and 89+12 vs 46+8 U/l,$p<0.001$). Diazoxide administration induced a significant decrease of plasma insulin levels in responders as compared to non-responders (44+12 vs 82+14 U/l,$p<0.01$). Tissue sensitivity to insulin was significantly reduced in both subgroups of insulinoma patients in comparison with healthy persons (22+5 and 25+6 vs 39+7 mmol/kg/min per U/l, $p<0.001$) whereas an improvement after diazoxide was found only in responders(22+5 vs 28+4 mmol/kg/min per U/l,$p<0.02$) Similar results were obtained in Clamp II. The amelioration of all the above variables was present after surgical removal of an insulinoma in both groups of patients. We conclude that euglycaemic clamps may distinguish responders to diazoxide therapy from those in whom only surgical removal of the tumor may be beneficial.

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INSULIN SECRETION AND ACTION IN VARIOUS POPULATIONS WITH TYPE 2 (NON-INSULIN-DEPENDENT) DIABETES MELLITUS.

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In order to better understand the natural history of Type 2 diabetes, we compared an index of insulin secretion (basal + post-glucagon plasma C-peptide levels ; n = 80) and an index of insulin sensitivity (glucose MCR during a euglycaemic hyperinsulinaemic glucose clamp ; n = 69) in six populations separated by the presence or absence of obesity, diabetes and insulin-requirement respectively. The four diabetic groups had similar basal blood glucose levels, around 8 $mmol.l^{-1}$. Non-diabetic obese subjects compensated a decreased insulin sensitivity ($p < 0.05$) by an increased insulin secretion ($p < 0.001$). In all diabetic groups, insulin sensitivity was markedly altered ($p < 0.001$) while insulin secretion was significantly impaired ($p < 0.05$) only when corrected for the higher basal blood glucose levels ; insulin sensitivity was more reduced ($p < 0.05$) while insulin secretion was less affected ($p < 0.001$) in the presence than in the absence of obesity. The transition to insulin-requirement was accompanied by a significant decrease in both insulin secretion ($p < 0.001$) and action ($p < 0.05$) in non-obese diabetic patients but only by a marked decrease ($p < 0.001$) in insulin secretion in obese diabetic subjects. This cross-sectional study suggests that insulin resistance plays a crucial role in early Type 2 diabetes but that deficient insulin secretion best explains the late evolution towards insulin-requirement.

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INDUCTION OF INSULIN RESISTANCE BY GROWTH HORMONE DEPENDS ON ITS PATTERN OF DELIVERY

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The mode of growth hormone administration influences its biological effects in the rat. Our aim was to determine the tissue specificity and dependence of mode of delivery of biosynthetic human growth hormone (GH) on induction of insulin resistance in the rat. Cannulated adult Wistar rats (groups n=6) were infused with saline (SAL), or GH (1 U/kg.day) administered continuously (CTS) or intermittently (PLS, 5 min pulse each 3 h). After 4 days euglycemic hyperinsulinemic (100mU/l) clamps were performed in the awake, 5 h fasted state. Significant whole body and liver insulin resistance (both $p<0.01$) were only present in CTS rats (clamp glucose infusion rates SAL 20.1±1.0, CTS 12.4±0.6, PLS 22.2±1.2; clamp hepatic glucose outputs SAL 0.8±0.5, CTS 11.1±3.8, PLS -0.1±0.7 mg/kg.min). Total clamp peripheral glucose uptake was not significantly different among groups. However tissue-specific insulin action (estimated from ^{14}C-2-deoxyglucose uptake) was significantly reduced ($p<0.05$) in 4/7 and 0/7 muscles of CTS and PLS GH-treated rats respectively compared with SAL rats. No significant differences were observed among groups in adipose tissue, heart or lung responses. We conclude that continuous but not pulsatile GH delivery produces in vivo insulin resistance in liver and muscle of the rat.

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GROWTH HORMONE INVOLVEMENT IN DIURNAL VARIATIONS OF INSULIN SENSITIVITY IN NORMAL SUBJECTS

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In order to evaluate the involvement of growth hormone (GH) in diurnal variations of insulin sensitiviy (IS), 2 groups of normal subjects were recruited for 2 experiments. In experiment I, 8 subjects underwent modified insulin suppression test (MIST, Somatostatin 500 mcg/hr, regular insulin 30 mU/M^2. min, glucose 6 mg/kg-min, iv infusion) in a diurnal setting (one at 8 am and one at 4 pm, each with a precedent 16 hr fasting). Steady state plasma glucose (SSPG) was obtained as an index of IS, insulin (SSPI) for insulin clearance (IC) calculation and RBC specimens for insulin binding(IB) measurements. In experiment II , another 5 subjects received similar procedures plus a subcutaneous injection of either GH (4 iu) or octreotide (OCT, 150 mcg) at 11 pm the night before study. The results showed: (1) SSPG was lower (118.0+43.6 vs 150.3+34.2 mg/dl, $p<0.05$) and IB higher (3.3+0.9 vs 2.7+0.8%, $p<0.05$) in am than in pm, respectively. There was no diurnal difference of IC at SSPI around 40-60 mcU/ml. (2) GH injection induced lower am SSPG (129.8+50 vs 166.9+28.0 mg/dl, $p<0.01$), whereas OCT the opposite (144.5+25.3 vs 110.6+47.7 mg/dl, $p<0.01$). It is concluded that there are indeed diural variatons of IS in normal subjects, i.e., better in the morning, and GH plays an important role in its mechanism.

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IMMUNOGOLD LABELING OF GLUCOSE- AND CAT-ION TRANSPORTERS IN HUMAN ADIPOCYTE MEMBRANES

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The in vitro insulin effect on the expression of different transporter proteins on the surface of adipocytes from normal individuals was quantitated. Adipocytes were isolated from subcutaneous abdominal biopsies obtained from 5 young, nonobese women, devoid of metabolic disorders and undergoing laparatomy for other reasons. The insulin effect on the transport of 3-O-methylglucose in adipocytes was determined, and native plasma membrane fragments, with the cytoplasmic face exposed, were prepared from the cells and incubated with different monoclonal antibodies directed against intracellular epitopes on the glucose transporter isoforms GLUT-1 or GLUT-4 or the Na/K-ATPase isoforms $\alpha 1$ or $\alpha 2$, followed by immunogold labeling and negative staining. The labeling of the various transporters was quantitated by counting on electron micrographs. It was found that insulin (100 nmol/l) increased 3-O-Methylglucose transport (at 10 mmol/l) from (mean ± SE) 0.045 ± 0.007 to 0.109 ± 0.011 (s^{-1}) and the GLUT-4 labeling in the plasma membrane from 29 ± 5 to 84 ± 13 (thousands of particles/cell), whereas labeling of GLUT-1, $\alpha 1$ and $\alpha 2$ was low and not significantly affected by insulin. In conclusion the relative, insulin induced increases of glucose transport over and of GLUT-4 transporters in the plasma membrane of human adipocytes are similar.

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DEFECT INSULIN RECEPTOR KINASE IN MUSCLE FROM YOUNG RELATIVES OF TYPE 2 DIABETIC PATIENTS

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Insulin resistance in Type 2 diabetes is associated with decreased activation of the insulin receptor kinase in skeletal muscle. To investigate whether this could be primary to the development of Type 2 diabetes we studied 10 young (27 ± 1 yr) non-obese first degree relatives of patients with Type 2 diabetes and 8 matched controls without a family history of diabetes. Insulin sensitivity was assessed by an euglycemic, hyperinsulinemic clamp. Insulin receptors were partially purified from muscle biopsies obtained in the basal and the insulin stimulated state during the clamp. Insulin binding capacity was decreased by 28% in the relatives in the basal biopsy ($p < 0.05$) but normal in the insulin stimulated biopsy. Mean receptor tyrosine kinase activity towards Poly(Glu:Tyr(4:1)) after stimulation with various insulin concentrations "in vitro" was reduced in the relatives in basal ($p < 0.005$) and insulin stimulated biopsies ($p < 0.01$) and also when expressed per binding capacity. Basal kinase activity correlated with non-oxidative glucose uptake in the fasting state ($r = 0.53$, $p < 0.03$), and "in vitro" insulin stimulated kinase activity with insulin stimulated non-oxidative glucose metabolism ($r = 0.61$, $p < 0.01$). We conclude that the marked defect in the insulin receptor tyrosine kinase activity may be a primary defect of significance for the development of Type 2 diabetes.

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RESPONSE OF PYRUVATE DEHYDROGENASE COMPLEX ACTIVITY TO A GLUCOSE LOAD IN OBESE MICE.

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In the gold-thioglucose (GTG)-obese hyperinsulinaemic mouse, peripheral tissues become insulin-resistant while liver remains insulin-sensitive. The aim of this study was to see if this difference is reflected in the changes in pyruvate dehydrogenase complex activity (PDHa) in response to an oral glucose load (3g/kg). PDHa was measured in mitochondrial preparations of heart, liver, quadriceps muscle, brown adipose tissue (BAT) and white adipose tissue (WAT) from 10week GTG-obese mice and age-matched controls killed 0'-120' post gavage. In obese animals, fasting PDHa was increased 3-fold in BAT ($p<0.001$), decreased in quadriceps (50%, $p<0.05$) and WAT (75%, $p<0.001$) and unchanged in heart and liver. Maximum PDHa response to glucose in controls was >5-fold in heart ($p<0.001$) and liver ($p<0.01$) at 30' and 2-fold in BAT ($p<0.01$) at 60'. In all tissues PDHa returned to fasting levels by 90'. Responses in muscle and WAT were not significant. In obese mice, there was no significant change to fasting PDHa in any tissue except liver where there was a 5-fold increase at 30' ($p<0.001$). We conclude that the ability of the PDH complex to respond to a glucose load is lost in peripheral tissues after the development of insulin resistance whereas liver PDHa remains insulin-sensitive.

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LONG-TERM CONTINUOUS AND PULSATILE INTRAVENOUS INSULIN TREATMENT OF DIABETIC RATS: EFFECT ON INSULIN-MEDIATED GLUCOSE UPTAKE AND LIPOLYSIS IN ISOLATED ADIPOCYTES.

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Short-term exposure of tissues to pulses of insulin leads in general to an enhancement of insulin action. We investigated the effects of 2.5 weeks of near-physiological continuous and pulsatile intravenous insulin treatment of streptozotocin (70 mg/kg) diabetic rats (D) on in vitro insulin action in isolated adipocytes. D received a continuous (Dc, n=6) or pulsatile (Dp, n=6)(6 min double infusion speed, 6 min off) insulin infusion. Control rats (C) received a continuous (Cc, n=7) or pulsatile (Cp, n=6) vehicle infusion. Data are means ± SE. At adipocyte isolation, fasting plasma glucose was elevated in Dc vs Cc (15.3 ± 3.0 vs 6.8 ± 0.1 mM, $p<0.05$) but similar in Dp and Cp (5.1 ± 2.1 vs 6.6 ± 0.1 mM). Body weight, mean adipocyte cell size (69.3 ± 2.8, 65.6 ± 2.7, 64.7 ± 2.9, 64.2 ± 1.1 μm), specific [^{125}I]-insulin binding per $4x10^5$ cells (2.3 ± 0.4, 1.7 ± 0.4, 2.1 ± 0.4, 2.2 ± 0.6%) and basal [U-^{14}C]-glucose uptake (96 ± 12, 94 ± 11, 75 ± 12, 93 ± 21 fmol/min.$4x10^5$ cells) were comparable in Cc, Dc, Cp, Dp and the dose-response curves for insulin-stimulated [U-^{14}C]-glucose uptake were identical. Isoproterenol (10^{-6} M)-stimulated glycerol output was 617 ± 117, 490 ± 120, 847 ± 167, 803 ± 160 ng/min.$4x10^5$ cells in Cc, Dc, Cp, Dp (p=n.s.) and the dose-response inhibition by insulin was identical in Cp and Dp (Vmax=48.6 ± 6.1 vs 42.3 ± 4.6%) but blunted in Dc (Vmax=8.2 ± 4.6%) vs Cc (44.0 ± 7.2%), $p<0.01$. In conclusion, in D, long-term near-physiological pulsatile intravenous insulin treatment results in normal insulin-mediated glucose uptake and lipolysis in isolated adipocytes, whereas continuous insulin treatment induces a post-binding defect in the antilipolytic action of insulin.

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IMPAIRMENT OF GYCOSYL-PHOSPHATIDYLINOSITOL-DEPENDENT INSULIN SIGNALLING SYSTEM IN HEPATOCYTES ISOLATED FROM OBESE *(fa/fa)* RATS.

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An inositol-phosphate glycan (IPG), generated by the phospholipase C-catalyzed hydrolysis of an insulin-sensitive glycosyl-phosphatidylinositol (GPI), mimics and may mediate some of the biological effects of this hormone in different types of cells. Changes in GPI-dependent cellular signalling system have been observed in different situations of insulin-resistance (IRes) (i.e. chronic dexamethasone treatment, streptozotocin-diabetes, aging). Now, we have investigated how the IRes expressed in genetically obese *(fa/fa)* rats affects the GPI-dependent signalling system in isolated hepatocytes. GPI was isolated by TLC after labelling of hepatocytes with (1-^{14}C)isethionyl acetimidate. The hepatocyte content of GPI was reduced by about 30% in obese rats, as compared to that measured in lean *(Fa/fa)* rats (2553 ± 138 vs 3334 ± 115 dpm/mg protein). In obese rats, the decreased level of GPI was accompanied by a blockade of insulin-mediated GPI hydrolysis, a decreased rate of hepatocyte IPG uptake (about 30%) and a significant reduction in the stimulatory effects of both insulin and IPG on glycogen synthesis and glycogen synthase activity ratio. Our results demonstrate that IRes associated with genetic obesity is accompanied by an impairment of the GPI-dependent insulin signalling system.

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INTRAPORTAL ADMINISTRATION OF AMYLIN INDUCES INSULIN RESISTANCE IN NORMAL RATS.

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Amylin has been proposed to be responsible for insulin resistance. The aim of our study was to examine if an intraportal administration of amylin could modify glucose disposal in vivo. Intraportal and intracardiac catheters were implanted into male Sprague-Dawley rats (3 months old). Three groups of animals formed the study : Group 1 (G1) received intraportal saline infusion ; G2, amylin at 2.6 nmol/kg/h ; and G3, amylin at 26 nmol/kg/h. An IVGTT was performed 30 min after the beginning of the infusion (total infusion time : 90 min). The rate of glucose disappearance was not altered at the lowest dose of amylin but it was dramatically decreased by the highest dose : $K(\times 10^{-2}) = 3.49 \pm 0.26$ for G1 vs 1.91 ± 0.22 for G3 ($p < 0.01$). Interestingly, this decrease in glucose tolerance existed despite a sustained elevated release of insulin in the G3 group throughout the last 20 min of the IVGTT (15.6 ± 1.4 for G3 vs 10.7 ± 1.3 µU/ml for G1, $p < 0.05$). Thirty minutes after stopping the infusion of amylin, insulin levels rapidly returned to basal values. Since there was a decreased glucose disposal in spite of elevated insulin secretion, we conclude that high dose of amylin infused directly into the portal vein provokes insulin resistance in vivo.

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EFFECT OF PHORBOLESTER ON CARRIER-TRANSLOCATION (GLUT-1, GLUT-4) IN THE RAT HEART.

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Insulin induces translocation of glucose carriers from intracellular pools to the plasma membrane in fat and skeletal muscle. As phorbolesters mimick the effect of insulin on GLUT-4-translocation in these tissues, a role of protein kinase C in insulin- induced carrier translocation was suggested. Aim of the study was to characterise the mechanism of the insulin effect on glucose transport in the heart.

Hearts from male wistar rats were perfused in Langendorff-technique with control medium, insulin (8×10^{-8}M), and the protein kinase C-stimulating phorbolester TPA (10^{-9}M). Glucose transport was measured using 3-O-methylglucose-efflux. Subcellular distribution of GLUT-4 and GLUT-1 in fractions enriched with plasma- and low density microsomal membranes was determined by western blot. Insulin stimulated glucose transport as well as GLUT-1- and GLUT-4-translocation in a similar order of magnitude (factor 3-4, n=6 in each group). In contrast, in TPA-treated hearts no alteration of glucose transport nor carrier-translocation was found, whereas both contractility and relaxation increased.

These data suggest that in perfused rat hearts both carrier-isoforms are present and translocated by insulin. In contrast to fat and skeletal muscle, protein kinase C is not involved in the insulin signal on glucose carrier translocation in the heart.

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PHOSPHODIESTERASE ACTIVATION IS IMPORTANT FOR THE EFFECT OF INSULIN TO STIMULATE GLUCOSE TRANSPORT

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To elucidate the role of the cGMP-inhibited low K_m phosphodiesterase (PDE) for insulin action, rat adipocytes were incubated at 37°C for 20 min with or without insulin, cAMP-analogues and the specific PDE-inhibitor OPC 3911. ^{14}C-3-O-methylglucose uptake rate was then determined. The maximal rate of glucose transport elicited by 1000 µU/ml insulin was ~10-fold higher than basal, non-stimulated uptake ($p < 0.01$). It was not significantly affected by the addition of the hydrolyzable cAMP-analogue 8-bromo-cAMP (4 mM). Maximal glucose uptake was suppressed (by ~20%, $p < 0.05$) by N^6-monobutyryl-cAMP (4 mM) which is not hydrolyzed by PDE. When both 8-bromo cAMP and OPC 3911 (10 µM) were present, the reduction in maximal insulin response was more pronounced (~45%, $p < 0.05$). A small impairment (~15%, $p < 0.05$) was also seen with OPC 3911 alone. The dose-response curve for insulin-stimulated glucose uptake was shifted ~2-3-fold to the right by either N^6-monobutyryl-cAMP or 8-bromo-cAMP + OPC ($p < 0.05$) whereas each of the latter agents alone was without significant effect.

Conclusions: Inhibition of PDE in rat adipocytes impairs the insulin response on glucose transport. This is only partially attributable to regulation of cAMP levels. Insulin sensitivity is impaired, probably through cAMP elevation. Thus PDE plays a role in the regulation of both insulin sensitivity and responsiveness.

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PHOSPHORYLATION OF GLUT-4 IMPAIRS ITS INTRINSIC ACTIVITY
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We examined the effect of phosphorylation on GLUT-4 function in isolated rat adipocytes. Adipocytes labeled with ^{32}P for 2 hrs were incubated with PTH (5 or 20 ng/ml for 60 min) and exposed to insulin (25 ng/ml) for an additional 30 min. ^{32}P-GLUT-4 was immunoprecipitated from the plasma membrane and low density microsomal fractions, and its degree of phosphorylation was determined by autoradiography and densitometry. The state of GLUT-4 phosphorylation was correlated with the ability of insulin: 1) to translocate GLUT-4 to the plasma membranes and 2) to stimulate GLUT-4 intrinsic activity. PTH significantly increased GLUT-4 phosphorylation. Western blotting with R820 polyclonal antibody revealed normal distribution of GLUT-4 before and after insulin stimulation in control and the PTH-treated cells, suggesting that phosphorylation of GLUT-4 does not interfere with its recruitment to the plasma membrane. Glucose uptake in plasma membrane vesicles isolated from control and PTH-treated cells exposed to insulin (25 ng/ml x 30 min at 37°C) was measured using ^{14}C-2-deoxyglucose. With increased phosphorylation of GLUT-4, we observed a progressive inhibition of 2-DOG uptake (50% inhibition with 20 ng/ml PTH, $p < 0.01$, $n = 10$). We conclude that phosphorylation of GLUT-4 significantly impairs the ability of insulin to stimulate its intrinsic activity.

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METFORMIN BLOCKS DOWN-REGULATION OF CELL-SURFACE GLUT4 IN CHRONIC-INSULIN-TREATED RAT ADIPOCYTES

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The effect of acute-and of chronic-insulin treatment and of treatment with metformin on the cell-surface distribution of Glut 4 and Glut 1 in cultured rat adipose cells has been studied using the impermeant photolabel [^{3}H]-ATB-BMPA. The cell-surface labelling has been compared with that obtained in digitonin-permeabilized cells. Following 24 hr in culture the proportion of GLUT 4 and GLUT 1 at the surface of basal cells were 18% and 26% of the total while the insulin-stimulated levels of GLUT 4 and GLUT 1 at the cell-surface were 49% and 37% of the total. Chronic, 24 h -insulin treatment reduced glucose transport activity to almost basal levels and the proportion of GLUT 4 at the cell surface to 25% of the total. The down-regulation of GLUT 4 with chronic-insulin treatment was alleviated by metformin and the proportion of GLUT 4 at the cell-surface was maintained at 60% of the total. Furthermore, cells which were chronically treated with insulin and which were then washed to remove insulin showed severe resistance to subsequent acute-insulin re-stimulation of transport and cell-surface recruitment of both GLUT 4 and GLUT 1. This effect was also alleviated by inclusion of meformin during the chronic-insulin treatment.

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GLUT4 mRNA CONCENTRATION IN WHITE ADIPOSE TISSUE FROM RECENT ONSET TYPE 1 DIABETIC PATIENTS.
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Patients with recent onset insulin-dependent diabetes mellitus present a marked insulin-resistance which is progressively corrected by intensified insulintherapy. Site(s) of this resistance at the cellular and molecular levels remain(s) unclear. A decrease in GLUT4-mediated glucose transport into insulin-sensitive cells could contribute to this resistance. Adipose tissue samples (3 g ; abdominal wall) were obtained from informed consenting recent onset patients and controls. Total RNA were extracted. The mRNA coding specifically for GLUT4 was revealed by the specific cDNA probe (Dr. G. Bell) and quantified by Northern blot analysis. A 50% decrease in GLUT4 mRNA was noted in patients treated for less than 7 days with insulin (1.2 ± 0.1 U/kg/d) normoglycemic (101 ± 4 mg/dl) with plasma insulin levels of 19 ± 2 μU/ml, in post absorptive state (controls : 86 ± 4 mg/dl glucose and 13 ± 2 μU/ml insulin). A marked insulin-resistance was present, quantified by the euglycemic hyperinsulinemic clamp. After four weeks of insulin treatment, the GLUT4 mRNA was normalized, a normal total body sensitivity to insulin was restored at that time. A deficient GLUT4 synthesis may contribute to the decrease of glucose transport into human adipocytes and to insulin-resistance in recent onset type 1 diabetic patients.

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EXPRESSION OF GLUT4 IN TYPE 1 DIABETIC PATIENTS IN POOR GLYCEMIC CONTROL: EFFECTS OF 24 HOURS OF INTENSIVE INSULIN THERAPY.
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Type I diabetic patients in poor glycemic control are characterized by insulin resistant glucose uptake in muscle. We have examined the effects of a near normalization of glycemia by 24 hours of intensive insulin therapy on the expression of GLUT4 protein and mRNA in vastus lateralis muscle of 8 Type 1 diabetic patients with poor long-term glycemic control (mean HbA_{1C} 10.3%). Good glycemic control was obtained in all patients after 24 hours of insulin therapy (plasma glucose, 20.8 ± 0.8 vs. 8.7 ± 0.8 mmol/l). Furthermore, serum insulin level was increased (0.06 ± 0.01 vs. 0.17 ± 0.03 nmol/l). However, the abundance of both GLUT4 protein (0.138 ± 0.019 poor glycemic control vs. 0.113 ± 0.009 arb.units, good glycemic control, NS) and mRNA (96432 ± 15904, poor glycemic control vs. 81394 ± 9002 arb.units, good glycemic control, NS) in muscle remained unchanged by near-normalization of glycemic control. In conclusion: 1) Physiological plasma levels of insulin plays no role in the acute regulation of GLUT4 expression in human skeletal muscle. 2) Impaired translocation or activation of GLUT4 may be involved in the pathogenesis of insulin resistant glucose uptake in muscle from Type 1 diabetic patients.

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COMPARATIVE STUDIES OF GLYCOGEN SYNTHASE GENE EXPRESSION AND ACTIVITY IN SKELETAL MUSCLE FROM ATHLETES AND SEDENTARY CONTOLS.

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In 7 competitive level athletes and 9 matched controls we have characterized the expression of the rate limiting enzyme in muscle glucose storage, the glycogen synthase (GS). Insulin-glucose clamp in combination with indirect calorimetry and biopsy sampling of vastus lateralis muscle were performed in the basal state and after 4 h of euglycaemia and hyperinsulinaemia (2 mU/kg/min). GS mRNA was studied using slot blots while GS protein was examined by immunoblotting using an antipeptide rabbit antibody specific for human muscle GS. In the basal state, total GS activity as well as GS activation by glucose-6-phosphat were increased in athletes by 39% ($p < 0.02$) and 52% ($p < 0.02$) respectively. In parallel the GS mRNA level in athletes was increased by 85% ($p < 0.02$) whereas the GS protein abundance was similar (athletes 0.87 $\pm$ 0.06 vs controls 0.85 $\pm$ 0.06 arb.units/100 μg protein). Following 4 h of insulin exposure nonoxidative glucose disposal was higher in athletes (12.9 $\pm$ 0.4 vs 10.1 $\pm$ 0.6 mg/kg FFM/min, $p < 0.02$) while levels of total GS activity, mRNA and protein remained unchanged in both groups when compared with basal findings. In conclusion: Athletes have increased insulin stimulated nonoxidative glucose metabolism associated with both pretranslational (mRNA) and posttranslational (enzyme activity) upregulations of GS.

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Effects of Starvation or Diabetes on Pyruvate Dehydrogenase Kinase require Protein Synthesis.

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Glucose oxidation is inhibited in diabetic or starved rats by phosphorylation and inactivation of pyruvate dehydrogenase (PDH) complex. A major factor is a slow (24-48h) and stable increase in the activity of PDH kinase. This effect of diabetes or starvation can be reproduced in cultured hepatocytes, cardiac myocytes, or soleus muscle by 24h exposure to cyclic AMP and/or n-octanoate. The factor responsible is a protein termed PDH kinase activator protein (KAP), separable from PDH complex by gel filtration, known to be a soluble PDH kinase, and which has been purified to homogeneity . The object of the study was to determine whether effects of 50µM-cyclic AMP/1mM-octanoate require protein synthesis. Results are mean ± SEM (n). In primary hepatocytes in culture (24h) 30µM-cycloheximide decreased incorporation of ^{14}C leucine into protein by 92 ± 1.2%. PDH kinase activities following culture were control 1.05 ± 0.03 (12); cycloheximide 0.97 ± 0.03 (12); dibutyryl cyclic AMP/ octanoate 2.2 ± 0.04* (12); dibutyryl cyclic AMP/ octanoate/ cycloheximide 1.1 ± 0.02† (12)(*P<0.001 for effect of octanoate + dibutyryl cyclic AMP; †P<0.001 for effect of cycloheximide). The results indicate that the effect of n-octanoate + dibutyryl cyclic AMP to increase PDH kinase activity requires protein synthesis.

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Purification and Characterization of Pyruvate Dehydrogenase Kinase Activator Protein

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Glucose oxidation is inhibited in diabetic or starved rats by phosphorylation and inactivation of pyruvate dehydrogenase (PDH) complex. A major factor is a slow (24h) and stable increase in activity of PDH kinase. Effects of diabetes or starvation are reproduced in cultured hepatocytes, cardiac myocytes, or soleus muscle with 24h exposure to cyclic AMP and/or n-octanoate. The factor responsible is a protein - PDH kinase activator protein (KAP), separable from PDH complex by gel filtration, and known to be a soluble PDH kinase. The objective was to purify KAP to homogeneity, show the effect of starvation on its specific activity, and obtain N-terminal amino acid sequence(s). This has been achieved with liver mitochondrial extracts (fed, starved rats) by successive poly(ethylene)glycol fractionation, gel filtration on Sephacryl S300 , and chromatography on matrex orange, and mono Q FPLC. The product showed a single band on SDS-PAGE of Mr = 45kDa. Sequence analysis was consistent with a single peptide chain of N-terminal sequence K.N.A.S.L.A.G.A.I.E. thus confirming purity. The specific activity of purified KAP from starved rats was fourfold greater than from fed rats; yields of protein were similar, ie starvation (and diabetes) increase specific activity of KAP and not its concentration.

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INSULIN SECRETION AND ACTION IN THE SPONTANEOUSLY HYPERTENSIVE RAT

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To explore mechanisms for the link between hypertension and insulin resistance with hyperinsulinaemia observed in man, insulin secretion and action were studied in spontaneously hypertensive rats (SHR). SHR (15-16 weeks, mean weight 311 g, systolic BP 219 mm Hg) were compared with an inbred normotensive strain of Donryu (DRY) rats (15-16 weeks, mean weight 297 g, systolic BP 136 mm Hg). Insulin secretory response to IV glucose (300 mg/kg) was lower in SHR than DRY (insulin area 2.4 $\pm$ 0.4 vs 6.2 $\pm$ 0.8 mU.min, n=7, $p<0.005$). Basal glucose turnover was similar (SHR 8.9 $\pm$ 0.5 vs DRY 8.0 $\pm$ 0.7 mg/kg/min) as was glucose disposal during insulin infusion (6 mU/min, IRI 183 $\pm$ 3 vs 160 $\pm$ 22 mU/l) with glucose clamp (SHR 23.9 $\pm$ 4.3 vs DRY 21.1 $\pm$ 3.0 mg/kg/min). Basal uptake of ^3H-2-deoxyglucose into isolated soleus muscle strips was greater in SHR than DRY (6690 $\pm$ 270 vs 4730 $\pm$ 380 dpm/100 mg/min, n = 13, $p<0.001$) but the increment with insulin stimulation (100 mU/ml) was similar (2730 $\pm$ 440 vs 2860 $\pm$ 420). Western and Northern blot measurements of glucose transporter showed similar skeletal muscle levels of GLUT 4 content and message in SHR and DRY rats. In this study, SHR secreted less insulin than control DRY rats and insulin action was not impaired. There was no evidence for insulin resistance or hyperinsulinaemia as factors in the pathogenesis of hypertension in this animal model.

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MEASUREMENTS BY MICRODIALYSIS OF THE SUBCUTANEOUS INTERSTITIAL INSULIN CONCENTRATION IN MAN

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The interstitial insulin concentration was measured in 9 healthy lean men (Age: 25±1 yrs, Mean±SE), Body Mass Index (BMI): 22.7±0.7 kg/m^2 fasting glucose 4.6±0.2 mmol/l, fasting insulin 4±1 mU/l). Two microdialysis catheters (polypropylene, pore diameter 0.2 μm) were placed in the abdominal subcutaneous adipose tissue and calibrated in situ during a euglycemic hyperinsulinemic clamp. After correction for binding of insulin to the catheter the absolute interstitial insulin concentration was calculated. In 5 subjects plasma insulin was then rapidly increased and kept at a new steady state level for 2 h. The insulin infusion was then stopped and the elimination monitored. Rise in interstitial insulin was markedly delayed (≥ 20 min) as compared with venous plasma insulin, whereas decline of interstitial insulin was not signficantly delayed. At steady state euglycemic clamp conditions interstitial and plasma insulin concentrations were 109±17 and 196±11 mU/l, respectively (n=9). With a similar study design, it was found that interstitial inulin was not lower than plasma inulin in 5 subjects undergoing an inulin clamp.
Conclusion: The data suggest the existence of an endothelial barrier for insulin but not inulin in the adipose tissue leading to lower (50-60%) levels of insulin and delayed insulin kinetics (≥ 20 min) in the interstitial fluid as compared with plasma.

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GLUCOSE TOLERANCE, INSULIN, TRIGLYCERIDES AND BLOOD PRESSURE LEVELS IN WOMEN WITH UPPER-BODY OBESITY AND LOWER-BODY OBESITY.

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Glucose intolerance, hypertriglyceridemia and hypertension have been shown to be more prevalent in obesity, the relationship being stronger with upper-body obesity, than with total obesity. To evaluate this association two groups of obese (BMI≥27) premenopausal women were compared. Group A with waist-to-hip girth ratio (WHR)<80 (n=19) and group B with WHR>80 (n=17). Within both groups, there were no significant differences with respect to age, body weight, BMI, smoking habit or alcohol consumption. Fasting glucose, insulin and triglycerides levels, total areas under the curve for glucose and insulin after a 75 gr oral glucose tolerance test and blood pressure were measured. Fasting glucose (5.0±0.5 vs 5.7±0.9 mmol/L; p<0.01), glucose area (773±118.4 vs 1021.4±229.6 mmol*L^{-1}*min; p<0.001), fasting insulin (114±52 vs 173±83 pmol/L; p<0.05), insulin area (52268±20472 vs 88352±57810 pmol*L^{-1}*min; p<0.05), and fasting triglycerides (1.03±0.35 vs 1.64±0.95 mmol/L; p<0.01) were all significantly higher in group B. Systolic blood pressure (119.7±14.4 vs 140.3±26.3 mm Hg; p<0.01), but not diastolic blood pressure (79.2±8 vs 85.3±12.2; ns), was also significantly higher in group B. We conclude that upper-body fat distribution in obese women is associated with lower glucose tolerance together with higher triglycerides and systolic blood pressure levels. The finding of higher insulin levels in this subset of obese women is in accordance with a pathogenic role for insulin in the development of diabetes, hypertriglyceridemia and hypertension in upper-body obesity.

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VISCERAL ABDOMINAL FAT AND THE METABOLIC DISTURBANCES OF OBESE SUBJECTS WITH TYPE 2 (NON-INSULIN-DEPENDENT) DIABETES MELLITUS.

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Glucose and lipid metabolism in the basal state and during a 4-h euglycemic insulin (20 mU/min.m2) clamp associated with [^{3}H]-3-glucose infusion and indirect calorimetry were compared in 20 nondiabetic and 20 sex-, age- and weight-matched type 2 (non-insulin-dependent) obese subjects. Visceral abdominal fat was estimated by magnetic resonance imaging. Obese diabetic subjects showed: higher basal (3.30 ± 0.16 vs 2.60 ± 0.06 mg/min.kgFFM, p<0.001) and insulin-suppressed (2.05 ± 0.29 vs 0.40 ± 0.10, p<0.001) hepatic glucose production (HGP); lower insulin-stimulated total (TGD, 3.60 ± 0.27 vs 4.33 ± 0.23 mg/min.kgFFM, p<0.05) and oxidative (GOx, 2.10 ± 0.17 vs 3.04 ± 0.13, p<0.001) glucose disposal; higher insulin-suppressed plasma free fatty acids (FFA, 0.41 ± 0.04 vs 0.21 ± 0.01 μmol/l, p<0.001) and lipid oxidation (1.15 ± 0.10 vs 0.61 ± 0.09 mg/min.kgFFM, p<0.001); higher amounts of visceral fat (211 ± 28 vs 133 ± 15 cm^2, p<0.025). In female subjects visceral fat correlated (p<0.05) with clamp HGP (r=0.468), TGD (r=-0.563), GOx (r=-0.472), FFA (r=0.423), LOx (r=0.459). Clamp FFA and LOx correlated to HGP (r=0.703, r=0.736), TGD (r=-0.437, r=-0.565) and GOx (r=-0.616, r=-0.901) and were intercorrelated (r=0.735). Similar correlations were observed in males and were independent of total body fat content. We suggest that obesity-associated diabetes is featured by an increased amount of visceral fat which further deteriorates glucose metabolism through an increased release of FFA and the consequent increase in lipid oxidation within the liver and the muscle.

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HYPERTENSION, BODY SHAPE, OBESITY, HYPERINSULINAEMIA AND DIABETES IN URBAN AFRICANS

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Hypertension, diabetes, hyperinsulinaemia, body shape and obesity were investigated and their relationships determined in a population survey of African subjects living in Cape Town, South Africa. There were 729 respondents (79% response rate). Blood pressure and anthropometric measurements were taken and a 75 g oral glucose tolerance test administered. WHO criteria were used to define diabetes, impaired glucose tolerance (IGT) and hypertension. Hyperinsulinaemia was defined as >75% centile of fasting and two hour insulin concentrations. The age standardised prevalence of hypertension was 29.5 % (CI 26-33%), diabetes 8% (CI 6-10%) and IGT 7% (CI 5-9%). 52% of diabetics and 21% with normal glucose tolerance had hypertension ($p<0.001$). 14% of hypertensives and 4% of normotensives had diabetes ($p<0.001$). Based on logistic regression, hypertension was significantly associated with age >45 yr, urbanisation, upper segment fat distribution and either obesity alone or obesity coexisting with diabetes. Hyperinsulinaemia was not associated with hypertension after correction of other factors. In urban Africans: (1) hyperinsulinaemia was not an independent risk factor for hypertension and (2) the frequent co-existence of hypertension and diabetes was accounted for by common risk factors.

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PLASMA INSULIN LEVELS AND PREVALENCE OF ARTERIAL HYPERTENSION.

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To study relationships between prevalence of arterial hypertension, degree of glucose intolerance and plasma insulin levels we have screened 2376 men, aged 20-69 years. All subjects underwent a 75,0 g oral glucose tolerance test. Fast plasma insulin, total and high-density lipoprotein cholesterol, triglyceride levels were measured. Blood pressure was measured in a sitting position by using random zero manometer. Hypertension and glucose intolerance were determined according to WHO criteria. The prevalence of hypertension in subjects with diabetes mellitus (DM) was 46,3% (v. s. 28,0%-in persons with normal glucose tolerance, $P<0,005$), with impaired glucose tolerance (IGT)-31,8% (v. s. 28,0%, $P<0,001$). In subjects with DM and IGT the high prevalence of hypertension was associated with high basal insulin levels. To confirm this association we analysed the hypertension prevalence according to insulin distribution in whole population. In the fourth quartile of insulin distribution the prevalence of hypertension was 30,9% while in the first quartile it was 19,9% ($P<0,01$). This difference was more significant when hypertension was associated with dyslipoproteinemia. Conclusion: there is a strong association between the prevalence of arterial hypertension and fast plasma insulin levels.

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ANTIHYPERTENSIVE THERAPY WITH ENALAPRIL IMPROVES GLUCOSE STORAGE AND INSULIN SENSITIVITY IN HYPERTENSIVE TYPE 2 DIABETIC PATIENTS

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A double-blind, placebo controlled 4 week trial was performed to determine the antihypertensive and metabolic effects of enalapril (20-40 mg/day) in 16 hypertensive type 2 diabetic patients. Glucose kinetics, oxidation and storage were determined basally and during euglycemic hyperinsulinemia (180 min) using [3-^{3}H]glucose and indirect calorimetry. Enalapril decreased systolic (161±3 vs 149±4, $p<0.05$) and diastolic (100±2 vs 92±2 mmHg, $p<0.01$) blood pressure and urinary albumin excretion (8±1 vs 4±1 µg/min, $p<0.05$). Peripheral insulin sensitivity, i.e. insulin stimulation of glucose utilization, increased by 4.3±1.7 µmol/kg.min (13.1±2.0 vs 17.4±3.5 µmol/kg.min, $p<0.05$, before vs after) by enalapril but remained unchanged during placebo (15.4±2.8 vs 15.3±2.7 µmol/kg.min). The increase in glucose utilization by enalapril was explained by a 4.1±1.7 µmol/kg.min increase in glucose storage (4.1±1.2 vs 8.1±2.9 µmol/kg.min, $p<0.05$). Enalapril increased HDL-cholesterol slightly by 8% ($p<0.05$) with no change in total cholesterol or triglycerides. HbA_{1c} improved slightly by enalapril (7.7±0.7 vs 7.3±0.7%, $p<0.05$) but not by placebo. We conclude that enalapril improves insulin sensitivity in hypertensive NIDDM patients. Thus, the favourable metabolic effects of ACE inhititors are not restricted to nondiabetic patients and captopril but can also be observed in hypertensive type 2 diabetic patients treated with enalapril.

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DOES A POSITIVE FAMILY HISTORY OF DIABETES CONVEY A MORE ATHEROGENETIC BLOOD PROFILE?

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Type-2 diabetes has a strong genetic component and is burdened with an excessive risk of mortality from CVD. The purpose of this cross-sectional study was to determine whether a positive family history (PFH) for type-2 diabetes is associated with adverse risk factors levels for CVD. We evaluated a population of about 1000 subjects who in the course of 1991 underwent a medical work-up in Taranto (South Italy). The subjects were divided (NDDG criteria) into normal (n=382), impaired glucose tolerance (n=144) and OGTT-diagnosed diabetes (n=103); a group of 120 subjects could not be put in any category (non-diagnostic OGTT). There was a continuous increase in fasting and 2-h glucose, HbAlc, insulin, triglyceride and blood pressure accross the categories of glucose intolerance (from normal to diabetic, F test, $p<0.05$-0.001), which persisted after adjustment for age, BMI and waist/hip ratio (analysis of covariance). All subjects with PFH were younger (4 years on average) than those without PFH. Risk factors for CVD were not significantly affected by PFH: only in OGTT-diagnosed diabetes the presence of PFH was associated with higher values of 2-h glucose and HbAlc, and lower C-peptide but not insulin. Thus, we found a graded increase in levels of several risk factors for CVD with increasing glucose intolerance even at levels which are considered normal according to the WHO classification. OGTT-diagnosed diabetics with PFH seem to proceed to metabolic decompensation at an higher speed.

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INSULIN SENSITIVITY, GLUCOSE SENSITIVITY, B-CELL SECRETION AND INSULIN METABOLISM IN HUMAN OBESITY.

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In this study we compared insulin sensitivity, glucose sensitivity, B-cell secretion (as assessed by plasma C-peptide), and insulin metabolism during intravenous glucose tolerance test in sex- and age-matched obese and nonobese subjects. Glucose, insulin and C-peptide kinetics were studied by the "minimal model" technique. As compared to nonobese subjects, obese patients showed: a) higher fasting levels of plasma C-peptide (0.88 ± 0.10 vs 0.51 ± 0.06 pmol/ml, $p<0.01$) and, to a greater extent, plasma insulin (112 ± 21 vs 42 ± 7 pmol/l, $p<0.001$); b) a B-cell response to i.v. glucose similar in the first phase ($\phi 1$ = 64 ± 14 vs 57 ± 12 pmol/min x mg/dl) and only slightly higher in the second phase ($\phi 2$ = 50 ± 12 vs 39 ± 5 pmol/min x mg/dl, p=NS); c) lower values of either insulin sensitivity (S_i = 2.36 ± 0.29 vs 5.62 ± 0.87 min^{-1} x U/L, $p<0.001$) and glucose sensitivity (S_g = 0.011 ± 0.001 vs 0.022 ± 0.002 min^{-1}, $p<0.001$); d) a lower metabolism of insulin (INS_{FCR} = 0.045 ± 0.007 vs 0.072 ± 0.009 min^{-1}, $p<0.05$) but not of C-peptide ($C\text{-}PEP_{FCR}$ = 0.024 ± 0.001 vs 0.028 ± 0.002 min^{-1}). In the whole population, S_i, S_g and INS_{FCR} were inversely related to either total body fat content or waist/hip ratio, while $\phi 1$ only directly correlated with total body fat. These results suggest that human obesity is featured by an impairment of both insulin and glucose sensitivity, a reduced insulin metabolism but a normal B-cell response to i.v. glucose.

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SERUM INSULIN LEVELS AND GLUCOSE TOLERANCE AS PREDICTORS OF CARDIOVASCULAR RISK FACTORS.

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In a random sample of 708 men (M) and 762 women (W) with unknown diabetes, and based on WHO criteria, 4.1% of M and W were newly diagnosed as diabetics (DM), while 6.8% and 4.7% of M and W respectively, were found to have impaired glucose tolerance (IGT). Subjects with DM and IGT were older and had higher body mass index (BMI) values compared to normal subjects. Mean serum insulin (SI) levels (pmol/L) post challenge were lowest among normals (6.13 in M and 6.41 in W), intermediate among subjects with DM (12.4 in M and 8.92 in W), and highest among subjects with IGT (15.74 in M and 11.56 in W). Subjects with IGT showed the highest values of total cholesterol (TC), LDL-cholesterol and triglyceride (TG), and the lowest HDL-cholesterol (HDL-C), as compared to non-diabetic subjects or subjects with DM. In M, after controlling for age, ethnicity, BMI and smoking, SI was a significant positive predictor of TG, and a significant negative predictor of HDL-C. DM and IGT were not independently associated with these variables. A similar association with HDL-C was observed among W. However, in W, IGT and insulin levels were independently positively associated with TG levels after controlling for the above-mentioned confounders. These findings point to the possible underlying mechanisms by which glucose intolerance and insulin resistance may be involved in the etiology of cardiovascular disease.

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THE EFFECTS OF HYPERGLYCEMIA ON MYOCARDIAL BLOOD SUPPLY, FUNCTION AND METABOLISM

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The experiments on 32 anesthetized dogs were performed for the purporse of investigation of regional coronary hyperglycemia (H) effects on the heart. Catheterization and extracorporal autoperfusion of coronary artery (CA), catheterization and continuous dreinage of coronary sinus (CS) were used. H (21,8±3,87 mmol/l) was induced by the glucose (G) infusion (I) into extracorporal perfusion system by means of ultramicroinjector. Systemic arterial G level increased insignificantly during 2-4 min GI. H reduced CA perfusion pressure (-5,5±1,31 kPa), raised left ventricular dP/dt (+120,0±25,63 kPa/s), decreased O_2 CS blood saturation (-1,9±0,42 %), increased coronary artery-venous blood difference (AVD) by O_2 saturation (+9,8±4,06 %) and augmanted AVD by G from 0,4±0,17 to 7,2±2,04 mmol/l. Myocardial contractility and O_2 consumption enhancement coincided with the increase of negative $T_{b,m}$ waves (+0,7±0,10 mV and 0,9±0,11 mV, respectively) and the ST j, m segment depression (-0,4±0,05 mV). The ECG changes were not accompanied by the myocardial lactate release, index lactate/pyruvate reduced (20 %) and myocardial FFA consumption (0,2±0,01 mmol/l) markedly decreased or even ceased. Just after the GI we observed significant enhancement of heart adrenergic reactions. Beta-adrenergic blockade failed to eliminate the H effects on heart contractility and ECG. We suggest that coronary H can exxert direct action on myocardial function, blood supply and metabolism independently from systemic metabolic disturbances in diabetes mellitus.

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IMPAIRED MYOCARDIAL PERFUSION IN PATIENTS WITH HYPERINSULINAEMIA AND MICROVASCULAR ANGINA.

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Hyperinsulinaemia and elevated C-peptide concentrations have previously been demonstrated in patients with angina and normal coronary angiograms. It has been suggested that increased insulin concentrations may lead to coronary microvascular dysfunction in such patients. We have studied coronary flow in 9 patients with typical effort angina, abnormal exercise Thallium scans and entirely normal vessels at coronary angiography, with no evidence of coronary artery spasm. None were hypertensive or diabetic. None were taking drugs known to affect carbohydrate metabolism. Patients had a standard 75g oral glucose tolerance test. Mean fasting, peak and 2-hourly glucose values were respectively 4.6 (±0.34), 7.2 (±1.3) and 6.0 (±0.9) mmol/L. Median fasting, peak and 2-hourly insulin concetrations with ranges were 11.6 (9.1-25), 99.9 (65.4-492) and 87.0 (32.9-128) mU/L respectively. Mean fasting, peak and 2-hourly C-peptide concentrations were 0.74 (±0.24), 3.37 (±1.32) and 2.86 (±0.98) mU/L respectively. Myocardial flow was measured by the Xenon clearance techniques. Resting flows were within normal limits. By atrial pacing to heart rates similar to that achieved in exercise, chest pain occurred in all patients. Distribution volume fell in at least one coronary distribution in all patients and this was associated with reduced coronary artery flow by a mean 28.6 ± 9.8%, $p<0.05$. This group of patients with angina, normal coronary angiography and hyperinsulinaemia have reduced myocardial flow presumably at the microvascular level.

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INSULIN SENSITIVITY IN MICROALBUMINURIC TYPE 1 (INSULIN-DEPENDENT) DIABETIC PATIENTS.

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Type 1 (insulin-dependent) diabetic patients with microalbuminuria are at risk of cardiovascular and renal disease. The aim of this study was to assess insulin sensitivity and its relationship to other cardiovascular risk factors in these patients. Insulin sensitivity, expressed as whole body glucose disposal rate (by an euglycaemic hyperinsulinaemic clamp with plasma insulin level of 100μU/ml), plasma lipids and lipoproteins, 24 hr ambulatory blood pressure (ABP) and waist/hip ratio (WHR) were measured in 9 microalbuminuric(DM)(albumin excretion rate (AER) [geometirc mean (95% C.I.)49.1(47.5-50.7)] and 8 normoalbuminuric (DN)(AER 8.83(7.6-10.1)μg/min) patients. The 2 groups were similar in age (mean±S.D.)(40±10 vs 40±7 yr), duration of diabetes (23±7vs22±6 yr) and body mass index (26.6±2.7 vs 24.6±1.4 kg/m2). Peripheral insulin sensitivity was significantly reduced in the DM group (glucose disposal rate 6.1±1.6 vs 9.5±0.7 mg/kg/min, p<0.05) who also had higher night-timeABP(97.1±7.4 vs 85.9±5.7mmHg, p<0.01), total cholesterol (5.38±1.1 vs 4.26±0.6, p=0.02) and apoB (0.94±0.2 vs 0.61±0.3g/l, p=0.02). The difference in WHR was not significant: 0.86±0.05 vs 0.82±0.02. In the DM patients, insulin sensitivity was significantly positively correlated with AER (R=0.79,p=0.01). In conclusion reduced insulin sensitivity is a feature of Type 1 (insulin-dependent) diabetic patients with microalbuminuria and contributes with other factors to the increased risk for renal and cardiovascular disease in these patients.

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ANALYSIS OF CAROTID ATHEROSCLEROSIS AND SILENT CEREBRAL INFARCTION IN JAPANESE NIDDM PATIENTS

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[Aim and Methods] Silent cerebral infarctions and carotid atherosclerosis were investigated by T2 image in MRI (Signa 1.5 Tesla, GE) or B-mode ultrasound-scopy (7.5MHz, Aloka) in 67 Japanese NIDDM patients (age:59+/-9 yr., duration:10+/-8 yr., mean+/-SD) without any history and symptoms of cerebral infarction. Relationships between two lesions and clinical data such as FPG(fasting plasma glucose), serum lipids and diabetic complications were tried to examine.
[Results] In NIDDM, silent cerebral infarctions were found in 29.9% (20/67), and carotid atherosclerosis were found in 23.9% (16/67). Co-occurrence rate of both lesions in NIDDM was 6.0% (4/67). There were no significant relationship between two lesion and FPG or serum lipid levels. However, in NIDDM with silent cerebral infarction, mean ages (66+/-10 vs. 58+/-9yr., p<0.05) and frequency of hypertension (63 vs. 28%, p<0.05) were significantly higher than without cerebral infarction.
[Conclusions] In NIDDM incidence of silent cerebral infarctions and carotid atherosclerosis were higher than expected. From this study, it was suggested that the clinical pathogenesis of these two lesions might be different.

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LOWER EXTREMITY ARTERIAL OCCLUSIVE DISEASE IN RELATION TO GLYCEMIC LEVEL IN A CAUCASIAN POPULATION

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The aim of this study was to evaluate the relationship between parameters of glucose metabolism and the occurrence of lower extremity arterial occlusive disease (LEAOD). We studied an age/gender stratified random sample (N=628) from a Caucasian population, aged 50-76 years (the Hoorn Study). Based on two Oral Glucose Tolerance Tests and WHO criteria, participants were classified into five categories of glucose tolerance, normal/normal (N=236), normal/IGT (N=94), IGT/IGT (N=89), IGT/DM (N=50), DM/DM (N=89), and the category of previously diagnosed DM (N=67). LEAOD was defined as an ankle/arm index <0.90 and/or a monophasic doppler-flow-velocity curve in any of the lower extremity arteries. In the glucose tolerance categories the crude prevalence of LEAOD was 19.9%, 14.9%, 21.3%, 18.0%, 31.5% and 41.8%, respectively. Controlling for age, gender, blood pressure, BMI, W/H-ratio and serum lipids, multiple logistic regression analysis showed, in four different models, significant contributions of HbA1c (p<0.0001), fasting blood glucose (p=0.0005) and category of glucose tolerance (p<0.0005) to the risk of LEAOD, whereas no such contribution of fructosamine was demonstrated. In conclusion, these cross-sectional data indicate that the glycemic level is positively associated with the risk for LEAOD in a Caucasian population.

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EFFECTS OF TYPE 1 DIABETES ON CARDIAC SIZE AND FUNCTION - INFORMATION FROM A STUDY OF IDENTICAL TWINS

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To define the nature of diabetes related cardiac dysfunction, we studied 38 young (mean age 25, range 10-37 years) normotensive identical twins discordant for Type 1 diabetes (mean duration 14 years) by 2D-Doppler echo. End-diastolic volume (EDV), end-systolic volume (ESV) and left ventricular mass (LVM) were calculated from echo images and corrected for body surface area. Diastolic function was measured as; 1) ratio of transmitral blood flow velocities (E/A); 2) time-velocity integrals of early (E-integral) and late (A-integral) LV filling; 3) diastolic filling contributed by atrial systole (A%). Diabetic, compared with non-diabetic twins, had lower EDV and ESV (72 ± 20 vs 76 ± 19 ml/m^2, p<0.001 and 31 ± 8 vs 36 ± 14 ml/m^2, p<0.02) and lower E/A ratio (1.4 ± 0.3 vs 1.6 ± 0.3, p<0.0005) but similar LVM and stroke volume. Despite similar total time-velocity integrals of diastolic filling, diabetic twins had higher A-integral (55 ± 18 vs 47 ± 10, p 0.005) and higher A%. (32 ± 5 vs 29 ± 5%, p<0.005). We conclude that these Type 1 diabetics have decreased LV chamber volume and subclinical diastolic dysfunction but retain normal systolic function, indicating a specific diabetic heart disease.

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CORONARY ARTERIOSCLEROTIC LESIONS IN TYPE I DIABETIC PATIENTS WITH END-STAGE RENAL DISEASE

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Mortality in patients with diabetes mellitus and end-stage renal failure (ESRF) is dominated by ischemic heart disease. Therefore we analyzed the extent and magnitude of coronary arteriosclerotic lesions in these patients. Coronary cineangiograms of 43 consecutive type I diabetic patients who were eligible for renal transplantation were analyzed quantitatively with automated edge detection techniques. Average age was 38 years (range:23-57); average duration of diabetes was 23 years (range:14-39). Angina was present in 4 patients; exercise tests revealed no evidence for ischemic heart disease in the remaining 39 patients. No lesions with a percent diameter stenosis (%-D) > 20% were found in 17 patients; 4 patients had complete obstruction of the right coronary artery. Therefore the results of 22 angiograms were further analyzed. This revealed a total of 61 obstructions, equally distributed over the three major branches. The average stenotic flow reserve, a measure for functional significance of obstruction, was decreased at 3.97 (range: 1.16-4.96). Average %-D was 44.2% (range: 20.2-74.4). Average percent cross-sectional area stenosis (%-A), which correlates well with the visually determined degree of the stenosis, was 67.1% (range: 36.3-93.4); Patients with angina had significantly larger %-A than those without: 82.9% vs 64.8% ($p=0.02$). However out of 18 patients without angina still 13 had at least 1 obstruction with %-A $\geq$ 70% . We conclude that the majority of type I diabetic patients with ESRF although asymptomatic, have developed at young age severe and multiple obstructions in the coronary arteries. Our results stress the importance of coronary angiography before renal transplantation in diabetic patients.

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THE RISK OF ATHEROSCLEROTIC LESIONS OF THE CAROTID ARTERIES IN YOUNG TYPE 1 DIABETIC PATIENTS

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Alterations of the vessel wall of carotid arteries (CA) can be detected by high resolution ultrasound technique. We studied the risk of atherosclerotic CA disease in young Type 1 diabetic patients (P) aged up to 40 years. We examined the CA of 90 P and looked for plaques and for thickening of the vessel wall by measuring of the reflection of the inner wall (R). 19 P with diabetes duration less than one year served as a control group (CG), the other 71 P were distributed to three groups as follows: A: normal R ($\leq$0.6 mm, equal with CG); B: broadened R (0.7-1.0 mm); C: plaques. Alterations of the vessel wall of CA occured in 24% of our P with diabetes duration > 1 year. The percentage of the following late complications or accompanying diseases was significantly higher in group B than in group A: nephropathy (stad. III and IV) 67% vs. 13% ($p<0.001$); hypertension (>140/90 mmHg) 33% vs. 6% ($p<0.01$); cheiropathy (scleroderma-like syndrome) 67% vs. 28% ($p<0.05$). There was no difference concerning hypercholesterolaemia (>200 mg/dl, 11% vs. 20%). In group C however hypercholesterolaemia played an important role (75% vs. 20% in A, $p<0.01$); all patients had a nephropathy (100% vs. 13%, $p<0.001$) and a half showed hypertension (50% vs. 6%, $p<0.001$); cheiropathy 63% vs. 28% ($p<0.05$). Nephropathy, hypertension, cheiropathy and hypercholesterolaemia are important indicators of early atherosclerotic lesions of CA in young Type 1 diabetic patients.

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ABNORMALITIES IN EXTRAHEPATIC INSULIN SENSITIVITY ARE A PECULIAR FEATURE OF NON INSULIN DEPENDENT DIABETIC PATIENTS WITH BUT NOT WITHOUT HYPERTENSION AND/OR MICROALBUMINURIA.

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Insulin resistance could be one mechanism linking non insulin dependent diabetes to hypertension and to cardiovascular mortality. Microalbuminuria is a further independent risk factor of cardiovascular mortality and hypertension. Little information is available on the relationship between microalbuminuria and insulin action. Our aim was to investigate the relationships among blood pressure levels, microalbuminuria and insulin resistance in non insulin dependent diabetes. Fortyfive non-insulin-dependent diabetic patients were located in four groups: Group 1) blood pressure levels lower than 140 and 85 mmHg and albumin excretion rate lower than 15 µg/min; Group 2) blood pressure levels higher than 145 and 90 mmHg and albumin excretion rate lower than 20 µg/min; Group 3) microalbuminuria and normal blood pressure levels and Group 4) microalbuminuria and hypertension. Eight normal subjects served as controls. Patients and controls underwent euglycemic multiple step insulin clamp. Results: Whole body glucose utilization, mainly an index of extrahepatic insulin action, was lower at all insulin infusion steps in Group 4) 3) and 2) patients than in Group 1) patients and controls. (Group 4) vs 3) vs 2) vs 1) vs controls: 5.70±0.56 vs 6.64±0.46 vs 5.72±0.70 vs 8.73±1.15 vs 9.35±1.19 $mg \cdot Kg^{-1} \cdot min^{-1}$ at 1500 µU/ml insulin concentrations). On the contrary hepatic glucose output (an index of insulin action in the liver) was less inhibited in all diabetics than in controls. Conclusions: 1) extrahepatic insulin sensitivity is normal in non insulin dependent diabetics without but not in those with hypertension and microalbuminuria, 2) hepatic insulin sensitivity is reduced in all non insulin dependent diabetics.

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DETERMINANTS OF INSULIN RESISTANCE AND THEIR RELATION TO LIPOPROTEINS IN TYPE 2 DIABETES.

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Determinants of insulin sensitivity and their relation to lipids, lipoproteins and blood pressure were investigated in 46 patients with type 2 diabetes (26F/20M, age 59.1±6.1 yrs, HbA1c 7.4±1.2%). To study the determinants of insulin-mediated glucose uptake (M-value), measured with the euglycemic hyperinsulinemic clamp, multiple linear regression was applied. Only sex, percent bodyfat, waist to hip ratio and resting energy expenditure (REE/kgLBM) emerged as significant independent factors, with a multiple r2 for the model of 44.1%. Age, HbA1c, VO2max, smoking, alcohol consumption and dietary habits did not contribute significantly. Furthermore, the M-value, the fasting insulin level and the above mentioned variables were included in multiple regression models predicting average levels of lipids, lipoproteins and blood pressure. The M-value was independently and inversely associated with triglyceride (TG) and VLDL-cholesterol concentration (multiple r2 of the models were 48.8% and 32.1%, respectively) and positively with HDL2-cholesterol and apolipoprotein-A1 (multiple r2 of the models were 52.9% and 59.4%, respectively), but not with blood pressure level. Moreover, fasting insulin contributed directly, independent of the M-value, to the variation of TG, while not to the other lipoproteins. In conclusion, the results implicate that insulin per se affects TG levels, while HDL2-cholesterol levels are predominantly influenced by the degree of insulin sensitivity.

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ISOLATED HYPERCHOLESTEROLEMIA IS NOT PART OF THE INSULIN RESISTANCE SYNDROME.

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Hyperlipidemia was described as part of the syndrome of insulin resistance. To evaluate insulin action in isolated hypercholesterolaemia a 180min hyperinsulinemic (420 pM) euglycemic (4.7 mM) clamp was performed in 5 (BMI=26±1 kg/m^2; age=47±4 yrs) hypercholesterolaemic patients (total Ch=7.2±0.3; HDL-Ch=1.3±0.05; LDL-Ch=4.7±0.3; Tg=2.4±0.2; VLDL-Tg=1.1±0.1 mM) with normal glucose tolerance and 5 control subjects (BMI=27±1; age=48±4; tot-Ch=5.0±0.1; HDL-Ch=1.0±0.08; LDL-Ch=3.2±0.3; Tg=1.5±0.2; VLDL-Tg=0.9±0.2 mM). Studies were performed with a hot (3-^{3}H) glucose infusion, to ensure steady state plasma glucose specific activity, and indirect calorimetry. Basal hepatic glucose production (HGP) and glucose utilization were similar in hypercholesterolaemics (10.4±1.2) and controls (10.1±0.7 µmol/min·kg). No difference was apparent in the basal rates of glycolysis (^3H_2O generation=8.7±1.2 vs 9.6±1.5), glycogen deposition (glucose disposal - glycolysis= 1.7±1.2 vs 0.5±1.5), glucose oxidation (8.5±2.0 vs 6.7±1.6), plasma FFA levels (3.4±0.7 vs 4.1±0.4 g/L), and lipid oxidation (2.6±0.8 vs 3.2±0.5 µmol/min·kg). In response to hyperinsulinemia, glucose disposal increased similarly in both groups (34.5±4.1 vs 33.4±3.1 µmol/min·kg). Also similar was the increment in the glycolytic flux (18.6±3.6 vs 15.3±3.8), glycogen deposition (15.9±3.5 vs 18.1±3.8), glucose oxidation (14.4±2.3 vs 14.4±2.9), and the reduction of FFA (0.6±0.1 vs 0.5±0.1) and lipid oxidation (0.8±0.6 vs 0.7±0.3). HGP suppression tended to be lower in hypercholesterolaemics (4.1±1.5) than in controls (1.6±1.4 µmol/min·kg) without reaching statistical significance (p=0.12). In conclusion, isolated hypercholesterolemia is not associated with altered insulin action. Lipid metabolism disturbances other than hypercholesterolaemia should be included in the syndrome of insulin resistance.

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ISOLATED HYPERCHOLESTEROLEMIA IS NOT PART OF THE INSULIN RESISTANCE SYNDROME (SYNDROME X).

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Recently it has been suggested that insulin resistance is a multifaceted syndrome that can manifest itself with different phenotypes, including obesity, glucose intolerance or type 2 (non-insulin-dependent) diabetes, hypertension and dyslipidemia. While it is well known that subjects with isolated familiar hypertriglyceridemia are less insulin sensitive than normal subjects, it has been just postulated, but not demonstrated, that subjects with isolated familiar hypercholesterolemia are insulin resistant. Aim of the present study was to evaluate whether subjects with isolated familiar hypercholesterolemia have or have not a diminished insulin sensitivity. 10 premenopausal nonobese, nondiabetic, not hypertensive women with familiar hypercholesterolemia and 10 age (38 ± 4 vs 35 ± 2) and BMI (22 ± 1 vs 24 ± 1) matched healthy women were studied. In the former hypolipidemic medications were discontinued at least 2 weeks before the study was performed. Pre-treatment serum cholesterol was at least 300 mg/dl. The study consisted in a 4-h euglycemic insulin clamp (20 mU/m^2 surface area), associated with 3-[^{3}H]-glucose infusion and indirect calorimetry. During insulin clamp the rates of glucose disposal (mg/min.Kg BW; mean ± SE) were not significantly different in hypercholesterolemic and normal women (total: 6.12 ± 0.48 vs 5.54 ± 0.69; oxidative: 3.12 ± 0.18 vs 2.80 ± 0.12; non-oxidative: 3.00 ± 0.44 vs 2.74 ± 0.65, respectively). These results suggest that isolated familiar hypercholesterolemia is not part of the insulin resistance syndrome.

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EFFECT OF GENDER ON INSULIN RESISTANCE ASSOCIATED WITH AGING

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To examine whether age-related changes in energy metabolism are influenced by gender 20 females (50±4 years, BMI 23.4±0.7 kg/m^2, weight 63.5±2.1 kg, fat percentage 33.4±2.1 %) and 20 males (48±3 years, BMI 24.1±0.5 kg/m^2, weight 78.4±2.1 kg, fat percentage 25.7±1.7 %) varying in age from 21 to 80 years were studied with euglycemic insulin clamp in combination with indirect calorimetry and infusion of ^{3}H-3-glucose. Lean body mass (LBM) was measured with the tritiated water technique. Fat percentage correlated with age only in males (r=0.60;p<0.05). Insulin-stimulated total glucose disposal and storage were similar in females (53.1±2.7 and 28.4±2.5 umol/kgLBM.min) and males (47.6±3.2 and 26.1±2.3 umol/kgLBM.min). Glucose storage expressed per kg body weight (r=-0.64;p<0.01) or per kg LBM (r=-0.46;p<0.05) correlated inversely with age only in males, but not in females (r=0.27;p=NS). In fact, the slopes relating glucose storage and age were significantly different in males and females (p<0.05). Basal metabolic rate expressed per kg body weight did not differ between females (63.7±2.2 (J/kg.min) and males (65.8±2.1 J/kg.min) and decreased with age in both sexes (r=-0.49;p<0.05 in females, r=-0.60;p<0.01 in males). No correlation with age was observed when data were expressed per kg LBM. These data clearly demonstrate different effects of age on energy metabolism in females and males.

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INSULIN RESISTANCE, HYPERTENSION AND NEUROPSYCHOLOGICAL PERFORMANCE IN THE ELDERLY

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Essential hypertension is known to be associated with an increased risk for cerebrovascular disease but studies indicating that cognitive function is disturbed in hypertensive subjects are scarce. Therefore, cognitive function was examined in a random sample of 978 elderly subjects of Kuopio town, eastern Finland. From 772 non-diabetic subjects altogether 396 were hypertensive and 376 were normotensive. The following neuropsychological screening tests were used to measure cognitive function: the Mini-Mental State Examination Test(MMSE), Heaton Visual Reproduction Test (HVRT), Trail Making Test (TMT), Buschke Selective Reminding Test (BSR) and Verbal Fluency Test (VFT). The hypertensive group scored more poorly than did the normotensive group in most screening tests , especially in the Trail Making Test (part B: 203.7± 5.2 vs. 188.6± 5.1, p<0.05; part C: 188.6± 4.3 vs. 171.2± 4.2, p<0.01) which evaluates ability to solve problems, and Verbal Fluency Test (P-words 11.4± 0.3 vs. 10.6± 0.3, p<0.05) which assesses long-time semantic memory. Within the hypertensive group, the subjects with high fasting plasma insulin (> 10.1 mU/l) systemically scored more poorly than did the non-hyperinsulinaemic (≤ 10.1 mU/l) hypertensive subjects, especially in the Verbal Fluency Test and Trail Making Test. This difference in the Verbal Fluency and Trail Making Tests between these three groups (normotensive, hypertensive normoinsulinaemic and hypertensive hyperinsulinaemic) remained statistically significant even after adjustment by ANCOVA for fasting plasma glucose, age, sex and education (TMT, part A, p= 0.009 ; VFT, p= 0.009). Thus, hyperinsulinaemia/insulin resistance seems to identify a subgroup of hypertensive subjects who have a particularly poor performance in neuropsychological screening tests.

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ABNORMAL CALCIUM HOMEOSTASIS IS ASSOCIATED WITH INSULIN RESISTANCE AND GLUCOSE INTOLERANCE.

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Glucose tolerance, insulin resistance and beta-cell function were studied in 19 non-obese subjects with primary hyperparathyroidism (PH) without hypertension, diabetes or family history of diabetes and 10 age and BMI matched controls. Plasma glucose and insulin were measured during a 1 hour continuous glucose infusion (5mg/kg ideal body weight/min) and insulin resistance and beta-cell function were derived by mathematical modelling. Median ionised calcium was higher in PH [1.49 (interquartile range 1.42-1.6) vs 1.21 (1.15-1.24) mmol/l, p<0.0001]. Parathyroid hormone (PTH) was 60.0 (41-80) pg/ml in PH and 20.0 (16.5-25.7) pg/ml in controls (p<0.0001). Achieved glucose at the end of the infusion was higher in PH [8.9 (8.1-9.8) mmol/l] than in controls [8.0 (7.2-9) mmol/l, p<0.05], and 7 PH subjects had impaired glucose tolerance. Glucose stimulated insulin was 23 (18-35) mU/l in PH compared to 14 (11-18) mU/l in controls (p<0.001). PH subjects were more insulin resistant [2.8 (1.7-3.4)] than controls [1.2 (0.9-1.7, p<0.001)] and had higher beta-cell function [160% (100-209%) vs 81% (62-100%), p<0.001]. Abnormal calcium homeostasis in PH is associated with peripheral insulin resistance even in the absence of hypertension and obesity. These subjects may be at higher risk for diabetes and coronary artery disease.

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ANTIGLUCOCORTICOID TREATMENT AMELIORATES HIGH-FAT FEEDING INDUCED INSULIN RESISTANCE IN RATS

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Hyperresponsivity of adrenal glucocorticoids to stress has been shown in genetic models of insulin resistance and obesity. Conversely adrenalectomy or treatment with antiglucocorticoids ameliorate many of the metabolic abnormalities. High-fat diets also induce insulin resistance and hyperresponsivity to stress. We aimed to determine whether the antiprogestin/antiglucocorticoid RU38486 prevented the insulin resistance induced by high-fat feeding. Four groups of adult, male Wistar rats were fed for 4 weeks on high-starch (70% of calories as starch) or high-fat diets (59% of calories as fat) with or without 30 mg/kg/day RU38486 in the diet. Insulin action was determined by euglycemic clamp at an insulin infusion rate of 0.25 U/kg/hr. Whole-body insulin action was defined as the glucose infusion rate (GIR) to maintain euglycemia over the last hour of the clamp. RU38486 did not affect food intake or body weight. GIR in starch-fed controls was 32.9±2.3 mg/kg/min and reduced to 12.3±2.2 in fat-fed controls. RU38486 did not influence GIR in the starch-fed animals (33.8±0.7 mg/kg/min) but significantly elevated GIR in the fat-fed group (22.2±2.6 mg/kg/min, p<0.01 compared to fat-fed controls). In conclusion, the results support the hypothesis that adrenocortical hyperactivity plays an important role in development of insulin resistance with high-fat feeding.

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POST-TRANSPLANTATION INSULIN RESISTANCE ASSOCIATED WITH CYCLOSPORIN A

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Post-transplantation diabetes mellitus is a reported complication of the immunosuppressive agent cyclosporin A (CsA). However, the contribution of concurrent corticosteroid therapy to this phenomenon remains unclear. In this study extended (300 min) 75 g oral glucose tolerance tests (OGTTs) were performed 7 $\pm$ 1 months (mean $\pm$ SEM) post-operatively in 10 clinically stable CsA-treated liver transplant recipients. Eight patients were also receiving azathioprine but prednisolone was completely withdrawn at least 4 weeks prior to study in each case. Ten healthy volunteers matched (CsA vs. controls) for age (46 $\pm$ 8 vs.49 $\pm$ 3 yr, p>0.1) and body mass index (23 $\pm$ 3 vs. 24 $\pm$ 1 kg/m^2, p>0.1) served as controls. Venous whole blood glucose concentrations at 120 min were significantly higher in the CsA patients (6.6 $\pm$ 0.5 vs. 5.2 $\pm$ 0.2 mmol/l, p<0.05) being diagnostic of impaired glucose tolerance (WHO, 1985) in 3/10 patients. Plasma immunoreactive insulin concentrations during the OGTTs were also significantly elevated (F=5.9, p<0.05) in the CsA patients. The combination of glucose intolerance in concert with hyperinsulinaemia suggests that immunosuppression with CsA is associated with post-transplantation insulin resistance.

PS 6
Signal Transduction

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ALTERED SECRETORY RESPOSES OF INS-1 AND RINm5F CELLS ARE ASSOCIATED WITH DIFFERENCES IN $InsP_3$ RECEPTOR FUNCTION.

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Mobilization of Ca^{2+} from inositol trisphosphate ($InsP_3$) - sensitive stores may potentiate insulin secretion partly by activating intramitochondrial Ca^{2+}-sensitive dehydrogenases. This hypothesis was examined by measuring (i) mitochondrial Ca^{2+} uptake and (ii) $InsP_3$-induced Ca^{2+} mobilization in electropermeabilized preparations of two insulin secreting cell lines: INS-1, which respond to glucose but poorly to Ca^{2+} mobilizing agonists, and RINm5f, where this situation is reversed. Mitochondrial Ca^{2+}uptake activity (s^{-1} 10^6cell^{-1}) was 60% higher in INS-1 (0.016 ± 0.001) than RINm5f cells (0.010 ± 0.001). However, the mitochondrial content (succinate dehydrogenase activity, pmol min^{-1} 10^6 $cell^{-1}$, n=3) was 50% higher in INS-1 (412 ± 49) than RINm5f cells (278 ± 28, $p < 0.01$). These findings could underlie the retained glucose sensitivity of INS-1 cells, but indicate that defective Ca^{2+} uptake by individual mitochondria in these cells does not explain the attenuated response to Ca^{2+} mobilizing agonists. By contrast, saturating $InsP_3$ released only 13.9 ± 3.4% (n=3) of the Ca^{2+} released from a nonmitochondrial store by the Ca^{2+}-ATPase inhibitor, thapsigargin, in INS-1 cells. This value was 69.8 ± 5.1% for RINm5f cells. In intact fura-2-loaded INS-1 cells vasopressin induced a peak rise in cytosolic Ca^{2+} which was 23.3 ± 1.7% (n=3) of the thapsigargin effect, compared with 41.7 ± 2.0% for RINm5f cells. Thus the poor secretory response of INS-1 cells to Ca^{2+} mobilizing agonists may be due to the partial absence of functional $InsP_3$ receptors.

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INSULIN BUT NOT IGFs PROMOTES THE HYDROLYSIS OF A GLYCOSYL-PHOSPHATIDYLINOSITOL IN RAT ASTROCYTES

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An inositol phospho-glycan (IPG) -the polar head group of a glycosyl-phosphatidylinositol (GPI)- is considered as a mediator of insulin action in peripheral tissues. In order to gain insight into the mechanism of insulin signal transduction on nervous system, the relationships between insulin, insulin receptors, GPI and IPG in cultured astrocytes were studied. Insulin receptors were partially purified by affinity chromatography and its α-subunit detected by SDS-PAGE after [^{125}I]-Insulin binding and chemical cross-linking. On autoradiography, a single band (Mr≈130 kDa) was identified, which was inhibited by insulin, IGF-II and IGF-I (IC_{50} 3 nmol/l, 25 nmol/l and >0.1 µmol/l, respectively). When cells, prelabelled at steady state with [^{3}H]-galactose, [^{3}H]-glucosamine or [^{3}H]-myristate, were exposed to 10 nmol/l insulin, a 50% decrease in GPI and 50% increase in dimyristoyl glycerol (DG) contents were observed into 1-2 min. By contrast, neither IGF-I and IGF-II (4-6 nmol/l) modified GPI level along a 20 min time course. However, IGF-II but not IGF-I rose DG content. These findings suggest that insulin may activate a GPI specific phospholipase C through its own receptor in cultured astrocytes and that insulin, IGF-I and IGF-II are probably using independent signal transduction pathways.

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ENHANCED Ca^{2+} INFLUX HYPERPOLARIZES THE ß-CELL MEMBRANE BY INACTIVATING A Ca^{2+} CONDUCTANCE

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Glucose depolarizes the ß-cell membrane and induces synchronous oscillations of membrane potential and intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$). $[Ca^{2+}]_i$ changes due to Ca^{2+} influx may, in turn, modulate the membrane potential, but the ionic mechanisms underlying this interaction are not currently understood. We have now investigated the effects of ionomycin and high extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) upon glucose-induced electrical activity and $[Ca^{2+}]_i$ under conditions where the ATP-dependent K^+ channel is blocked (100 µM tolbutamide or 4 µM glibenclamide). Membrane potentials and $[Ca^{2+}]_i$ were recorded from single mouse islets using high-resistance microelectrodes and a FURA-2 fluorescence ratio microscopy technique. Raising $[Ca^{2+}]_o$ from 2.6 to 10.2 or 12.8 mM turned continuous electrical activity into an oscillatory pattern. Ionomycin (10 µM) typically induced sustained hyperpolarizations of the ß-cell membrane. The ionomycin- and high $[Ca^{2+}]_o$-induced hyperpolarizations were blocked by nifedipine (10 µM), but were insensitive to charybdotoxin (40 nM) and tetraethylammonium (2 mM). High $[Ca^{2+}]_o$ induced large $[Ca^{2+}]_i$ transients followed by fast oscillations, the latter frequently overshooting the $[Ca^{2+}]_i$ recorded in presence of tolbutamide. Ionomycin evoked a $[Ca^{2+}]_i$ rise followed by a decay towards a plateau. The data indicate that the $[Ca^{2+}]_i$ rises due to stimulation of Ca^{2+} influx cause membrane hyperpolarization by inactivating an L-type Ca^{2+} conductance.

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THE INFLUENCE OF CULTURE DURATION ON B CELL CYTOPLASMIC Ca^{2+} AND INSULIN RELEASE

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It is unclear why the oscillations of cytoplasmic Ca^{2+} (Ca^{2+}_i) occurring in glucose–stimulated B cells have been found to be highly variable. In this study, Ca^{2+}_i was measured in whole mouse islets loaded with the Ca^{2+} indicator fura–2 after 1–4 days of culture in RPMI medium containing 11 mmol/l glucose. Insulin release was measured in parallel experiments. After 1 day, stimulation of the islets with 15 mmol/l glucose induced a biphasic rise in Ca^{2+}_i, with a long initial peak and rapid oscillations (2–3/min) in the steady state. The initial increase was little affected by the duration of culture but the oscillations became progressively longer and slower, and eventually disappeared after 4 days, Ca^{2+}_i remaining elevated at a higher average level than after 1 day. Stimulation by arginine, in the presence of glucose, also caused a slightly larger rise in Ca^{2+}_i after 4 days. The first phase of glucose–induced insulin release was not affected by the duration of culture but the second phase was larger after 4 days, as was also the response to arginine. In conclusion, the characteristics of the increase in B cell Ca^{2+}_i induced by glucose change with the duration of islet culture. A higher average Ca^{2+}_i is accompanied by a larger secretory response.

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COMPLEX EFFECTS OF ACETYLCHOLINE ON CYTOPLASMIC Ca^{2+} AND Na^+ CONCENTRATIONS IN MOUSE B CELLS

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The effects of acetylcholine (ACh) on the concentrations of cytosolic Ca^{2+} (Ca^{2+}_i) and Na^+ (Na^+_i) were studied in whole islets and single B cells loaded with the fluorescent probes fura-2 and SBFI respectively. In the presence of 15 mmol/l glucose, 0.1–1 µmol/l ACh caused a sustained rise in Ca^{2+}_i, whereas 10–100 µmol/l ACh caused a large initial increase followed by a progressive decrease. When Ca^{2+} influx was prevented (no external Ca^{2+}, D600 or diazoxide), ACh triggered a concentration-dependent (1–100 µmol/l) transient peak of Ca^{2+}_i followed by a small sustained increase above basal levels. The initial peak was decreased after pretreatment with thapsigargin, an inhibitor of the Ca^{2+}–ATPase of the endoplasmic reticulum. The previous proposal that muscarinic agonists depolarize B cells by increasing membrane Na^+ conductance was supported by the observation that ACh increased Na^+_i in B cells, at least in the presence of extracellular Na^+. In a Na^+–free medium, ACh affected Ca^{2+}_i in a similar way as when Ca^{2+} influx was prevented. Atropine blocked all ACh effects. In conclusion, ACh mobilizes Ca^{2+} from the endoplasmic reticulum and stimulates Ca^{2+} influx through voltage-dependent Ca^{2+} channels activated by a Na^+-dependent depolarization. The first effect is directly concentration-dependent, but the second one paradoxically decreases at high ACh concentrations.

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REGULATION OF SERINE/THREONINE PROTEIN PHOSPHATASES IN RINm5F INSULINOMA CELLS.

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Reversible protein phosphorylation is an important mechanism for control of hormone secretion. Whereas stimulation of phosphorylation by protein kinases is relatively well characterized, considerably less is known about protein phosphatases that regulate dephosphorylation. Most protein dephosphorylation reactions are catalyzed by type 1 and 2A serine/threonine phosphatases (PPases). We studied PPase regulation by various second messengers in RINm5F insulinoma cells. Addition of prostaglandins, cAMP or cGMP to cell homogenates failed to affect PPase activity. ATP caused 50 % inhibition of PPase-1 activity at 1 mM and PPase-2A at 0.1 mM, while ADP was less potent and AMP and adenosine were inactive. Of inositol polyphosphates, IP_6 produced 50 % inhibition of PPase-1 activity at 6 µM and PPase-2A at 2 µM. Ca^{2+} and TPA slightly elevated PPase-2A activity, while Ca^{2+} suppressed PPase-1. Polyamines suppressed PPase-1 (spermine>spermidine>putrescine), while having no consistent effects on PPase-2A. All differences are $P<0.05$. When PPases were assayed subsequent to addition of TPA or forskolin + IBMX to intact cells, no changes were detected. Possible effects of other secretagogues are being investigated. We conclude that RINm5F cell PPases are regulated *in vitro* by certain intracellular second messengers and suggest that PPases may be as important as protein kinases in regulation of stimulus-secretion coupling.

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mRNA OF INSULIN RECEPTOR AND INSULIN RECEPTOR SUBSTRATE IN RODENT TISSUES

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Receptor and post-receptor defects have been suggested to impair signal transduction and thus to cause insulin resistance. Splicing of insulin receptor (InsR) transcripts has been found to be altered in skeletal muscle of type 2 diabetics. Factors in the insulin signaling pathway are likely to be involved, too. We studied the alternative splicing of InsR transcripts and the mRNA levels of InsR and insulin receptor substrate (IRS-1) in mice (ob/ob, db/db, NZO and normal NMRI) and rats (normal and STZ-Wistar and fa/fa). RNA was analysed by Northern blots and polymerase chain reactions. The tissue-specific splice pattern of the InsR mRNA in mouse and rat is similar to man. However, there is a difference between these patterns in mouse and rat heart, showing a muscle-type pattern with predominant $Ex11^-$ and a mixed type, respectively. In contrast to findings in type 2 diabetics, there are no changes in splice patterns of InsR mRNA in the animal models. The ratio of InsR to IRS-1 mRNA is increased in skeletal muscle of STZ-diabetic rats. IRS-1 expression is tissue-specific in the rat with highest levels in skeletal muscle and kidney.

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PROTEIN KINASE C MODULATES GLUCOSE-INDUCED OSCILLATIONS IN CYTOPLASMIC FREE CALCIUM IN THE PANCREATIC B-CELL.

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The role of Protein Kinase C (PKC) in the regulation of glucose-induced oscillations in cytoplasmic free Ca^{2+}-concentration, $[Ca^{2+}]_i$, was investigated in mouse pancreatic B-cells. $[Ca^{2+}]_i$ was measured in small aggregates of cells in a microscopic system, using the fluorescent Ca^{2+}-indicator fura-2. At intermediate glucose concentrations (7-12 mmol/l), up to 40% of the B-cells responded with slow (2-5 minutes) oscillations in $[Ca^{2+}]_i$. Acute stimulation of PKC inhibited these oscillations. In PKC-depleted cells, glucose induced transients in $[Ca^{2+}]_i$ lasting approximately 10 seconds. $[Ca^{2+}]_i$-transients did not occur in the absence of extracellular Ca^{2+}. Typically, transients were preceded by a slow increase in $[Ca^{2+}]_i$ and the $[Ca^{2+}]_i$ immediately after a transient was lower than just before. The lower post-transient $[Ca^{2+}]_i$ possibly reflects attenuated influx of Ca^{2+} due to repolarization-induced closure of voltage-gated Ca^{2+}-channels. This repolarization may be explained by activation of Ca^{2+}-dependent K^+-channels accounted for by intracellular mobilization of Ca^{2+}. The inhibitory effect of PKC on glucose-induced oscillations in $[Ca^{2+}]_i$ is most likely explained by both activation of the plasma-membrane Ca^{2+}-pump and inhibition of the phospholipase C-system. The rapid Ca^{2+}-transients in PKC-depleted cells probably reflect withdrawal of such mechanisms. These data emphasize the modulatory role of PKC in the regulation of B-cell $[Ca^{2+}]_i$-oscillations.

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EFFECT OF PROTEIN KINASE C ON THE PLASMA MEMBRANE CALCIUM PUMP IN PURIFIED BETA CELL.

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The activity of the plasma membrane calcium pump is of critical importance to the maintenance of cellular Ca^{2+} homeostasis. It has been suggested that activation of protein kinase C (PKC) stimulates Ca^{2+} efflux from beta cells. Using inside-out vesicles (IOV) prepared from a purified plasma membrane fraction of a glucose-responsive insulinoma, the effect of PKC activation on the Ca-pump activity was studied. Purified beta cells were incubated at 37°C in Hank's buffer containing 5.5 mM glucose for 10 min in the presence or absence of 100 nM TPA. The cells were washed, homogenized, and purified plasma membranes were prepared using sucrose gradients. ATP-dependent $^{45}Ca^{2+}$-uptake into IOV was measured using a rapid filtration method. The $^{45}Ca^{2+}$ uptake in the presence of TPA at varying Ca^{2+} concentrations had a Km for Ca^{2+} of 79.0 $\pm$ 19.1 nM, and the maximal velocity was 1.68 $\pm$ 0.43 nmol/min*mg protein. In the absence of TPA , the Km for Ca^{2+} was 71.3 $\pm$ 16.5 nM, and the maximal velocity was 1.59 $\pm$ 0.39 nmol/min*mg protein (n=6). It is concluded that PKC activation does not seem to regulate Ca^{2+}-pump activity directly.

PS 7
Metabolism In Vitro

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EFFECT OF SULFONYLUREAS ON GLUCOSE UPTAKE BY SKELETAL MUSCLE

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It has been referred that sulfonylureas enhance the action of insulin on peripheral tissues. The aim of the study was to know whether glicazide, a second-generation sulfonylurea, has a direct effect on glucose uptake by skeletal muscle, at concentrations similar to those producing a hypoglycemic effect in the intact rat. Hindquarter preparations from wistar rats (200 g) were perfused with Krebs-Henseleit medium, pH 7.4, containing 5.5 mM glucose, 0.15 mM pyruvate, 4% BSA and 30% washed bovine erithrocytes. After a period of 30 min (basal glucose uptake) glicazide (50-1000 μg/ml) or insulin (10^{-9} and 10^{-7}M) were added to the perfusion medium. Samples were taken every 5 min for glucose determination, from which values the glucose uptake was calculated. Basal glucose uptake was 3.9±0.3 μmol/g/h, (n=30, mean±SE). The addition of glicazide inmediately increased the dissapearance rate of glucose from the medium in a dose-dependent manner, achieving a plateau at a concentration of 300 μg/ml (10.2±0.8 μmol/g/h, n=7). The half maximal effect (ED_{50}) was obtained with 100 μg/ml of glicazide (6.8±0.4 μmol/g/h, n=7). The maximum effect on glucose uptake produced by glicazide (300 μg/ml) was similar to the one obtained with 10^{-9}M insulin (8.7±1.1 μmol/g/h, n=4, $p>0.05$) and lower to that achieved with 10^{-7}M insulin (14.4±1.4 μmol/g/h, n=4, $p<0.05$). In conclusion glicazide has an inmediate effect on glucose uptake by the skeletal muscle and this effect does not require the presence of insulin.

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INVOLVMENT OF THE SYMPATHETIC NERVOUS SYSTEM IN WHITE ADIPOSE TISSUE METABOLISM.

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In rats, the development of obesity is associated with hyperinsulinemia and decreased sympathetic nervous system (SNS) activity. To determine the potential importance of this decrease on white fat, retroperitoneal fat pad was surgically denervated on one side, the contralateral pad being used as control. Experiments were performed 7 days after denervation which was assessed by a decreased noradrenaline content.

The weight of the denervated pad was increased over the control value. In vivo glucose utilization, measured by [1-3H] 2 deoxyglucose, was similar in the two pads, in both basal and insulin-stimulated conditions (euglycemic-hyperinsulinemic clamps). However, Glut 4 mRNA and protein were decreased by about 50% in denervated fat pads when compared to controls. FAS mRNA and activity were also decreased respectively by 75% and 50% in denervated pads, suggesting that the weight gain was not due to an increased lipogenesis. Current studies on lipolysis (Hormone Sensitive Lipase expression), indicate that this pathway could be affected by denervation and could account for the weight gain.

In conclusion, in white adipose tissue 1) the SNS could have a role in the modulation of Glut 4 and FAS expression 2) a decreased of SNS activity could be involved in an abnormal development of this tissue.

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EFFECTS OF FRUCTOSE FEEDING ON GLUCOSE METABOLISM IN ADIPOCYTES OF NORMAL AND DIABETIC RATS.

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The effect of fructose feeding on glucose metabolism in epididymal adipocytes was evaluated in 27 normal and 27 diabetic male Sprague-Dawley rats. Diabetes was induced by streptozotocin 2 days after birth. At the age of 5 weeks rats were fed on a diet containing 57% CHO either as fructose (F), dextrose (D) or starch (S). After 6 weeks of diet, fructose feeding induced glucose intolerance in normal rats ($p<0.05$, ANOVA) and aggravated that of diabetic ones ($p<0.05$). Plasma triglycerides and cholesterol were increased in F ($p<0.0001$) and were higher in diabetic than in normal rats (TG: $p<0.001$; cholesterol: $p<0.0001$). Fructose fed rats showed decreased insulin stimulated ^{14}C-glucose incorporation into total lipids (normals: F=2.53±0.47 nmol/10^5 adipocytes/h, D=4.33±1.01, S=3.90±0.55; diabetics: F=1.56±0.27, D=1.79±0.59, S=5.46±2.18, $p<0.05$) and also decreased insulin stimulated oxidation of ^{14}C-glucose into CO2 (normals: F=1.95±0.31, D=2.81±0.90, S=2.96±0.35; diabetics: F=1.29±0.23, D=1.68±0.43, S=2.65±0.94). There was no significant change in the apparent insulin sensitivity (ED_{50}). **In conclusion**, in both normal and diabetic rats, a chronic fructose-rich diet induced: 1) hypertriglyceridemia and hyper-cholesterolemia; 2) glucose intolerance; 3) insulin-resistance at adipocyte level.

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ROLE OF GLUCOSE-6 PHOSPHATASE IN THE INCREASED RENAL GLUCONEOGENESIS OF LONG-TERM STARVATION.

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It is known that the renal gluconeogenesis which contributes for 5-10 % of total gluconeogenesis in the fed state, may represent up to 45 % of the total glucose output during long-term starvation in man. We studied the role of the microsomal glucose-6 phosphatase (Glc-6Pase), the last enzyme of gluconeogenesis, in this phenomenon. With this aim, we assayed the enzyme in microsomes of rat kidney and liver in the course of a 96h fast. We report that the liver Glc-6Pase increases up to 48h of fasting and significantly decreases from 72h of fasting (Vm = 0.31±0.04 ; 0.50±0.04 ; 0.54±0.08 ; 0.44±0.07 ; 0.44±0.03 µmol/min/mg prot., mean±S.D. n=6, at 0, 24, 48, 72 and 96h of fasting, respectively). Concomitantly, the kidney Glc-Pase progressively increases throughout the 96h fast (Vm = 0.21±0.02; 0.26±0.01; 0.3±0.03; 0.37±0.05; 0.4±0.04 µmol/ min/mg prot.). Since the other gluconeogenic enzymes follow a parallel evolution in liver and kidney during long term starvation, we propose that the antiparallel evolution of Glc-6Pase in both tissues constitutes an important factor determining the shift from a principally hepatic gluconeogenesis to a hepatic and renal gluconeogenesis during long-term fasting.

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UDP-GLUCOSE ACCURATELY REFLECTS GLUCONEOGENESIS DURING ENTERAL GLUCOSE IN FASTED DOGS.

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We have shown that in fasted dogs infused with [U-^{14}C]lactate, specific activities (SA) of intrahepatic UDP-[^{14}C]glucose (hepatic glycogen's immediate precursor) as sampled by acetaminophen glucuronidation are nearly identical to the plasma [^{14}C]glucose SA, suggesting a common intrahepatic glucose-6-P source. To determine whether during an enteral glucose infusion, gluconeogenesis would also be equally reflected in endogenously produced glucose and UDP-glucose synthesized via gluconeogenesis, mongrel dogs, after fasting overnight or for 2½ days, received a 5-h infusion of glucose at 22.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ along with [3-^3H]glucose, [U-^{14}C]lactate, and [1-^{13}C]galactose followed by IV acetaminophen. The amount of UDP-glucose flux coming from direct uptake of glucose was similar on both days (12.9 ± 3.4 vs 13.8 ± 3.6 $\mu mol \cdot kg^{-1} \cdot min^{-1}$), while the amount from gluconeogenesis increased ($p<0.05$) (8.8 ± 2.0 vs 15.3 ± 1.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) with 2 more days of fasting. The estimated ^{14}C-SA of the glucose moiety from gluconeogenesis entering plasma and intrahepatic UDP-glucose were similar on each day (overnight: 178 ± 58 vs 143 ± 36 $dpm \cdot \mu mol^{-1}$; fasted: 939 ± 129 vs 894 ± 35 $dpm \cdot \mu mol^{-1}$). In conclusion, in mongrel dogs: 1) During fasting or an enteral glucose infusion, endogenously produced glucose and intrahepatic UDP-glucose from gluconeogenesis seem to come from a common pool; 2) The importance of the indirect pathway to glycogen synthesis increases with a longer period of fasting.

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MASS SPECTROMETRIC ANALYSIS OF GLUCOSE ^{13}C LABELING PATTERN : APPLICATION TO THE STUDY OF HEPATIC GLUCOSE METABOLISM.

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Determination of the ^{13}C labeling pattern of glucose during infusion of labeled bicarbonate, acetate or gluconeogenic precursors is an important feature in studies of gluconeogenesis or glycogen synthesis. Using four derivatives of glucose (permethyl, aldonitrile pentacetate, butylboronate acetate, methyloxime trimethylsilyl) and selective mass-spectrometric analysis of fragment ions retaining specific carbon atoms, we developed a method allowing the redundant determination of the ^{13}C enrichment of each of the six carbon atoms of glucose. The method was first tested by performing standard curves of natural glucose enriched (0.5 to 5 % MPE) with glucose labeled in different positions ($1^{13}C$, $2^{13}C$, $3^{13}C$, $4^{13}C$, $6,6^2H_2$, $U^{13}C$-glucose). Next the analysis of various mixtures of natural and labeled glucose showed for all labeling positions a good agreement ($r>0.98$) between the expected and measured enrichments. Lastly, isolated livers of rats (48 hr starved) were perfused with lactate, pyruvate and either $NaH^{13}CO_3$, [$2^{13}C$]acetate, [$3^{13}C$]KIC, [$3^{13}C$]pyruvate or [$U^{13}C$]pyruvate. In each case, the labeling pattern of glucose produced agreed with patterns previously reported in experiments using ^{14}C-labeled substrates and stepwise degradation of glucose molecule or ^{13}C-labeled substrates and NMR. Thus, the present method appears reliable, avoids radioactive tracers, requires only small amount of samples, and will be useful for in vivo studies of gluconeogenesis.

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POSITIONAL ISOTOPIC ANALYSIS OF STABLE ISOTOPE LABELED GLUTAMATE BY MASS SPECTROMETRY.

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Determining the labeling pattern of Krebs cycle intermediates during infusion of labeled $NaHCO_3$, acetate or gluconeogenic substrates allows to estimate some parameters of Krebs cycle activity and to correct apparent gluconeogenic rates. Hepatic glutamine, whose labeling reflects that of α ketoglutarate, can be safely sampled in humans by conjugation with phenylacetate to form phenylacetylglutamine. Its labeling pattern is determined after purification of phenylacetylglutamine from plasma or urine and isolation of the glutamine moiety as glutamate. To avoid radioactive tracers, we developed a mass-spectrometric method to determine the labeling pattern of glutamate by stable isotopes. The dimethylaminomethylene methylester derivative of glutamate yields fragment ions retaining different glutamate carbons i.e., C_1-C_5 (m/z 230),C_2-C_5 (171), C_2-C_4 (111), C_1-C_3 (157) and C_1-C_2 (143), making possible calculation of enrichment in each position. Standard curves of glutamate enriched (0.5 to 5 % MPE) with 1-^{13}C, 5-^{13}C, U-^{13}C or [2,3,3,4,4-$^{2}H_5$] glutamate are linear. Next, we analyzed glutamate isolated from rat livers (48hr starved) perfused (open circuit) with lactate, pyruvate and either $NaH^{13}CO_3$, [1-^{13}C]octanoate, [2-^{13}C]acetate or [1,2$^{13}C_2$]acetate. The labeling patterns obtained agreed with theoretical predictions or patterns reported with ^{14}C labeled tracers. This method appears useful for in vivo studies of hepatic Krebs cycle activity and gluconeogenesis.

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INFLUENCE OF GLIBENCLAMIDE AND HB-699 ON HEPATIC GLUCOSE METABOLISM: A COMPARATIVE STUDY.

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Sulphonylureas may increase the cytoplasmic concentration of Ca^{++} in different types of cells. In isolated hepatocytes, these hypoglycemic agents cause a paradoxical Ca^{++}-dependent activation of glycogen phosphorylase (GPh) which is accompanied by an increase in the cellular concentration of fructose 2,6-bisphosphate (F-2,6-P_2). Here, we have compared, in isolated rat hepatocytes, the effect of glibenclamide (GBC) and that of HB-699 (a benzoic acid derivative similar to the non-sulphonylurea moiety of GBC) on GPh activity, on F-2,6-P_2 levels and on cytoplasmic Ca^{++} concentration measured with FURA-2. Hepatocytes were isolated from fed male Wistar rats by perfusion of the liver with collagenase. Both GBC and HB-699 caused a dose-dependent activation of GPh, the half maximal effects corresponding to 1.4 and 5.2 μmol/l, respectively. The enzyme activation occurred without significant changes in hepatocyte cyclic AMP levels and was accompanied by an increase in the cytoplasmic concentration of Ca^{++}. Parallel to these effects, GBC raised hepatocyte F-2,6-P_2 levels and inhibited the conversion of a mixture of (^{14}C)lactate/ pyruvate (4/0.4 mmol/l) to glucose. Under these conditions, HB-699 caused a significant decrease in F-2,6-P_2 and stimulated hepatocyte gluconeogenesis. This comparative study may help to elucidate which among the hepatic effects of GBC are exerted specifically by the sulphonylurea moiety.

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GLUCOCORTICOID INCREASES GLUCOSE CYCLING AND INHIBITS INSULIN RELEASE IN PANCREATIC ISLETS OF Ob/Ob MICE

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The mechanisms involved in the diabetogenic effect of steroids were investigated in isolated pancreatic islets from normoglycemic ob/ob mice treated with dexamethasone (25μg/day) or saline. Islets were incubated with $^{3}H_2O$ or [U-^{14}C]glucose or [5-^{3}H]glucose, at 5.5 and 16.7mM glucose. Incorporation of ^{3}H from $^{3}H_2O$ into carbon- 2 of glucose and the yield of $^{14}CO_2$ from [U-^{14}C]glucose and $^{3}H_2O$ from [5-^{3}H]glucose were measured. Dexamethasone treatment for 48 hours significantly increased islet glucose cycling at both 5.5 (16% vs 24%) and 16.7mM (36% vs 56%) glucose, while glucose oxidation and utilization were unaffected. Dexamethasone also inhibited insulin release by about 60% at 5.5 and 16.7mM glucose, either in the presence or absence of 10mM arginine. Moreover, 24 h treatment with dexamethasone significantly increased glucose cycling at low and high glucose concentrations and inhibited insulin responsiveness to glucose and arginine. In conclusion, the inhibitory effect of glucocorticoid on insulin release in vitro may be due to enhanced glucose cycling. This may constitute an important aspect of the diabetogenic effect of steroids in addition to decreased glucose uptake and increased gluconeogenesis.

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HYPERGLYCAEMIA-INDUCED ENDOTHELIAL CELLS ALTERATIONS ARE REVERSED BY ANTIOXIDANTS

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Exposure to hyperglycaemia induces delay in the cell cycle of human endothelial cells (EC) in culture,and induces an overexpression of laminin mRNA.Recently,it has been reported that glucose may autoxidize generating free-radicals.To test the involvement of free-radicals in the delayed cell replication and laminin overexpression in hyperglycaemic conditions,EC from umbelical vein were incubated for 9 days in 5 or 20 mmol glucose with or without three different antioxidants: SOD,CAT and glutathione.EC grown in medium with 20 mmol glucose,without antioxidants,yelded a lower growth rate (GR),and showed an overexpression of mRNA for laminin and for the adhesion molecule VCAM-1,while with antioxidants they almost recovered to a normal GR and expression for laminin and VCAM-1.EC grown in 5 mmol glucose,with or without antioxidants,yelded the same GR and laminin and VCAM-1 expression.These data suggest that glucose may induce EC alterations consistent with micro and macroangiopathy development by generating free-radicals.

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TOLRESTAT BLOCKS THE GLUCOSE-INDUCED DOWN REGULATION OF NA^+/MYO-INOSITOL COTRANSPORTER MRNA

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The depletion of cellular free myo-inositol (MI) in lens retina, kidney and peripheral nerves has been implicated in the etiology of the chronic complications of diabetes. We have studied the mechanism of MI depletion in established primary cultures of lens epithelial (LE) cells by quantitating (1) cellular MI by gas chromatography, (2) the rate of MI uptake by measuring the accumulation of ^{3}H-MI, and (3) the abundance of Na+/MI cotransporter mRNA using a cDNA probe from MDCK cells. MI is transported into LE with a K_m of 52 µM, against a steep concentration gradient and is rapidly depleted by 95% within 24h when cells are exposed to medium with 30mM glucose. This depletion is due to the non-competitive inhibition of MI uptake by glucose, Ki= 17mM, and to the rapid accumulation of sorbitol. This non-perturbing organic osmolyte appears to mediate its effect by reducing the level of SMIT mRNA. When sorbitol accumulation was blocked with tolrestat, both MI and SMIT mRNA return toward normal levels. The observed effect of sorbitol on SMIT mRNA may be related to osmotic stress since the incubation of LE in hypertonic media (150mM NaCl) resulted in a ten-fold increase in SMIT mRNA.

PS 8
Glucose Turnover

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GLUCOSE UPTAKE IN TISSUES OF HYPERINSULINAEMIC, HYPERGLYCAEMIC, GOLD-THIOGLUCOSE-OBESE MICE

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Gold-thioglucose (GTG) obese mice exhibit hyperlipogenesis, hyperinsulinaemia and hyperglycaemia. The aim of this study was to determine if glucose uptake in tissues of obese mice is different from controls at the ambient glucose and insulin levels of the fed state. GTG-obese mice (4 weeks post-injection) and age-matched controls were fitted with jugular catheters 5 days before experimentation. All mice were infused with a bolus of [1-^{14}C] 2-deoxyglucose and blood samples taken from the catheter 2,5,10,15 and 30 min later. At 30 min mice were sacrificed and heart, quadriceps muscle, white adipose tissue (WAT) and brown adipose tissue (BAT) analysed for [1-^{14}C] 2-deoxyglucose-6-phosphate content for calculation of glucose uptake. In the fed state plasma glucose was significantly higher in GTG-obese mice (20.0±0.9 vs 14.3±0.5mM, P<0.005) as was plasma insulin (210.8±47.5 vs 48.7±4.5µU/ml, P<0.005). Glucose uptake was not different in heart, quadriceps muscle and WAT of fed obese and control mice, but was significantly lower in BAT of obese mice (0.56±0.10 vs 2.40±0.23µmoles /min/g tissue, P<0.005). These results indicate that the hyperglycaemia and hyperinsulinaemia of obese mice compensate for insulin resistance in some peripheral tissues such that glucose uptake is the same as in control mice.

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EFFECTS OF THE ANTIDIABETIC α_2-ADRENOCEPTOR ANTAGONIST SL 84.0418 ON PLASMA GLUCOSE AND INSULIN LEVELS FOLLOWING EXERCISE IN NORMAL AND TYPE-II DIABETIC RATS.

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We have studied the effects of α_2-adrenoceptor blockade by the potent, selective, peripherally active antihyperglycemic and antidiabetic compound, SL 84.0418, on glycemic control and insulin secretion in normal and type-II diabetic rats following exercise on a treadmill. Type-II diabetes was induced in neonatal rats (day 1 of birth) by the treatment with streptozotocin (90 mg/kg ip). Short exercise induced a significant reduction of plasma insulin levels in both normal and diabetic rats (of 28.2 ± 1.6 and 25.2 ± 2.3 µU/ml, respectively), which was however of longer duration in diabetic rats, compared to normal controls. SL 84.0418 (1 and 3 mg/kg ip), which by itself only slightly reduces glycemia in normal rats, markedly reduces glycemia in diabetic rats, without significant changes in insulin secretion. Following exercise, however, a 2 -3 fold potentiation of insulin release is observed both in normal and diabetic rats pretreated with SL 84.0418, while the glycemic response to exercise was not modified. These results suggest that the inhibition of insulin secretion, induced as a result of the sympathetic activation by exercise, is mediated via α_2-adrenoceptors, while the glycemic responses are largely α_2-adrenoceptor independent.

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EFFECTS OF IN-VIVO ADMINISTRATION OF IGF-I ON GLUCOSE UTILISATION IN RAT SKELETAL MUSCLE.

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The effects of IGF-I and its interaction with insulin were studied. IGF-I was given to rats in-vivo (200µg/12h s.c.) and soleus muscles were isolated: A) 1 h after one injection or B) after 10 days treatment. Glucose phosphorylation (G), lactate formation (GL) and glycogen synthesis (GS) were measured. Serum level of IGF-I increased by 100% in (A) and 30% in (B). In (A), IGF-I vs Control increased (in µmol/h/g): G (7.84±0.93*, 8.57±0.95*, 8.06±0.53 vs 4.00±0.31, 6.6±2.44, 9.20±0.36), GL (8.48±1.50*, 8.89±1.21*, 8.39±0.84 vs 3.50±0.40, 5.65±0.78, 8.02±0.65), GS (4.24±0.24*, 5.15±0.83, 4.35±0.34 vs 2.24±0.14, 3.80±0.19, 5.19±0.14); in (B), IGF-I vs Control increased (in µmol/h/g): G (6.92±0.46*, 8.70±0.69*, 13.65±1.07 vs 5.01±0.57, 6.93±0.70, 10.69±0.77), GL (7.82±1.00*, 7.78±0.84, 11.77±1.44 vs 4.35±0.57, 6.37±1.40, 7.6±1.08), GS (3.00±0.15*, 4.81±0.35*, 7.77±0.61 vs 2.1±0.21, 3.74±0.12, 7.29±0.50) at 10, 100 and 1000 mU/l insulin, respectively (* p<0.05 vs controls). In (B), responsiveness of glucose utilisation (GU) to insulin was similar in muscles isolated from treated and control rats. **Conclusions:** 1) acute or chronic increases in serum IGF-I concentrations increase basal GU in skeletal muscle independently of insulin; 2) the effects of insulin on GU are not additive to those of IGF-I; however, this may depend on the level of IGF-I in serum.

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INFLUENCE OF THE DURATION OF NORMOGLYCEMIA ON GLUCOSE-STIMULATED β-CELL RESPONSIVENESS IN TYPE 2 DIABETIC SUBJECTS.

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To answer the question whether the ß-cell responsiveness is affected by the duration of normoglycemia we have on 4 separate days studied 7 patients with Type 2 diabetes (age 49-70 yr, BMI 24-33 kg/m^2) before and during a 120 min hyperglycemic clamp followed by an IV bolus of 1 mg of glucagon. The tests were performed at usual fasting p-glucose level: 10.7 ± 1.6 mM (test A), and after IV infusion of soluble insulin resulting in normoglycemia for 30 min (test B), 120 min (test C) and 240 min (test D). Basal C-peptide level was: A: 0.73 ± 0.09, B: 0.61 ± 0.15, C: 0.35 ± 0.05, D: 0.49 ± 0.09 nM. Both C and D were lower than A ($p<0.05$), and C was lower than B ($p < 0.05$). First phase C-peptide response was not significantly different: A: - 0.8 ± 0.7, B: -0.08 ± 0.23, C: 0.24 ± 0.21, D: -0.01 ± 0.2 nM x min. Second phase C-peptide response was: A: 45.85 ± 17.2, B: 38.96 ± 17.8, C: 39.5 ± 14.0, D: 61.31 ± 16.39 nM x min. C was different from D ($p < 0.05$). C-peptide level after glucagon: A: 2.59 ± 0.46, B: 2.18 ± 0.44, C: 1.61 ± 0.27, D: 2.63 ± 0.53 nM. C was significantly different from both A and D ($p < 0.05$). The C-peptide response to glucose in Type 2 diabetics depends not only on the ambient glucose level, but also on the duration of preceeding normoglycemia. Interpretation of ß-cell responsiveness is highly dependent on the experimental design.

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SUBTYPE OF MUSCARINIC RECEPTOR INVOLVED IN INSULIN AND GLUCAGON SECRETION *IN VIVO*

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The muscarinic receptor-subtype involved in cholinergically-induced insulin and glucagon secretion is not yet characterized. We therefore compared the effects of the subtype-selective muscarininc receptor antagonists pirenzepine (M_1-receptor antagonist), AFDX-116 (M_2) and 4-DAMP (M_3) with the effect of methylatropine ($M_{1,2,3}$) on carbachol-stimulated insulin and glucagon secretion *in vivo* in mice. Antagonists (0.0021-650 µmol/kg) or saline were administered i.p. 15 min prior to carbachol (0.16 µmol/kg, i.v.). Blood was sampled 2 min later. We found that basal plasma levels of insulin or glucagon were not affected by the antagonits. However, carbachol-stimulated insulin release was dose-dependently inhibited by 4-DAMP or methylatropine. A complete inhibition was observed at 2.1 µmol/kg of 4-DAMP (+34±8 vs. +2±6 µU/ml, P<0.01) and at 0.21 µmol/kg of methylatropine (+34±5 vs. +8±5 µU/ml, P<0.01). In contrast, the M_1- and M_2-selective antagonists less potently inhibited the insulin response. Thus, a partial inhibition was observed at 65 µmol/kg (pirenzepine) and 650 µmol/kg (AFDX-116) (P<0.05). Glucagon secretion stimulated by carbachol was dose-dependently inhibited by either of the antagonists. The order of potency for total inhibition was: methylatropine (0.21 µmol/kg) > 4-DAMP (0.65 µmol/kg) > pirenzepine (65µmol/kg) ≥ AFDX-116 (65 µmol/kg) (P<0.001).
It is concluded that cholinergically-stimulated insulin and glucagon secretion *in vivo* are mediated by muscarinic receptors of the M_3-subtype.

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EFFECT OF GROWTH HORMONE-RELEASING HORMONE ON SERUM INSULIN AND GLUCOSE IN HYPOPHYSECTOMIZED RATS

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Growth hormone (GH) is diabetogenic. However, both GH and growth hormone-releasing hormone (GHRH) can stimulate insulin release *in vitro*. The physiological relevance of this is unclear. Female Sprague-Dawley rats were hypophysectomized at 40 days of age. At day 188, fed rats received 10 µg GHRH-(1-29)/kg i.v., a dose known to induce near maximal stimulation of GH release. In sham operated rats, GHRH induced a rise in serum GH from 36.5 ± 1.9 ng/ml (n=6) at time of injection to 560 ± 13.3 ng/ml after 5 min. GH was not detectable in serum from hypophysectomized rats. Serum insulin was 5.9 ± 1.0 ng/ml in sham operated and 5.8 ± 0.9 ng/ml in hypophysectomized rats. Serum insulin was unaffected by GHRH in both groups (up to 60 min after injection). Serum glucose was lower in hypophysectomized rats, 6.3 ± 0.1 mmol/l (n=6) vs. 7.3 ± 0.4 mmol/l (n=6) in sham operated, but GHRH had no effect on serum glucose levels in either group. In isolated rat islets, 0.1 - 10 nmol/l GHRH stimulated insulin release at 3 mmol/l glucose. It is concluded that, neither growth hormone-releasing hormone, nor a GHRH-induced rise in serum growth hormone levels, have any acute effects on serum insulin and glucose levels.

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IMPAIREMENTS IN GLUCOSE DISPOSAL IN PATIENTS WITH RECENT-ONSET TYPE 1(INSULIN-DEPENDENT) DIABETES

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In 14 patients with recent-onset Type 1(insulin-dependent) diabetes we aimed to evaluate insulin-stimulated(insulin sensitivity,S_I) and insulin-independent glucose disposal (glucose effectiveness,S_G) by Bergman's minimal model of glucose kinetics,first in insulin-requiring state(IRS)and then in non-insulin-requiring state(NIRS)(clinical remission) and compared them with 7 healthy controls.S_I was decreased only in IRS(1.6± 0.5 vs 3.2± 0.9 $min^{-1}/(\mu U/ml)$, $p< 0.05$) while S_G remained diminished both in IRS and NIRS (1.5±0.4;1.3±0.5 vs 3.2±0.8x100/min^{-1},$p< 0.01$).To elucidate the observed defects,we analysed oxidative and non-oxidative glucose disposal by continuous indirect calorimetry combined with insulin clamp(somatostatin used to block endogenous insulin secretion).During hyperglycemic clamp(glycemia targeted to 200 and 275 mg% sequentially, insulin infusion rate 0.25mU/kg/min) we detected persistently decreased nonoxidative glucose disposal both in IRS(3.6±0.3;2.9±0.7)and in NIRS(3.4±0.5;2.7±0.4 vs 4.6±0.7 4.9±0.6 mg/kg/min in controls,$p< 0.01$).The oxidative disposal was also decreased in IRS(2.0±0.2;1.7±0.1 vs 2.7±0.2 2.4±0.3 mg/kg/min in controls,$p< 0.05$) but was normalized in NIRS. During euglycemic hyperinsulinemic clamp(glycemia targeted to 90mg%,insulin infusion rate 1.5 mU/kg/min)both oxidative and nonoxidative disposal were decreased but returned to normal in NIRS.Our results revealed the persistent defect in insulin-independent glucose uptake in recent-onset Type 1(insulin-dependent)diabetes,presumably based on impaired non-oxidative glucose disposal.

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ACTION OF INSULIN ON GLUCOSE METABOLISM : WHAT IS DEPENDANT OF ITS ACTION ON LIPID METABOLISM

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Insulin stimulates glucose metabolism but also inhibits lipid oxidation. In order to test what in insulin action on glucose metabolism is dependant of its action on lipid metabolism, we have realized an insulin dose-response (0.1, 0.2, 0.7 mU/kg/min of insulin) using the euglycemic clamp with an infusion of Ivelip (IV) to maintain the lipid oxidation rate constant. This test was realized in 5 normal subjects and was compared to the control test (C) without Ivelip. Glucose and free fatty acids (FFA) fluxes(fx) were measured using 6,6 $^2H_2$ glucose and 1 ^{13}C palmitate. Oxidation (Ox) was determined using indirect calorimetry and $^{13}CO_2$. FFA Fx , FFA ox and lipid ox were maintained throughout the test in IV whereas they decreased in C. Insulin action on glucose metabolism was altered in IV when compared to C with, at the last insulin rate, a decreased **utilisation** (C 6.8±0.6, IV 3.9±0.4 mg/kg/min C $p<0.01$), **storage** (C 3.8±0.5, IV 1.9±0.2 mg/kg/min $p<0.01$) and a lack of stimulation of **oxidation** (C 3.9±0.3, IV 2.0±0.4 mg/kg/min $p<0.01$) which was unchanged when compared to basal (IV 1.7±0.5 mg/kg/min). A defect in **glucose production** inhibition was also significant for the 2nd (C 1.25, IV 2.03 mg/kg/min $p<0.01$) and 3rd insulin rate (C 0, IV 0.45±0.17 mg/kg/min $p<0.01$). Thus all insulin effets on glucose metabolism appears to be partly or mostly dependant of its effects on lipid metabolism.

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PRIMARY ROLE OF HEPATIC GLUCOSE OUTPUT IN MAINTAINING GLUCOSE HOMEOSTASIS

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The role of changes in hepatic glucose output (HGO) in maintaining glucose homeostasis was investigated in normal subjects by determining glucose turnover, using a primed-constant infusion of 6-^{3}H-glucose, before and during low-dose intravenous glucose infusions of 5.3 (Group A,n=12) and 11.1 μmol/kg/min (Group B, n=10). In Group A basal glucose (5.17 ± .08 mmol/l) and insulin levels (20.4 ± 2.4 pmol/l) rose minimally during the infusion to 5.33 ± .07 mmol/l($p<.01$) and 26.4 ± 3.0 pmol/l($p<.02$) respectively. Concurrent total glucose appearance (Ra : 10.7 ± .3 μmol/kg/min) and disappearance (Rd) remained unchanged during the infusion suggesting that sustained suppression of HGO was the sole compensatory homeostatic response. In Group B basal glucose (5.16 ± .11 mmol/l) and insulin levels (19.8 ± 3.6 pmol/l) rose during the infusion to 6.11 ± .11 mmol/l($p<.001$) and 56.9 ± 7.8 pmol/l($p<.001$) respectively. Ra rose from 9.7 ± .3 μmol/kg/min basally to peak levels of only 13.6 ± .8 μmol/kg/min($p<.001$) at 30 min indicating concurrent sustained suppression of HGO. Additionally, Rd rose by .42 ± .04 μmol/kg/min($p<.001$). Six studies employing 2-min sampling suggested increased glucose and insulin and decreased glucagon concentrations were the combined cause of HGO suppression. Conclusions : (1) Decreased HGO is the primary sole homeostatic response to small challenges to the basal steady state (2) HGO is exquisitely sensitive to suppression by minimal changes in glucose, insulin and glucagon levels (3) only following greater glucose challenges is increased Rd an additional homeostatic response.

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HEPATIC GLUCONEOGENIC HYPERSENSITIVITY TO SUBSTRATE AVAILABILITY IN TYPE II DIABETES

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Increased gluconeogenesis (GN) is considered to be mainly responsible for the increased hepatic glucose output (HGO) in type II diabetes. To determine whether this is due to increased hepatic GN efficiency, we compared the effect of Na-lactate infusion (30 μmol/kg/min) in 8 nonobese normal volunteers (NV) and 5 age- weight- sex- matched type II diabetic subjects (DS) during which plasma insulin, growth hormone and glucagon were clamped at indentical levels in both groups. Control Na-bicarbonate infusion (30 μmol/kg/min) studies were performed under identical conditions in both groups. Despite comparable hyperlactatemia (4.6 mmol/liter), lactate GN (umol/kg/min) increased by 4.9±0.3 during the lactate infusions in the DS compared to only 2.7±0.5 in the NV ($p<0.05$); HGO (μmol/kg/min) increased by 2.8±0.8 in the DS vs. only 1.1±0.4 in the NV. Moreover, the percent of HGO derived from lactate increased by 32±2% in the DS compared to 22±4% in the NV. We, therefore, conclude that in type II diabetes, there is increased hepatic sensitivity to substrate availability and this may account in part for the increased GN found in this condition.

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REDUCED ENERGY EXPENDITURE AFTER ORAL FRUCTOSE IN CIRRHOSIS

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Fructose metabolism is insulin independent. As cirrhotics are insulin resistant and glucose intolerant, fructose might confer advantages in terms of carbohydrate utilisation. We studied 8 cirrhotics (54±11 [±SD] y) and 6 controls (49±13 y). Fasting glucose levels and rates of 6-3H-glucose appearance (Ra) were similar. Basal NEFA and glycerol levels were higher in cirrhotics ($p<0.05$) but whole body lipid (LOX), carbohydrate oxidation (COX) and energy expenditure similar. After 75 g fructose, fructose and insulin levels were higher in cirrhotics (both $p<0.001$) and the incremental 4 h plasma glucose AUC greater (154±17 vs 74±26 mmol.l^{-1}h, $p<0.05$), due to a higher glucose Ra and reduced MCR ($p<0.001$). Plasma glycerol fell in controls (basal, 77±11; post-fructose, 49±6 μmol/l, $p<0.05$) but not in cirrhotics (basal, 122±16; post-fructose, 116±20 μmol/l); NEFA were suppressed to similar low levels. Suppression of LOX and stimulation of COX paralleled changes in fructose levels rather than serum NEFA suggesting that fructose metabolism inhibits intracellular lipid oxidation. Total lipid oxidised in 4h after fructose (cirrhotics, 7.0±1.5, controls, 8.6±2.1 g, NS) was similar but the suppressed LOX and elevated COX were sustained for longer in cirrhotics. Overall oxidative metabolism and storage were similar, but the increase in energy expenditure after fructose 46% lower in cirrhotics ($p<0.005$). The energy cost of glycogen synthesis from fructose in liver is ~x2 that in muscle. High systemic fructose levels secondary to reduced hepatic uptake in cirrhotics would favour muscle glycogen synthesis.

PS 9
Insulin Synthesis

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IDENTIFICATION OF THE CHROMOGRANIN A-PROCESSING ENDOPEPTIDASE IN INSULIN SECRETORY GRANULES AS THE MAMMALIAN SUBTILISIN-RELATED PROTEASE, PC2

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Two endopeptidase activities are required for the conversion of proinsulin to insulin: one of these (type 1) cleaves on the C-terminal side of $Arg^{31}Arg^{32}$ in proinsulin and the other (type 2) cleaves C-terminally to $Lys^{64}Arg^{65}$. The type 2 enzyme has been identified recently as PC2, a member of the eucaryotic subtilisin-like family of proteases. Endopeptidase activity is also required for processing of the insulin secretory granule protein chromogranin A. N-terminal sequencing of the products of bovine chromogranin A conversion by insulin secretory granules reveals that the precursor is cleaved at $Lys^{114}Arg^{115}$ and $Lys^{330}Arg^{331}$. To determine if chromogranin A processing requires the same endoproteolytic enzyme(s) as proinsulin, the type 1 and 2 activities were separated by ion-exchange chromatography and the resultant fractions tested for chromogranin A- and proinsulin-converting activity. Cleavage of chromogranin A coincided with type 2 endopeptidase activity and was distinct from the two peaks of type 1 activity. Immunoblot analysis of the fractions with anti-PC2 serum showed that the chromogranin A processing activity also coincided with PC2 immunoreactivity. These results indicate that PC2 is the enzyme responsible for cleavage of chromogranin A and potentially other propolypeptide precursors containing LysArg sites in the insulin secretory granule such as pro-islet amyloid polypeptide.

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CONVERSION OF NATIVE AND MUTANT (Met^{B29}) HUMAN PROINSULIN IN TRANSFECTED AtT20 CELLS

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We have previously postulated that Lys^{B29} is implicated in conversion at the B-chain/C-peptide junction of rat proinsulins. To verify this and to simulate the difference between rat proinsulins I and II, we have transfected AtT20 cells with native (hPI) or a mutant human proinsulin with a Met for Lys replacement at B29 (Met^{B29} hPI). Cells were labelled (10 min, ^{3}[H]leu) followed by chases up to 120 min. Radioactivity in proinsulin, conversion intermediates and insulin was determined by HPLC. At 60 min 57±7% labelled hPI remained, with 26±5% insulin and 17±2% of des-31-32 split hPI; des-64-65 split hPI was not detectable. The kinetics of conversion of hPI with transient accumulation of des-31-32 split hPI in AtT20 cells is comparable to conversion of rat proinsulin I in isolated islets. By contrast, conversion of Met^{B29} hPI showed no accumulation of des-31-32 split intermediate, and, unlike conversion of rat proinsulin II in islets, no accumulation of des-64-65 split Met^{B29} hPI was detectable. These data support the hypothesis that conversion at the B-chain/C-peptide junction is facilitated by a basic amino-acid at B29. Extrapolation from AtT20 to B-cells may however be complicated by reported differences in expression of PC2/PC3, the two conversion endoproteases.

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DIFFERENTIAL EXPRESSION OF PROINSULIN C-PEPTIDE I AND II IMMUNOREACTIVITIES IN THE MOUSE INSULINOMA CELL LINE, ß-TC.

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We have previously reported that clonal rat insulinoma cultures (MSL-cells) differentially express the two non-allelic insulin genes when passaged *in vitro*. This was shown by immunocytochemistry and *in situ* hybridization to be controlled at the level of transcription. These heterogeneous cell cultures preferentially express the rat insulin I gene. This is in contrast to the normal rat or mouse islets where both insulin gene products are expressed at comparable levels in all ß-cells.

By using specific antibodies raised against mouse proinsulin C-peptide I and II we have analyzed the insulinoma culture, ß-TC, derived from transgenic mice with heritable ß-cell tumors. We now show a remarkable differential expression of mouse C-peptide I and II immunoreactivities where a considerable fraction (approx. 20%) of insulin positive cells are devoid of C-peptide I immunoreactivity. C-peptide II staining indicate that this subpopulation express proinsulin II, only. We conclude that *in vitro* murine insulinoma cultures have the capacity to differentially express the two non-allelic insulin genes, but with a species specific difference, since mouse and rat cultures preferentially express insulin II and I, respectively. The nature of such an aberrant tumor specific process of "non-allelic" exclusion remains unclear.

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PREFERENTIAL STIMULATION OF PROINSULIN BIOSYNTHESIS BY SUCCINIC ACID METHYL ESTERS. I. Valverde, M.L. Villanueva-Peñacarrillo and W.J. Malaisse*. Fundación Jiménez Díaz, Madrid, Spain and *Laboratory of Experimental Medicine, Brussels Free University, Brussels, Belgium.

Succinic acid methyl esters are efficient insulin secretagogues. Their effect on rat islet biosynthetic activity was now examined. Over 90 min incubation in the presence of 2.8 mM D-glucose, both succinic acid monomethyl ester (SAM) and dimethyl ester (SAD), when tested at a 10 mM concentration, increased 10-fold at least the incorporation of L-[4-^{3}]phenylalanine into TCA-precipitable material, non-hormonal protein, proinsulin and insulin. Moreover, the ratio between tritiated (pro)insulin and total protein was increased by SAM and SAD from a control value of 22.3 ± 1.6 to 33.4 ± 4.0 and 35.9 ± 3.5 percent, respectively. A rise in D-glucose concentration to 16.7 mM increased the latter ratio to 43.9 ± 3.8 percent. Succinic acid (10 mM) also increased the incorporation of L-phenylalanine into TCA-precipitable material, as well as non-hormonal and hormonal peptides. However, this increase was less marked than that evoked by SAM or SAD and failed to coincide with an increased ratio between (pro)insulin and total protein biosynthesis. None of the agents tested affected the islet pool of TCA-soluble radioactivity. Likewise, the ratio between tritiated insulin and (pro)insulin was not affected by succinic acid and its esters, being only increased at the high concentration of D-glucose. Thus, succinic acid methyl esters, like D-glucose, stimulate preferentially the biosynthesis of proinsulin.

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INSULIN SECRETION IN INSULIN RESISTANT SUBJECTS WITH AND WITHOUT FAMILY HISTORY OF DIABETES

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First-degree relatives of patients with Type 2 diabetes (REL) are at increased risk for diabetes. Although insulin resistance is a frequent early finding in these individuals, defect in insulin secretion is required for manifest diabetes. To investigate whether insulin resistant REL are characterized by a concomitant ß-cell defect we measured first- (FPI) and second-phase (SPI) insulin secretion with a +7mmol/l hyperglycemic clamp and total glucose disposal (TGD) with a +80µU/ml euglycemic insulin clamp. ß-cell capacity was measured after administration of 0.5 mg glucagon at the end of the hyperglycemic clamp (GSI). 8 insulin resistant REL (age 45 ± 3 yrs, BMI 26.9 ± 1.0, TGD 4.2 ± 0.4 mg/kgxmin), 7 kidney transplanted patients (Tx) with steroid induced insulin resistance (age 41 ± 6yrs, BMI 26.8 ± 0.9, TGD 4.6 ± 0.4 mg/kgxmin) and 7 healthy control subjects (Con) without a family history of diabetes (age 47 ± 5 yrs, BMI 25.3 ± 0.4, TGD 6.1 ± 0.5 mg/kgxmin;$p<0.05$ vs Tx and REL) participated in the study. All groups had normal glucose tolerance. FPI, SPI and GSI did not differ significantly between REL and Con (103 ± 19 vs. 128 ± 58 µU/mlx10min and 457 ± 122 vs. 366 ± 147 µU/mlx110min and 515 ± 66 vs. 540 ± 102 µU/mlx30min). Tx tended to have higher FPI and SPI than REL (156 ± 49 µU/mlx10min and 531 ± 102 µU/mlx110 min) whereas GSI was significantly higher (723 ± 56 µU/mlx30min;$p<0.05$). Conclusion: REL seem to have decreased capacity to increase insulin secretion in relation to the degree of insulin resistance.

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DIFFERENT MECHANISMS OF LOW INSULIN RESPONSE IN PREDIABETIC AND ENDURANCE-TRAINED SUBJECTS

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Subjects with a low insulin response (LIR) to a standardized 60 min glucose infusion test (GIT) are considered prediabetic. Endurance-trained subjects (ET) also demonstrated low insulin levels after the same glucose challenge. Peripheral plasma insulin responses to GIT in LIR ($n=6$) were therefore compared to those in high insulin responders (HIR, $n=6$) and ET ($n=6$). We also measured insulin secretion rates with a two-compartmental model for C-peptide distribution and degradation. The parameters defining C-peptide kinetics were obtained in each subject after an i.v. bolus of biosynthetic human C-peptide. During GIT, blood glucose levels plateaued at 18-22 mmol/l. In peripheral plasma, mean insulin responses were 362 ± 32 pmol/l in HIR, 136 ± 14 pmol/l in LIR (38% of HIR, $p<0.05$), and 188 ± 14 pmol/l in ET (52%, $p<0.05$). Insulin secretion rates were 705 ± 60 pmol/min in HIR, 327 ± 53 pmol/min in LIR (46% of HIR, $p<0.05$), and 594 ± 30 pmol/min in ET (84%). In conclusion, an impaired insulin response in prediabetics (LIR) could mainly be accounted for by a lower insulin secretion rate than in HIR. In contrast, a normal insulin secretion rate and increased metabolic clearance rate of insulin are characteristics of ET.

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Defective beta cell function in subjects with impaired glucose tolerance (IGT).
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51 subjects with IGT matched to 51 controls with respect to age, sex, and BMI (mean [SD] 60.7 [7.9] vs 60.2 [7.4] yrs, 26.8 [3.4] vs 26.1 [2.8] kg/m^2), underwent a 75 gram OGTT. Fasting, 30 and 120 minute insulin and intact proinsulin, and fasting and 120 minute 32/33 split proinsulin, were measured by two-site immunoradiometric assays. The subjects with IGT had higher basal and 120 minute intact (mean [SEM] 5.0 [0.6] pmol/l vs 3.3 [0.25] ; $p < 0.02$, 27 [2.1] vs 17.2 [1.7] ; $p < 0.0001$) and 32/33 split proinsulin, (4.1 [0.34] vs 2.2 [0.26] ; $p < 0.0007$, 23 [2.8] vs 10.8 [2.2] ; $p < 0.0001$). Despite hyperglycaemia, the IGT group had similar fasting insulin, (58 [5.4] pmol/l vs 45.3 [3.3] ; $p = 0.3$), lower 30 minute insulin (248 [18.2 vs 304 [19.5] ; $p < 0.02$), and lower 30 minute insulin/glucose ratio, (23.7 [14.2] vs 34.8 [15.7] ; $p < 0.002$). The fasting percentage of proinsulin to total insulin-like molecules was higher in those with IGT, (15% [0.08] vs 11.6% [0.05] ; $p < 0.04$). After 6 months, at repeat OGTT, the same 51 subjects with IGT were classified as 'persisters' or 'reverters'. In the reverters 27 (52.9%) there was a reduction in 120 minute intact and 32/33 split proinsulin, (26.3 [2.4] vs 20.2 [1.6] ; $p < 0.02$, 21 [2] vs 14.2 [1.2] ; $p < 0.006$), and an increase in fasting insulin, (46.3 [5.7] vs 56.8 [5.7] ; $p < 0.02$) despite no change in fasting glucose after 6 months (5.7 [0.8] mmol/l vs 5.6 [0.65] ; p = NS). These findings show that IGT is strongly associated with beta cell dysfunction, with reduced early insulin secretion. In some subjects with IGT, beta cell function shows improvement in the short term.

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Negligible Insulinotropic Action of Exogenous Glucagon-like Peptide-1 [7-36 Amide] at Basal Plasma Glucose Concentrations in Healthy Volunteers
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Animal studies suggest that the incretin hormone glucagon-like peptide-1 [7-36 Amide] (GLP-1 [7-36 A]) might - in contrast to GIP - stimulate insulin secretion also at basal plasma glucose concentrations, thus possibly leading to hypoglycaemic episodes. In order to test this in man, six healthy male volunteers received incremental doses of intravenously infused GLP-1 [7-36 A] (Fa. Bissendorf) (0.3, 0.9, 2.7 pmol/kg/min, for 30 min each). Plasma glucose (capillary, glucose-oxidase-method), insulin, C-peptide and GLP-1 [7-36 A] (RIA) were measured over 2 hours. GLP-1 [7-36 A] steady-state plasma levels of 23 ± 2, 72 ± 18 and 178 ± 15 pmol/l (mean ± SEM) were reached at the incremental infusion levels (basal concentration 9 ± 2 pmol/l). After start of the infusion and at each dose increment small, short-lived insulin- (maximum, +44%) and C-peptide- (maximum, +26%) peaks as well as a small but significant reduction of plasma glucose levels from 4.9 ± 0.1 to a minimum of 4.0 ± 0.1 mmol/l at the highest dose were seen. At basal plasma glucose concentrations, even high doses of exogenously infused GLP-1 [7-36 A] do not stimulate insulin secretion to an extent that causes relevant hypoglycaemia.

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STRUCTURE OF HUMAN GLP-1(GLUCAGON-LIKE PEPTIDE-1) CONTAINING PEPTIDES.
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The potent insulinotropic hormone, GLP-1, has been isolated from human intestinal mucosa and demonstrated by sequencing and mass spectrometry to correspond to proglucagon (PG) 78-107amide. The occurrence of variant forms (PG 78-108, PG 72-108 or 72-107amide) and their source remain controversial. Based on synthetic fragments we developed highly specific radioimmunoassays against the N-terminus, the mid region and the C-terminus of PG 78-107 amide and analysed fresh human pancreas and intestinal mucosa (n = 6), extracted without loss of peptides, after analytical gel filtration on Sephadex G 50. Pancreas contained exclusively a large peptide identified by N-terminal and mid-region assays (same amounts as glucagon) and very small amounts (0-5 %) of a peptide reacting with C-terminal and mid-region assays, coeluting with PG 72-107amide. The small intestine contained a single peptide, coeluting with PG 78-107amide, reacting equally in all three assays (same amounts as glicentin). Conclusions: In humans the GLP-1 sequence is contained in major proglucagon fragment corresponding to PG 78-158 and very small amounts of PG 72-107amide, both from the pancreas. The intestinal form corresponds exclusively to PG 78-107amide. This insulinotropic hormone may be measured specifically by C-terminal assays.

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TRAINING AND ß-CELL SECRETION IN TYPE 2 (NON-INSULIN-DEPENDENT) DIABETES.

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In healthy subjects physical training induces a reduced glucose stimulated ß-cell response. To test if the same effect of training is seen in type 2 diabetics we studied eight type 2 diabetics, age 55 ± 2 years (mean ± SE), height 174 ± 2 cm, weight 89.5 ± 4.5 kg, and body fat 24 ± 1 %. The participants were examined before (UT) and after (T) 12 weeks ergometer bicycle training. Maximal aerobic capacity increased from 2.5 ± 0.2 to 3.0 ± 0.3 l/min ($p < 0.05$). During a mixed meal test, plasma glucose concentration and ß-cell function were not affected by training as areas under concentration curves (AUC) for insulin and C-peptide were similar: Insulin: UT: 10388 ± 1960, T: 9710 ± 1892 pmol·min/l, C-peptide: UT: 357 ± 61, T: 355 ± 54 nmol·min/l, both $P > 0.1$. During graded hyperglycemic (11, 18, and 25 mM) clamps steady state insulin and C-peptide concentrations were similar before and after training: Insulin: UT 19.95 ± 5.02, 30.47 ± 6.98, 41.23 ± 11.17; T: 20.24 ± 6.59, 32.67 ± 11.58, 51.04 ± 20.45 pmol/l; C-peptide: UT: 0.98 ± 0.19, 1.35 ± 0.25, 1.97 ± 0.35; T: 1.04 ± 0.19, 1.44 ± 0.29, 1.96 ± 0.43 nmol/l, at each clamp step respectively. It is concluded that the betacell function in type 2 diabetics, unlike in healthy young subjects, is unaltered by training.

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IMPAIRED INSULIN SECRETION PRECEDES INSULIN RESISTANCE WHEN DIABETES DEVELOPS IN CYSTIC FIBROSIS

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Diabetes mellitus accompanying cystic fibrosis (CF) is insulinopenic but not ketosis-prone. Peripheral sensitivity to insulin has been scarcely studied in CF patients, results are divergent, and, as for Type 2 diabetes, it is unknown whether changes in insulin sensitivity precede beta cell dysfunction or vice versa. We used an oral glucose tolerance test (75 g) and a previously validated model to simultaneously analyse the initial insulin response to glucose (IRG) and the peripheral sensitivity to insulin (SI) in adult CF patients with normal (NGT; N=14), impaired (IGT; N=4), and diabetic (DM; N=12) glucose tolerance, and in 10 age-matched normal subjects. The incremental area of the glucose curve above basal level correlated negatively with IRG (R(S)= -0.69) and SI (R(S)= -0.90), which were positively correlated (R(S)= -0.58) (all $p<0.001$). As compared with normal subjects (100%), median IRG (NGT:47%; IGT:36%; DM:10%) and SI (NGT:93%; IGT:75%; DM:41%) decreased with decreasing glucose tolerance; IRG in all CF groups and SI in CF patients with IGT and DM were significantly lower than in normal subjects. Thus, a progressive decrease in insulin response is seen in CF patients with decreasing glucose tolerance, whereas insulin sensitivity is normal in CF patients with NGT, but decreased at IGT and DM. Consequently, impaired insulin secretion precedes insulin resistance when diabetes develops in cystic fibrosis.

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INDUCTION OF MHC CLASS II ANTIGENS ON PANCREATIC ß-CELLS OF BB/OK RATS

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The expression of MHC class II antigens on pancreatic ß-cells in response to cytokines is still a matter of discussion. Hypothetically, ß-cells with increased MHC antigen expression could be a sensibilized target for cytotoxic lymphocytes.
To evaluate whether ß-cells are able to express MHC class II antigens, we cultivated isolated BB/OK rat islets (8 - 12 d of age) for 4 or 8 d in the presence of 1000 U/ml rIFNγ (control without IFNγ). After culture insulin and DNA content, glucose-stimulated insulin secretion, protein and DNA synthesis of the islets were determined. MHC antigen expression was determined on single cells by FACS analysis (double-staining) using the monoclonal antibodies OX 18 (class I) or OX6 (class II) and SB14D10-FITC (pancreatic ß-cells).
The proportion of ß-cells in cell suspensions ranged between 80 and 90%. IFNγ induced expression of MHC class II antigens on ß-cells: 18.2 ± 2.3% $OX6^+$ß-cells (control 4.5 ± 0.5%). The proportion of $OX\ 18^+$ islet cells was increased from 29.3 ± 5.8% to 68.5 ± 7.0%. Whereas the majority of functional parameters were not affected, glucose-stimulated insulin secretion was significantly reduced by IFNγ-culture (4d). Cytotoxicity of lymphocytes did not differ when using ^{51}Cr-labeled IFNγ-cultured or control islets as target.
Pancreatic ß-cells obtained from BB/OK rats do express MHC class II antigens associated with a reduced insulin-secretion. However, consequences for an increased vulnerability to cytotoxic lymphocytes could not yet be observed.

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THE INDIVIDUAL ISLET RESPONDS TO GLUCOSE WITH AMPLITUDE MODULATION OF RAPID INSULIN TRANSIENTS

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When islets are stimulated with glucose there is a synchronization of cytoplasmic Ca^{2+} cycles among the cells. What is the secretory counterpart for this coordinated pattern? The problem was approached by perifusing a single mouse islet and analyzing the perifusate for insulin with an ELISA technique capable of detecting as little as 0.4 pg insulin. Basal insulin release in a medium containing 3 mmol/l glucose was 0.02 ± 0.01 ng/µg dry weight/min. When the perifusate was collected in 18 s periods, an oscillatory insulin release with a periodicity of 2-3 min was revealed in the presence of 5.5 mmol/l glucose. After further increase of the glucose concentration to 11 and 20 mmol/l the height of the oscillations increased substantially. The rate of the oscillations, however, remained unaffected. By decreasing the sampling period to 2-3 s it became possible to resolve the oscillations into 10-20 s transients, often originating from the basal level and sometimes reaching peak values exceeding 10 ng/µg dry weight/min. Accordingly, the secretory response of an isolated islet to a glucose challenge is determined by fast insulin transients varying more than 1000-fold in their amplitudes. These rapid transients generate the slow 2-3 min oscillations, modulated by glucose in amplitude but not in frequency.

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INTRACELLULAR LOCATION OF THE SULPHONYLUREA RECEPTOR

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Sulphonylureas have been used for many years in the treatment of Type 2 diabetes and stimulate insulin release from pancreatic β-cells. However the location of sulphonylurea binding sites has not been characterized. The rat insulinoma cell line CRI-G1 is an in vitro model with which to study insulin secretion. Secreted immunoreactive insulin increased by up to 320% ($p<0.005$) in the presence of 50 µM ADP. The effect of ADP was also studied on the binding of [^{3}H]-glibenclamide to microsomal membranes. The binding was decreased 72% ($p<0.001$) by 1 mM MgADP. ADP alone decreased [^{3}H]-glibenclamide binding by only 32% ($p<0.001$). Proteolytic digestion of microsomal membranes and a crude cellular homogenate with trypsin reduced [^{3}H]-glibenclamide binding by 80 ± 6% and 83 ± 7% respectively, whereas identical proteolysis of whole cells did not affect binding. These studies indicate that although extracellular MgADP, like sulphonylureas, stimulates insulin secretion and MgADP inhibits glibenclamide binding to microsomal membranes, the site of these two effects differs. Glibenclamide binding is destroyed by trypsin when applied directly to microsomes but not when applied extracellularly, suggesting that the sulphonylurea receptor is intracellular.

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DUAL EFFECTS OF PROTEIN PHOSPHORYLATION ON THE BINDING ACTIVITY OF THE SULPHONYLUREA RECEPTOR

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The effects of protein phosphorylation on the binding activity of the sulphonylurea receptor, a putative ATP-sensitive K-channel, were investigated using the pancreatic ß-cell line, HIT T15. MgATP inhibited [^{3}H]glibenclamide binding and revealed inhibition of [^{3}H]glibenclamide binding by diazoxide, which opens the ATP-sensitive K-channel. These effects of MgATP were time-dependent and their disappearance was correlated with the time-course of ATP hydrolysis by endogenous ATPase. Diazoxide inhibited [^{3}H]glibenclamide binding even when added after MgATP was completely hydrolyzed, suggesting that diazoxide did not affect ATP metabolism or kinase activity which phosphorylates the receptor. ATP-γS had a long-lasting effect to reveal diazoxide-induced inhibition although this analogue elicited little inhibition of binding. The sulphonylurea receptor solubilized by CHAPS retained its affinity for sulphonylureas. Inhibition of [^{3}H]glibenclamide binding by MgATP was also observed after solubilization, but the revealing effect on diazoxide-induced inhibition disappeared. Incorporation of [γ-^{32}P]ATP into HIT cell membranes confirmed the presence of endogenous kinases and phosphatases. These findings suggest that the sulphonylurea receptor may change its binding properties via protein phosphorylation at two distinct sites.

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EFFECT OF CHRONIC HYPERGLYCEMIA ON PROINSULIN PROCESSING IN RAT AND *PSAMMOMYS OBESUS*

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Diabetics show increased plasma proinsulin, but the mechanism underlying this phenomenon is unclear. Our aim is to evaluate the role of hyperglycemia in the development of impaired proinsulin processing. Two animal models are used: rat and *Psammomys obesus*. Monolayer cultures of rat islets were exposed to RPMI 1640 medium (11.1 mM glucose), or to medium containing 33.3 mM glucose for 2-3 weeks. Insulin immunoreactive (IRI) peptides were seperated by reversed-phase HPLC and identified by pulse-chase experiments. Chronic exposure to high glucose had no effect on islet proinsulin content (7-10% of total IRI peptides), or medium proinsulin (<3%). In *Psammomys obesus*, a rodent model of type 2 diabetes, insulin constituted 90% of total IRI peptides in pancreatic extracts of nondiabetic animals, but only 70% in diabetic ones. In addition to proinsulin, other unidentified IRI peptides were detected in both groups, but more so in diabetic pancreata. **In summary:** 1. Chronic exposure to high glucose *in vitro* does not affect proinsulin processing and secretion in normal rat islets, 2. Pancreata of diabetic *Psammomys obesus* contain considerable amounts of proinsulin and unidentified IRI peptides. This may reflect an intrinsic defect of proinsulin processing in diabetes-prone animals, amplified by hyperglycemia.

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INSULIN RELEASE OF GLUCOSE-UNRESPONSIVE RAT ISLET B-CELLS IS STIMULATED BY LEUCINE.

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Rat pancreatic B-cells differ in individual responsiveness to glucose. Cells with higher threshold for glucose-induced changes in metabolic redox require higher glucose levels for induction of insulin biosynthesis and release. This study compares the secretory response of B-cell subpopulations with different glucose sensitivity during perifusion at high glucose (20 mM) or leucine (10 mM). Cells with glucose-induced increase in intracellular redox (*G7.5-responsives*) were separated by flow cytometry from those with unchanged levels (*G7.5-unresponsives*). At 1.25 mM glucose, basal insulin release was comparable in both preparations. Glucose 20 mM induced a higher first phase insulin response in *G7.5-responsives* (0.05% of cellular content/min) than in *G7.5-unresponsives* (0.02%/min, $p<0.05$), but second phase releases were comparable. Leucine 10 mM elicited a similar secretory response in both subpopulations. Addition of glucagon (10 nM) during the second phase amplified glucose-induced release to 0.21%/min in *G7.5-responsives* vs 0.10%/min in *G7.5-unresponsives* ($p<0.05$). This dissociation was not observed during glucagon potentiation of leucine-induced insulin release. Thus, islet B-cells with different threshold for glucose-induced functions differ in secretory responsiveness to glucose but not to leucine. Cellular heterogeneity in glucose-induced insulin release appears attributable to intercellular differences in glucose-specific factors rather than in secretory capacity.

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EFFECTS OF OKADAIC ACID ON INSULIN SECRETION FROM RAT ISLETS OF LANGERHANS.

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Okadaic acid (OA) is a potent inhibitor of protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A), two of the four major cytosolic protein phosphatases that dephosphorylate proteins at serine and threonine residues. As protein phosphorylation is thought to be involved in the regulation of insulin secretion,we have investigated the effects of okadaic acid on insulin secretion from both intact and electrically permeabilised rat islets of Langerhans. Basal insulin secretion from intact islets was unaffected by 10μM okadaic acid (2mM glucose, 0.80±0.11; +OA,0.70± 0.09ng/islet/hr, mean±SEM, $n=8$, $p>0.2$), whereas glucose-induced secretion was significantly reduced with this concentration of phosphatase inhibitor (20mM glucose, 10.5±1.03; +OA, 5.1±1.07ng/islet/hr, $p<0.001$). Insulin secretion from intact islets stimulated with 20mM glucose plus 500nM PMA or 20mM glucose plus 10μM forskolin (F) also showed a marked inhibition in the presence of 10μM okadaic acid (PMA, 22.39±1.32, +OA 8.41±0.79ng/islet/hr, $p<0.001$; F, 19.20±1.17, +OA 5.34±0.49, $p<0.001$), suggesting that PP1 and/or PP2A may be involved in the mechanism of glucose-induced insulin secretion. In contrast, studies with electrically permeabilised islets (5 exposures to 3.4kVcm^{-1}) revealed that okadaic acid had no effect on calcium-induced secretion (10μM Ca^{2+}, 2.30±0.22; +OA, 2.13±0.39ng/islet/hr, $p>0.2$) but enhanced both basal (50nM Ca^{2+}, 0.94±0.17; +OA 3.10±ng/islet/hr, $p<0.001$))and cAMP-induced secretion (500μM, 2.28±0.21; +OA, 2.64±0.21ng/islet/hr, $p<0.001$). In electrically permeabilised islets, okadaic acid enhanced incorporation of ^{32}P from [γ^{32}P]ATP into a number of endogenous substrates (MW 17, 18.5, 19.5) under basal conditions (50nM Ca^{2+}, 1 min), and enhanced cAMP-induced (100μM) phosphorylation of proteins of MW16.5 and 23kDa. These results demonstrate that rat islets contain PP1 and/or PP2A activity. Our results in permeabilised islets suggest that phosphatase inhibition enhances the insulin secretory response to some kinase activators, unlike the studies in intact islets which suggest that okadaic acid inhibits glucose-induced secretion at a site proximal to calcium entry.

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OKADAIC ACID INHIBITS INSULIN RELEASE AND PROTEIN PHOSPHATASES OF RINm5F CELLS

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Protein phosphatases play an important role in a variety of cellular functions. Okadaic acid (OA) is a potent and specific inhibitor of the protein phosphatases 1 (PP-1) and 2A (PP-2A). Among phosphatases, only PP-1 and PP-2A possess significant phosphorylase *a* phosphatase activity. Here, we studied the effect of OA on phosphorylase *a* phosphatase activity and insulin secretion of RINm5F cells. In homogenates of RINm5F cells, OA inhibited phosphorylase *a* phosphatase activity in a concentration dependent manner ($IC_{50} \approx 2$ nM). In intact cells, half maximal inhibition was achieved with about 500 nM OA. OA (2 µM) did not affect basal insulin release (2.60 ± 0.37 µU IRI/µg protein), but significantly inhibited insulin secretion stimulated by 25 mM KCl (10.94 ± 0.39 vs. 6.87 ± 0.28 µU/µg, $p < 0.001$) or 10 mM L-alanine (5.38 ± 0.34 vs. 3.74 ± 0.32 µU/µg, $p < 0.01$). Our data indicate that RINm5F cells contain phosphorylase *a* phosphatase activity, which can be inhibited by OA. The concentration necessary for inhibition of phosphorylase *a* phosphatase activity in intact cells was about 300-fold higher than in homogenates. Since OA (≥ 1 µM) also inhibited insulin release stimulated by KCl or L-alanine, we suggest that PP-1 and/or PP-2A are involved in the mechanism of insulin release of RINm5F cells.

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GLUCOSE - INDUCED B-CELL DESENSITIZATION: MECHANISMS OF PROTECTIVE EFFECT BY DIAZOXIDE.

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Sustained hyperglycemia desensitizes B-cells to glucose; diazoxide (D) protects against this effect. Mechanisms behind protection were investigated. Desensitization was achieved by perifusing rat islets 180 min with 27 mmol/l glucose and 0.1 mmol/l 3-Isobutyl-1-Methylxanthine (IBMX). Restimulated response to glucose + IBMX, 30 min, was only 20 % of 30 min initial glucose-induced stimulation (IGIS); it was completely restored by previous D. 40 mmol/l K+ (180 min + IBMX) markedly stimulated secretion both during 6 and 27 mmol/l glucose; these conditions desensitized restimulated responses to 27 mmol/l glucose + IBMX + normal K+ to 24 % and 27 % respectively of IGIS. D with 40 mmol/l K+ completely failed to protect against restimulation-assessed desensitization. - Cooling (18-20°C) with 2.5 mmol/l calcium (blocking exocytosis but not calcium influx) only partly prevented desensitization (restimulated response, 37°C, 63 % of IGIS). Previous D during cooling exerted 1.7-fold higher effect. Cooling during omission of calcium totally prevented glucose-induced desensitization ; D during cooling did not alter this. - Specificity of D protective effect was tested by restimulating with non-glucose secretagogues. Previous D (co-perifused with 27mmol/l glucose) enhanced response to 10 mmol/l L-arginine and 2.5 mmol/l barium 2.8- and 5.0-fold respectively. Conclusions: Neither induction nor expression of D protective effects are directly coupled to drug interaction with ATP-sensitive K+-channels. Desensitization is partly induced distal to the calcium influx event.

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NORADRENALINE INHIBITION OF INSULIN SECRETION IS RELIEVED BY PHORBOL MYRISTATE ACETATE AND ARACHIDONIC ACID

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The mechanisms by which catecholamines inhibit insulin release remain obscure. We have investigated the effects of the protein kinase C (PKC) activator, 4ß phorbol myristate acetate (PMA) and the fatty acid, arachidonic acid (AA) on the inhibition of insulin secretion from rat islets by noradrenaline (NA). NA (10µM) fully inhibited both phases of glucose-stimulated insulin release. The secretory response to PMA, in the absence of exogenous glucose was also abolished by NA (0 glucose, 0.19±0.05; +500nM PMA, 0.61±0.04; +500nM PMA + 10µM NA, 0.28±0.04 ng/islet/h, n=8-9, $p<0.01$), but under the same conditions NA did not inhibit AA-stimulated insulin release (0 glucose, 0.24±0.05; +100µM AA, 0.64±0.07; +100µM AA +10µM NA, 0.61±0.09ng/islet/h, n=9, NS). At 20mM glucose PMA partially, but significantly ($p<0.01$), alleviated both the inhibitory effect of NA on insulin release (20mM glucose, 1.0±0.04; +500nM PMA, 1.9±0.1; +10µM NA, 0.5±0.04; +500nM PMA + 10µM NA, 0.8±0.05 ng/islet/15min, n=10) and on cAMP generation (20mM glucose, 54.1±4.8; +500nM PMA, 65.6±7.7; +10µM NA, 28.2±2.2; +500nM PMA + 10µM NA, 42.7±3.0 fmol/islet/15min, n=9-10). AA did not potentiate insulin secretion at 20mM glucose, but in its presence NA no longer exerted a significant inhibitory effect on insulin release (20mM glucose, 1.61±0.06; +100µM AA, 1.74±0.13; +100µM AA +10µM NA, 1.25±0.22ng/islet/h, n=9, NS). These results suggest that activation of PKC by PMA reduces the inhibitory capacity of NA at stimulatory concentrations of glucose, perhaps in part by reducing NA inhibition of cAMP generation. In addition, the inhibitory site of NA appears to lie largely proximal to the site of the secretory pathway at which AA exerts its effects.

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IS PROTEINKINASE C-ACTIVATION INVOLVED IN THE MEDIATION OF THE INCRETIN SIGNAL?

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The insulinotropic hormone glucagon-like peptide 1(7-36)-amide (GLP-1) is a potent candidate as incretin. It remains questionable whether other gastrointestinal hormones are additive to GLP-1 in eliciting the full insulin release after oral glucose. After the demonstration of the GLP-1 action in RINm5F-cells and isolated islets and the receptor characterisation we aimed to elucidate the intracellular signalling. Insulin release was determined in short term incubations. Membrane potential and cytosolic free calcium concentrations were monitored fluorometrically. While GLP-1 leads to a concentration dependent rise of cAMP levels in parallel to insulin release membrane potential and cytosol calcium remain unaffected. Thus, involvement of inositol phophate breakdown seems unlikely. Since GLP-1 leads to a maximal increase of cAMP levels the action of other incretins must be transduced via other signalling pathways. Therefore, proteinkinase C involvement was further investigated. At low and at maximal stimulatory concentrations of GLP-1 addition of 10^{-8} to 10^{-6} mol/l phorbol-myristate-acetate (PMA) leads to an additive stimulation of insulin release in RINm5F-cells and islets. Pretreatment with 10^{-8} mol/l PMA during 48 h downregulates proteinkinase C completely and abolishes PMA-induced insulin secretion while GLP-1-stimulated insulin relese is unaffected. In conclusion, the insulinotropic action of GLP-1 is intracellularly transduced by the cAMP-pathway. Proteinkinase C-activation is not involved and may be used for signal transduction of additional hormones.

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FREE ARACHIDONIC ACID INACTIVATES PROTEIN KINASE C IN MOUSE PANCREATIC ISLETS

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The role of protein kinase C in arachidonic acid (AA)-induced insulin secretion was investigated. In the absence of extracellular Ca^{2+}, exogenous AA (100 µmol/l) stimulated insulin secretion. AA-induced insulin secretion was not potentiated by the protein kinase C activator 12-O-tetradecanoylphorbol 13-acetate (0.16 µmol/l) and was not prevented by the protein kinase C inhibitor staurosporine (1 µmol/l). AA-induced insulin secretion in Ca^{2+} free medium was on the other hand potentiated by addition of extracellular Ca^{2+} (2.5 mmol/l). Stimulation of insulin secretion by exogenous AA was associated with inactivation of protein kinase C, and after 60 min of incubation with AA (100 µmol/l) islet protein kinase C activity was reduced to 53.5 ± 8.4 (11)% of control values ($P < 0.005$). In islet homogenate AA (100 µmol/l) stimulated protein kinase C activity by substituting for phosphatidylserine. Inactivation of protein kinase C was, however, also observed in islet homogenate after preincubation with AA in the absence of $MgATP^{2-}$. In conclusion, it is suggested, that free AA by inactivating protein kinase C may exert toxic effects in islets, and that AA may stimulate insulin secretion by a nonphysiological mechanism, involving an ionophoretic effect of AA to induce Ca^{2+}-mediated insulin release.

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INHIBITION OF INSULIN RELEASE BY DIAZEPAM-BINDING INHIBITOR (DBI) INVOLVES INTERACTIONS WITH PROTEIN KINASE C AND AN INHIBITORY G-PROTEIN

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The mechanism of inhibition of insulin release by diazepam-binding inhibitor (DBI) was investigated in a homologous system using rat DBI and isolated rat islets or electropermeabilised (2.5 kV/cm) rat clonal RINm5F cells. In rat islets, insulin response to 16.7 mmol/l glucose (1.9 ± 0.3 ng/islet/h; mean ± SEM, n=5) was inhibited by 24-40% ($P< 0.05$) by rat DBI (10-100 nmol/l). Using electropermeabilised RINm5F cells (incubated at 37°C in potassium glutamate buffer supplemented with ATP and ATP-regenerating system), rat DBI (10 nmol/l) decreased by 6-34% ($P <0.001$) the insulin response evoked by an increase in the ambient free Ca^{2+} concentration from 10^{-8} to 10^{-4} mol/l (3.2-fold insulin response ($P<0.001$) from basal: 9.1 ± 0.3 ng/10^6 cells/20 min, n=5). Protein kinase C (PKC) activation by 10 nmol/l 12-O-tetradecanoylphorbol-13-acetate (TPA) induced an approximate 3.1-fold stimulation ($P<0.001$) of insulin release from electropermeabilised RINm5F cells. The inhibitory effect of 10 nmol/l rat DBI at 10^{-8} mol/l to 10^{-4} mol/l Ca^{+2} was reduced 27-34% ($P<0.001$)' by 10 nmol/l TPA or after down-regulation of PKC by overnight pretreatment of RINm5 cells with 200 nmol/l TPA. The inhibitory effect of 10 nmol/l DBI on electropermeabilised RINm5F cells was abolished by overnight pretreatment with pertussis toxin (0.1 ug/ml). These results indicate that DBI-inhibition of insulin release involves PKC-induced phosphorylation and a pertussis toxin-sensitive inhibitory G-protein.

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NON-METABOLIZABLE α-AMINOISOBUTYRIC ACID EVOKES A GLUCOSE-DEPENDENT INSULIN SECRETORY RESPONSE IN β-CELLS.

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Although non-metabolizable analogues of the amino acid leucine induce an insulin secretory response, uncertainties existed as to whether the dissimilar α-aminoisobutyric acid (AIB) could influence insulin secretion. Studies have shown that Na^+-dependent transport systems, accessible to AIB, are present in insulin-producing β-cells. The effects of AIB were analysed both by column perifusion of adult mouse β-cells together with polyacrylamide beads, at a rate of 0.8 ml/min, and by intracellular sodium measurements using integrating flame photometry. The highly sensitive perifusion technique, made it possible to demonstrate a short-lived, repeatable insulin secretory response to AIB in the presence of 11 mmol/l but not 3 mmol/l glucose. Separate additions of 1 mmol/l and 10 mmol/l AIB resulted in peak increases of secretion of 50 and 500% respectively. Further analysis showed this effect to be physiological in its total suppression by both addition of 1 µmol/l clonidine and lowering of temperature to 22°C. The observed induction of insulin secretion was paralleled with a rise of intra-islet sodium. The importance of sodium uptake was further highlighted by complete suppression of the response by removing Na^+ from the extracellular media. It is concluded that a non-metabolizable amino acid co-transported with Na^+ can induce an instantaneous insulin secretory response from mature β-cells.

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GLUCOSE INDUCED B-CELL DESENSITIZATION: EVIDENCE AGAINST A MAJOR ROLE OF PROTEIN KINASE C.

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Prolonged hyperglycemia or high glucose in vitro induces B-cell insensitivity by unclarified mechanisms. Possible involvement of protein kinase C (PKC) was investigated by studying effects of previous exposure to phorbol myristate acetate (PMA). Rat pancreatic islets were cultured 16-18 hours in RPMI, 11 mmol/l glucose and 200 nmol/l PMA. They were then perifused 180 min with 27 mmol/l glucose and 0.1 mmol/l 3-Isobutyl-1-Methylxanthine (IBMX). Initial 0-30 min stimulated response was similar for previously PMA-exposed (95 ± 11 µU/islet/30 min) and control islets (110 ± 18 µU/islet/30 min). Subsequent secretion was lower in PMA-exposed (131 ± 29 µU/islet/150 min) than in control islets (216 ± 26 µU/islet/150 min, $P<0.05$). Following 20 min with 3.3 mmol/l glucose islets were restimulated 30 min with 27 mmol/l glucose + IBMX. PMA-exposed islets showed marked desensitization (14% of initial stimulation); corresponding desensitization for control islets was 20%. Islet insulin content at end of perifusions was 40% less in PMA-treated versus control islets. - Diazoxide during first stimulation blocked glucose-induced insulin secretion by 82% in PMA-exposed and by 93% in control islets, $P<0.05$ for difference. Previous diazoxide prevented restimulation-assessed desensitzation both in PMA-exposed (87% of initial stimulation) and in control islets (95% of initial stimulation) and increased islet insulin content 1.9- and 2.2-fold respectively. - Accelerated desensitization in PMA-treated islets may be secondary to decreased insulin reserves rather than modulation of PKC-activity.

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PARASYMPATHETIC INVOLVEMENT IN MEAL-ASSOCIATED CONDITIONED INSULIN SECRETION.

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During food intake vagally mediated insulin secretion plays a role in reducing blood glucose levels. The present study aims to investigate whether this insulin secretion can be conditioned. By clock-activated opening of doors in front of the food hopper rats were habituated to a feeding schedule of 6 meals in comparison with a schedule of 2 meals per day. Blood glucose and plasma insulin concentrations were measured in blood sampled via a permanently implanted cardiac catheter in freely moving rats. After opening of the doors insulin rapidly increased in the first minute during feeding in both conditions (16 ± 4 versus 33 ± 6 mU/L, $p < 0.05$, $n = 6$, for respectively the 6 and 2 meal schedule). After presenting an empty food hopper, insulin rose significantly in the first minute after opening of the door (31 ± 10 mU/L, $p < 0.05$) in the 2-meal/day condition but not in the 6-meal/day condition. This response was abolished following pharmacological blockade of nicotinic receptors by hexamethonium and muscarinic receptors with atropine. The present study shows that rapid conditioned insulin secretion can be evoked within 1 minute by a meal-associated stimulus. These results further indicate that this conditioned insulin secretion is vagally mediated and that its occurrence is dependent on the nature of the feeding schedule.

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ROLE OF THE CENTRAL NERVOUS SYSTEM IN INCREASED PANCREATIC ISLET BLOOD FLOW OF OBESE RATS.

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We have previously shown that pancreatic islet blood flow (IBF) was significantly higher both in the basal state and after an intravenous glucose load in obese than in lean Zucker rats. The aim of the present work, was to investigate whether the effect of glucose could be mediated by the central nervous system. Glucose (9 mg/kg) was injected towards the brain via a carotid artery in anesthetized lean and obese rats. IBF was determined by using the non radioactive microspheres technique. Glucose injection resulted in: 1) no significant changes of peripheral plasma glucose in both groups, 2) a similar increase above basal values of plasma insulin level (ΔI μU/ml lean 35±5, obese 46±6), 3) a higher increase of IBF in obese than in lean rats (ΔIBF μl/min: 203±38 vs 48±20). Both glucose-induced increase of plasma insulin and IBF were abolished by prior bilateral subdiaphragmatic vagotomy in lean rats and decreased significantly in comparison to basal values in obese rats. These data suggest that 1) the increased glucose induced IBF is mediated at least in part by a direct action of glucose in the brain 2) this central effect of glucose is abnormal in obese rats.

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THE EFFECT OF ADRENODEMEDULLATION AND TRAINING ON INSULIN SECRETION AND GLUCOSE METABOLISM IN PANCREATIC ISLETS.

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Chronical epinephrine treatment reduces the insulin response to glucose. Accordingly, we studied whether adrenomedullary hormones are responsible for the diminished glucose induced insulin secretion seen after training. To further elucidate the mechanism for this adaptation we also measured beta-cell glucose metabolism. Rats were adrenodemedullated (ADM) or shamoperated (C) and either swimtrained for 10 wks (T) or sedentary (S). At sacrifice body weight was lower ($p < 0.05$) in T than in S groups (412 ± 11 (SE) g (ADMT, $n = 8$), 378 ± 10 (CT, $n = 8$), 476 ± 20 (ADMS, $n = 8$), 509 ± 20 (CS, $n = 8$). Pancreatic islets were incubated at 3,10 and 20 mM glucose and insulin, $^{3}H_2O$ (from 5-^{3}H-glucose, overall glycolysis) and $^{14}CO_2$ (from U-^{14}C-glucose, glucose oxidation) were measured. In ADMS rats insulin secretion was increased ($p < 0.05$) at 3 mM (68 ± 7 ng/ml/5 islets/2 h vs 37 ± 5 (CS), 26 ± 8 (ADMT), 32 ± 7 (CT)). Insulin response was similarly depressed ($p < 0.05$) in both trained groups (at 10 mM: 49 ± 10 (ADMT), 50 ± 8 (CT) vs 69 ± 7 (CS), 62 ± 9 (ADMS). Glycolysis was higher ($p < 0.05$) in CT (3-10-20 mM: 914 ± 110, 2594 ± 415, 3764 ± 578 pmol/10 islets/2 h) than in CS (567 ± 134, 1173 ± 322, 1968 ± 264) rats and never affected by demedullation. Glucose oxidation showed the same pattern. Conclusions: Adrenomedullary hormones normally exert a trophic depression on basal beta-cell secretion but do not account for the training induced decrease in glucose stimulated insulin secretion. The latter is, surprisingly, accompanied by a training induced increase in beta-cell glucose metabolism.

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DIETARY RESTRICTION AMELIORATES IMPAIRED INSULIN SECRETION IN ISOLATED ISLETS OF AGING RATS.

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To explore the influence of a dietary restriction known to increase longevity in rodents (intermittent feeding), on the impairment in insulin secretory responsiveness of aging rats, islets were isolated from 25-mo-old Sprague-Dawley rats, fed either ad libitum (controls) or every other day (IF) over 22 months. During a 60-min static incubation, insulin release in IF islets was lower than in control islets at 2.8 mmol/l glucose (2.4 ± 0.2 versus 3.6 ± 0.5 ng/islet, $p < 0.05$) and higher at 16.7 mmol/l glucose (11.0 ± 1.8 versus 4.3 ± 0.7 ng/islet, $p < 0.01$). Furthermore, a clear-cut improvement of the secretory effectiveness was found in IF islets, in comparison with control islets, stimulated by either 20 mmol/l 2-ketoisocaproate (12.8 ± 1.2 versus 4.5 ± 0.4 ng/islet, $p < 0.01$, at 2.8 mmol/l glucose and 17.2 ± 1.8 versus 6.0 ± 1.3 ng/islet, $p < 0.01$, at 16.7 mmol/l glucose), or 20 mmol/l arginine plus glucose (13.3 ± 0.7 versus 5.6 ± 0.8 ng/islet, $p < 0.01$) or 1 mmol/l isobutylmethylxanthine plus glucose (20.6 ± 2.3 versus 7.1 ± 0.9 ng/islet, $p < 0.01$). Indeed, the feature of insulin scretory response of islets from IF aging rats is similar to that occurring in young animals. Immunoreactive insulin and glucagon content were higher in IF than in control islets (IRI, 446 ± 12 versus 356 ± 16 ng/islet, $p < 0.01$; IRG, 8.4 ± 0.6 versus 5.1 ± 0.2 ng/islet, $p < 0.01$). In conclusion, IF prevents the decline in insulin secretory efficiency of aging rats.

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AMYLIN TO INSULIN SECRETORY RATIOS IN OBESE SUBJECTS. W BLACKARD*, J CLORE*, D SCHROEDER, AND J KELLUM, Medical College of Virginia, P.O. Box 155, RICHMOND, VA 23298

Evidence suggests that insulin and amylin are co-secreted from the pancreas. The purpose of the present study was to determine the relative amylin and insulin secretory rates in man using portal vein catheterization. Four morbidly obese patients underwent portal vein catheterization at the time of gastric banding surgery. Baseline portal amylin levels were approximately 40% higher than peripheral (6.9±1.7 vs 4.9±1.0 pmol/L), in contrast to the two-fold elevation in insulin levels in portal blood reflecting the slower metabolic clearance rate of amylin. By subtracting peripheral amylin and insulin concentrations from portal vein amylin and insulin concentrations respectively, instantaneous relative amylin to insulin secretory rates can be determined. Insulin and amylin secretory rates were compared in each patient at eight time points before and after administration of 25 gm intravenous glucose. Insulin and amylin secretion rates were highly correlated in each patient ($r=0.77$ to 0.94, $p<0.05$), but large inter-subject variations in the ratio of amylin to insulin secretion were observed (.004 to .014). Thus, we conclude that the amylin secretory response to glucose is tightly coupled to the insulin response in any individual patient, but that the relative rate of amylin to insulin secretion may be determined by constitutional factors.

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ON THE MECHANISM OF THE INHIBITORY EFFECT OF AMYLIN ON INSULIN SECRETION. STUDY IN THE PERFUSED RAT PANCREAS.

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The study of the effect of amylin on insulin secretion has yielded conflicting results. In this work, we have investigated the influence of amylin on insulin release (IRI) as evoked by secretagogues which act on the B-cell via different mechanisms, i.e., a phosphodiesterase inhibitor [isobutyl-methyl-xanthine (IBMX), Sigma Chem. Co., 75 μmol/l)], and a dihydropyridine derivative, Ca^{2+}-channel activator (BAY K 8644, Bayer AG, 10 μmol/l). The study was performed 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). Amydated rat amylin (Peninsula Labs.) was infused at 75 pmol/l, so far the lowest concentration reported to inhibit glucose-induced insulin output. First, the inhibitory effect of 75 pmol/l amylin on the insulin response to glucose (9 mmol/l) was confirmed [incremental areas: 16±5 ,SEM,(N=5) vs. 57±9 ng/20 min (N=7) in control experiments; $p<0.05$]. Amylin reduced the insulin responses to both IBMX [incremental areas: 45±15 (N=6) vs. 182±63 ng/20 min (N=5) in control experiments; $p<0.05$] and BAY K 8644 [incremental areas: 28±11 (N=7) vs. 78±20 (N=5) ng/20 min in control experiments; $p<0.05$]. The present results would indicate that the inhibition of amylin on insulin secretion is, at least in part, mediated by cAMP as well as Ca^{2+} dependent mechanisms.

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IN VITRO RELEASE OF C-PEPTIDE AND INSULIN IS NOT EQUIMOLAR.

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Equimolar quantities of C-peptide and insulin are expected to be produced during proinsulin processing in the B-cell. To determine the proportion of these two peptides released during stimulation and inhibition of B-cell secretion, we cultured neonatal (3-5 days old) rat pancreata in monolayers and then incubated them in 16.7 mM glucose with or without epinephrine 10^{-7} M. C-Peptide and insulin immunoreactivity were measured in the medium following static incubations of 15 to 120 minutes. Molar ratios of C-peptide to insulin released were:

Time (min)	Glucose	Glucose + Epinephrine
15	28±3%	44±1% *
30	30±2%	40±4%
60	33±1%	44±3% *
90	33±1%	40±6%
120	29±1%	45±1% *

(x±sem; n=4 per condition; * $p<0.05$ vs glucose)

Despite expectations, three times as much insulin as C-peptide was released in response to glucose. Thus, although the molar ratio remained constant over time, C-peptide and insulin were not released in equimolar quantities. With epinephrine, both C-peptide and insulin release were inhibited but this inhibition was less for C-peptide (72%) than insulin (80%), so that the molar ratio increased over that observed in glucose alone. Based on these data we conclude that variable proportions of C-peptide and insulin exist in different B-cells or granules.

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NICOTINAMIDE, A POLY(ADP-RIBOSE)SYNTHETASE INHIBITOR, PROMOTES REGENERATION OF PANCREATIC B-CELLS.

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To investigate the effect of a poly(ADP-ribose)synthetase inhibitor, nicotinamide, on regeneration of pancreatic B-cells, 7-wk-old male Wistar rats received 90% pancreatectomy (Px), and a part of them were treated with nicotinamide (ND, 0.5 g/kg bw, ip) every day for one wk before and 12 wk after Px. Plasma glucose levels in fed ND rats were significantly lower than those in Px rats without ND treatment (8.4±0.5 vs. 10.2±0.5 mmol/l, $p < 0.05$). IPGTT (2 g/kg) showed marked hyperglycemia in Px rats, and ND treatment effectively ameliorated diabetes (120 min values; 20.8±1.5 vs. 9.9±1.4 mmol/l, $p < 0.005$). In vitro perfusion of the isolated pancreas, insulin secretion induced by 16.7 mmol/l glucose was blunted whereas insulin secretion induced by 19 mmol/l arginine was rather exaggerated in Px rats. In contrast, insulin secretion from the ND rat pancreas was not different from that in sham-operated animals. The dry weight of the pancreas was significantly higher in ND rats than in Px rats (0.13± 0.01 vs. 0.10± 0.00 g, $p < 0.01$). The insulin content in ND rats was not different from that of sham-operated rats (7.74±2.32 vs. 7.18±0.79 mU/mg dry wt). Histological examination revealed fibrotic degeneration and degranulation in the islets of Px rats, whereas the normal structure was retained in most islets of ND rats. These results suggest that ND promotes regeneration of pancreatic B-cells and ameliorates islet function.

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THE POTENTIATION OF EXOCYTOTIC INSULIN RELEASE BY GTP IS GLUCOSE-CONCENTRATION DEPENDENT

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To investigate the role of GTP in insulin secretion, isolated rat islets were cultured overnight in the presence of mycophenolic acid (MPA) or mizoribine, selective inhibitors of *de novo* GTP synthesis. Pre-exposure to either agent inhibited glucose-induced insulin secretion (by 54% at 25 μg/ml MPA); in parallel, either agent reduced GTP content (measured by HPLC) by 68-81%, from 2.71$\pm$0.08 to 0.52$\pm$0.03 pmol/islet. Both effects were prevented by co-culture with guanine (but not adenine, hypoxanthine or xanthine) to circumvent the GTP synthetic block by entering into the nucleotide "salvage" pathway. Although ATP was also moderately reduced (-39%), adenine (150 μM) increased ATP nearly to normal without restoring GTP content or insulin secretion. In contrast to these data from islets cultured at 11.1 mM glucose, secretion from islets cultured at 4.4 mM glucose was virtually insensitive to inhibition by MPA (-16%); correspondingly, their basal GTP content was lower and less inhibitable by MPA than that of islets exposed to the higher glucose concentration. Thus, glucose appears to activate the *de novo* pathway for GTP synthesis and thereby primes or potentiates subsequent insulin release. These studies provide the first direct evidence of a physiologic, regulatory role for GTP in exocytotic insulin release.

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MECHANISMS BY WHICH MASTOPARAN STIMULATES INSULIN SECRETION FROM RAT ISLETS OF LANGERHANS

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The amphiphilic peptide mastoparan (MP) is a component of wasp venom which activates GTP-binding (G-) proteins and stimulates secretion from a number of cell types. We have now investigated the effects of MP on insulin secretion from isolated rat islets. MP caused a temperature-dependent and dose-related stimulation of insulin secretion from intact islets at a substimulatory concentration of glucose (2mM glucose, 1.0$\pm$0.08 ng/islet/30min; +10μM MP, 4.6$\pm$0.7; +20μM MP, 6.4$\pm$1.2; +30μM MP, 6.2$\pm$0.6, mean$\pm$SEM, n=6, $p<0.01$) and enhanced glucose-induced secretion (20mM glucose, 7.4$\pm$0.7ng/islet/30 min; +30μM MP, 12.2$\pm$0.5, n=6, $p<0.01$). Although glucose-induced insulin secretion was dependent on the presence of extracellular Ca^{2+} (20mM glucose, 2mM Ca^{2+}, 4.3$\pm$0.6 ng/islet/30min; 20mM glucose, 1mM EGTA, 0.7$\pm$0.1, n=6, $p>0.2$), MP induced secretion was not inhibited by the omission of extracellular Ca^{2+} (30μM MP, 2mM Ca^{2+}, 8.7$\pm$0.6 ng/islet/30min; 30μM MP, 1mM EGTA, 13.4$\pm$1.4). In experiments using electrically permeabilised islets, MP stimulated insulin secretion at a substimulatory Ca^{2+} concentration (50nM Ca^{2+}, 230$\pm$15 pg/islet/30min; +30μM MP, 987$\pm$68, $p<0.01$, n=6), and potentiated Ca^{2+}-induced secretion (10μM Ca^{2+}, 697$\pm$91; +30μM MP, 1122$\pm$102, n=6, $p<0.05$). Ca^{2+}-induced insulin secretion from permeabilised islets was ATP-dependent (10μMCa^{2+}, 5mM MgATP, 434$\pm$27 pg/islet/min; 10μM Ca^{2+}, 0 ATP, 147$\pm$19, n=9, $p>0.2$). In contrast, MP stimulated insulin secretion from permeabilised islets in the absence of exogenous MgATP (+15μM MP, 490$\pm$33 % basal; +30μM MP, 629$\pm$44%, n=9, $p<0.01$). MP-induced insulin secretion (30μM) was not inhibited by the protein kinase inhibitor, staurosporine (SP), even at concentrations as high as 500nM SP (2mM glucose, 0.9$\pm$0.2 ng/islet/30min; +MP, 7.3$\pm$0.7; +MP, +500nM SP, 8.8$\pm$0.5, n=8, $p>0.2$). These results suggest that MP stimulates insulin secretion by a mechanism that is independent of changes in cytosolic Ca^{2+} or protein kinase activation, and are consistent with MP acting as a fusogen itself, or generating a fusogen by interaction with a G-protein within ß-cells.

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CLOSURE OF K-ATP CHANNELS IN ß-CELLS IS PRECEDED BY AN INCREASE IN NAD(P)H FLUORESCENCE AND HYPERPOLARIZATION OF THE MITOCHONDRIAL MEMBRANE

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The aim of this study was to determine the relative magnitude and time course of nutrient-induced changes in ß-cell metabolism, K-ATP channel activity and intracellular calcium. We used autofluorescence (450nm) to monitor NAD(P)H levels and the fluorescent dyes rhodamine 123 and flo3 to measure mitochondrial membrane potential and intracellular free Ca^{2+}, respectively. K-ATP channels were recorded from cell-attached patches using patch-clamp methods. Experiments were carried out at 37°C on isolated mouse ß-cells. Glucose, or ketoisocaproate, caused a dose-dependent hyperpolarization of the mitochondrial membrane. Metabolic inhibitors depolarized the mitochondrial membrane, indicating it is significantly polarized in the absence of exogenous substrate. Glucose produced a dose-dependent increase in NAD(P)H autofluorescence. The mean latency of the response to an increase of glucose from zero to 20mM was 40-80s for autofluorescence, 60-80s for mitochondrial hyperpolarization, and 2-2.5 min for the appearance of action potentials and the rise in $[Ca]_i$. Simultaneous recordings showed the increase in NAD(P)H is paralleled by K-ATP channel closure. These results are consistent with mitochondrial metabolism playing an important role in the regulation of K-ATP channel activity.

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MECHANISM OF ACTION OF AG-EE 623 ZW, A NOVEL INSULINOTROPIC AGENT.

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AG-EE 623 ZW (((S)-(+)-2-ethoxy-4-[2- ((3-methyl-1-[2-(1-piperidinyl)phenyl]-butyl)amino)-2-oxoethyl)benzoic acid) is a new insulinotropic agent for use in treatment of Type-2 (non-insulin-dependent) diabetes. It has a short plasma half life (< 1 hour) and is excreted predominantly via the bile. The aim of the present study was to determine the action and potency of AG-EE 623 ZW applying patch-clamp techniques to single ß-cells isolated from newborn rats (cell-attached, outside-out and whole-cell experiments). Thirty min prior to experiments cells were transferred to a Ringer's solution containing (in mM): Na+: 140, K+: 4, Ca++: 2, Mg++: 1, Cl-: 150, Hepes: 10, glucose and drugs in varying concentrations; pH: 7.4. All experiments were performed at room temperature. Glucose alone (16.7 mM) totally blocked the ATP-sensitive K+-channels (~70 pS in symmetrical K+) within approx. 8 min. Further, it was found that AG-EE 623 ZW, like glibenclamide, closed the ATP-sensitive K+-channels within approximately one min (90% closure at 10 nM AG-EE 623 ZW as compared to 90% closure with glibenclamide at 20 nM in the presence of 0 or 1 mM glucose). AGEE 623 ZW did not affect neither the Ca++-sensitive K+-channels (~200 pS) nor the smaller (~15 pS) K+-channels. In conclusion: AG-EE 623 ZW acts by closure of the ATP-sensitive K+-channels in ß-cells like the sulfonylurea glibenclamide and with approximately the same potency.

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EFFECTS OF TRYPSIN ON ATP-DEPENDENT K^+ CHANNELS FROM PANCREATIC ß-CELLS.

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Regulation of ATP-dependent K^+ channels, which play a key role in ß-cell stimulus-secretion coupling, is complex and poorly understood. We have studied the decline of channel activity (rundown) which occurs in inside-out patches, using patch-clamp methods and mouse ß-cells. Trypsin (type II and XI) at concentrations of 10 - 50 μg/ml prevents run-down of ATP-K^+ channels: considerable channel activity remains after 2 hours. Trypsin treatment does not affect the single channel conductance, voltage-dependent block by internal Mg^{2+} ions or decrease in channel activity produced by 1 mmol/l Mg^{2+}. Furthermore, trypsin treatment restores channel activity after complete rundown. Exposure to low pH (6.2) inhibits channel activity; this inhibition remains after return to control solution, but activity can be restored by trypsin treatment. All effects of trypsin were absent in the presence of trypsin inhibitor (type II-O, 20 μg/ml). Chymotrypsin (20 μg/ml), was unable to reactivate the channel. These results suggest that trypsin modifies part of the ATP-K^+ channel, or an associated protein, which regulates rundown.

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GLYCINE POTENTIATION OF INSULIN RELEASE INVOLVES Na^+-DEPENDENT INCREASE OF CYTOPLASMIC Ca^{2+}

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In the individual pancreatic ß-cell, the glucose response is manifested either as large amplitude oscillations of cytoplasmic Ca^{2+} $[Ca^{2+}]_i$ or as a sustained increase. Studying how amino acids cotransported with Na^+ modulate the ß-cell function, it was analyzed how glycine and its N-methyl analogue sarcosine affect $[Ca^{2+}]_i$ and insulin release. $[Ca^{2+}]_i$ was monitored in mouse ß-cells with dual wavelength microfluorometry using the indicator fura-2, and insulin release was measured by perifusing ß-cells mixed with polyacrylamide beads. At 3 mmol/l glucose, glycine and sarcosine had only marginal effects on $[Ca^{2+}]_i$ and secretion. However, in the presence of 11 mmol/l glucose, 1-10 mmol/l of the amino acids triggered immediate and dose-dependent responses. The $[Ca^{2+}]_i$ oscillations were transformed into a sustained elevation with increase of time-average $[Ca^{2+}]_i$ paralleled by a pronounced potentiation of insulin release. The initial peak and the following sustained stimulation represented maximal increases to 700 and 250 % respectively of the secretory activity induced by glucose alone. The effects of glycine on $[Ca^{2+}]_i$ and insulin release were strictly dependent on extracellular Na^+. It is suggested that glycine potentiation of insulin secretion reflects influx of Na^+, which results in elevation of time-average $[Ca^{2+}]_i$ by increased entry and reduced elimination of Ca^{2+} from the cytoplasm.

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A highly sensitive ELISA for total proinsulin in human serum based on two monoclonal antibodies.

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Present proinsulin immunoassays have a detection limit in serum of 1-2 pmol/l. At least 25 % of normal individuals have fasting values below this limit. A new two site proinsulin sandwich ELISA was developed using an anti-C-peptide antibody (PEP-001) and a biotin labelled anti-insulin antibody (HUI-001). Detection limit (3xSD above zero value) in buffer is 0.05 pmol/l corresponding to 0.25 pmol/l in serum (diluted 1:5). Calibrator range is 0.05-20 pmol/l. Analysis time is 36-48 hours and 150 μl serum is required for analysis. Interassay coefficient of variation (CV) is 4.7 % at a median (range) of 2.25 pmol/l (0-3 pmol/l, N=8), 6.7 % at 5.1 pmol/l (4-7 pmol/l, N=8) and 8.7% at 10.0 pmol/l (8-12 pmol/l, N=10). The assay correlates with the formerly used proinsulin ELISA based on polyclonal antibodies. R=0.96, $p<0.0001$, N=49, serum range 1.3-70 pmol/l, new = 1.09 x old -1.02. Mean recovery of added human proinsulin (2, 5, and 10 pmol/l) to serum is 84% (range 68-128). Human C-peptide and human insulin do not crossreact at 1000 pmol/l. The four major proinsulin intermediates crossreacted almost 100%. Serum from one total pancreatectomised patient shows absorbance values not distinguishable from background absorbance.
Conclusion: We have developed a simple, precise and highly sensitive ELISA for total proinsulin immunoreactivity useful for evaluation of remaining beta-cell secretion in patients with insulin-dependent diabetes mellitus.

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Islet Cells

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IMMUNOLOGICAL CHARACTERISATION OF PERTUSSIS TOXIN-SENSIT -IVE G-PROTEINS IN ISOLATED ISLETS AND CULTURED INSULINOMA CELLS

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Receptor-mediated inhibition of insulin secretion involves a class of G-proteins that are substrates for Pertussis toxin. Since both G_i and G_o belong to this class, we have studied the expression of these proteins in freshly isolated rat islets and in cultured HIT-T15 cells. Specific anti-G-protein antisera were used to probe islet or HIT cell proteins immobilised on nitrocellulose. Both cell types were immunoreactive for G_{i2}, but G_{i1} was not detected in rat islets. By contrast, up to 7 proteins, ranging in molecular mass from 30 KDa to 56 KDa, were labelled by a specific anti-G_{i1} antiserum in HIT cell membranes. When islets and HIT cells were probed with anti-G_o antisera, both were found to express two immunoreactive protein bands (39 and 40 KDa). Treatment of islet membranes with pertussis toxin and ^{32}P-NAD, resulted in radiolabelling of G_o (demonstrated by immunoprecipitation) revealing that these proteins do act as pertussis toxin substrates. These studies reveal the presence of multiple G-proteins in the endocrine pancreas and suggest that there may be differences in expression between rat islets and cultured HIT cells.

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GENERATION OF A SOMATOSTATINOMA CELL LINE DERIVED FROM A PLURIPOTENT TRANSFORMED ISLET CULTURE. P. Serup, F.G. Andersen, E.E. Petersen, and O.D. Madsen. Hagedorn Research Laboratory, DK 2820, Gentofte, Denmark.

MSL-G2 cells have previously been described as a highly heterogenous clone with multiple hormone/neurotransmitter expression. MSL-G2 cells preferentially express glucagon, cholecystokinin (CCK), and islet amyloid polypeptide (IAPP), but display small populations of CGRP, insulin, and somatostatin positive cells. Also the neurotransmitters serotonin and GABA are expressed in the majority of MSL-G2 cells. MSL-G2 is known to change its phenotypical composition when passaged in vivo and stable insulinomas as well as glucagonomas has been established. We now report the establishment of a highly stable somatostatinoma culture (MSL-G2-Tu6) which has been maintained in continous culture for more than a year. Almost 100% of the cells express high levels of immunoreactive somatostatin. Few insulin, glucagon, CCK, and IAPP cells are still present. Interestingly, a significant fraction of the cells expressed β-endorphin immunoreativity, implicating activation of the POMC gene. The derivation of the somatostatinoma cell line MSL-G2-Tu6 further support the stemcell nature of MSL cells. MSL-G2-Tu6 cells constitutes an ideal cell line in which to study tissue-specific expression of the somatostatin gene.

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ESTABLISHMENT OF AN ENDOCRINE CELL LINE FROM A HUMAN INSULAR PANCREATIC TUMOR

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A human insular cell line has been established from the primary culture of the liver metastasis of an insular tumor of the pancreas. The primary tumor and metastasis were positive for insulin and gastrin. Nine colonies of small epithelial cells with islet cell morphology were expanded and one of them cloned. The cell line was designated "Pancreatic endocrine line-1" (Pel-1). In the initial screening of the culture supernatants 12/29, 21/28 and 11/28 of the clones were positive for glucagon, gastrin and insulin respectively (RIA). Indirect immunofluorescence staining of monolayer cultures from both the cell line an some of the clones showed clear positivity for cytokeratin, glucagon, Glutamic Acid Decarboxilase (GAD, MoAb GAD-6, Developmental Studies Hybridoma Bank) and synaptophysin (Boehringer Manheim). Western blots demonstrated the presence of both forms of GAD (65 and 67 Kd). The electronmicroscopy revealed endocrine type of granules in the two clones studied. In conclusion, we have established a human insular cell line of potential interest as a source of native GAD and other putative islet autoantigens.

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INTRACELLULAR ISLET AMYLOID POLYPEPTIDE-DERIVED AMYLOID IN HUMAN INSULINOMAS AND IN EXTREME INSULIN RESISTANCE

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Islet amyloid polypeptide (IAPP)-derived amyloid deposits are characteristic of type 2 diabetes mellitus and have been reported in insulinoma. However, the pathophysiologic and cellular mechanisms of islet amyloidogenesis *in vivo* are unknown. To address this we studied human insulinomas (n=12) by immunohistochemistry and electron microscopy. 11/12 tumors contained amyloid (by Congo red) which showed IAPP immunoreactivity, and 12/12 tumors stained for IAPP intracellularly. Dense intracellular staining for IAPP suggested intracellular formation of IAPP-derived amyloid. This was supported by electron microscopy demonstrating widely distributed dense intracellular amyloid deposits. Since IAPP-derived fibril formation is concentration dependent we hypothesized that intracellular IAPP fibril formation in insulinoma is due to unregulated IAPP synthesis exceeding the maximal cell clearance rate. If this hypothesis is valid IAPP-derived amyloid would be anticipated under conditions of extreme beta-cell stimulation. To test this we examined the pancreas of a patient with chronic severe insulin resistance secondary to an insulin receptor antibody. The islets demonstrated nesidioblastosis, extensive extracellular IAPP-derived amyloid, and dense intracellular IAPP deposits. Thus we conclude that under conditions of excessive unregulated, or, massive stimulated IAPP synthesis, IAPP derived amyloid deposits form intracellularly. The mechanism of amyloidogenesis in diabetes mellitus remains to be determined.

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EXPRESSION OF A NOVEL SRC HOMOLOGY 2 PROTEIN IN PROLIFERATING INSULIN PRODUCING CELLS.

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To understand the cellular mechanisms regulating β-cell proliferation, changes in gene expression during the G_1 phase of the cell cycle was studied by a cDNA library subtraction screening procedure. For this purpose, mRNA was prepared from serum deprived (G_0 cells) or serum fed (G_1 cells) insulin producing βTC cells. Library subtraction screening using these two mRNAs yielded a positive cDNA clone, which contains a reading frame coding for amino acids with a strong homology to src homology 2 (SH2) domains of several other proteins. SH2 domains are amino acid sequence motifs targeting binding of the SH2-proteins to receptor tyrosine kinases. This property may allow the SH2-proteins to function as messengers from the receptors to their target systems. The novel SH2 gene is expressed in RINm5F cells, spleen and kidney, and at lower levels in adult islets and heart. It is postulated that the novel SH2 protein serves a role in coupling growth factor receptor activation with stimulation of β-cell replication.

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ISLET AMYLOID POLYPEPTIDE PLASMA CONCENTRATIONS IN PATIENTS WITH INSULINOMA

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Islet amyloid polypeptide (IAPP) is synthesized in normal pancreatic B-cells; it is cosecreted and coreleased with insulin. In insulin-producing endocrine tumours this process may be abnormal. An elevated IAPP content in tumour tissue as well as in plasma has been reported. We studied IAPP plasma concentrations in five patients (age 21-80 yr) with proven benign insulinoma,and in one patient with metastases from a malignant insulinoma. A sensitive and specific RIA was performed, using polyclonal rabbit anti-human antiserum. Preoperative basal plasma concentrations ranged from 1.7 to 10.5 pmol/l (mean 6.7 pmol/l; reference value 2-12 pmol/l. Simultaneous insulin concentrations ranged from 12 to 157 mU/l, ratio insulin/IAPP from 2.2 to 23.4. No constant relation between mRNA content and immunoreactive IAPP in tumour tissue has been found. The presence of amyloid depositions was not related to IAPP plasma levels. In conclusion, up to now we have found no elevated IAPP plasma concentrations in patients with sporadic or familial insulinoma. It is not known whether in every insulinoma tumour B-cells release IAPP into the blood. Whether circulating IAPP is mainly produced by tumour cells, with suppression of insulin and IAPP release by the normal islet B-cells remains to be elucidated.Insulin resistance in the hyperinsulinemia of insulinoma patients is not caused by high concentrations of plasma IAPP.

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SELECTION OF POTENTIAL ENDOCRINE PRECURSOR CELLS FROM THE HUMAN FETAL PANCREAS BY NEUROENDOCRINE MARKERS AND CELL SORTING

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Human fetal pancreata (n=11) and single cell preparations (n=15) of 11-17 weeks gestation were studied for the co-expression of endocrine pancreatic hormones and chromogranin-A, synaptophysin, A2B5, and HNK-1. Chromogranin-A was found in a proportion of insulin and glucagon containing cells in endocrine cell clusters and in single cells. Synaptophysin was expressed in the majority of all four endocrine cell types. A2B5 was not localized in endocrine cells. HNK-1 reactivity was present in delta cells in the periphery of endocrine cell clusters, but not in alpha, beta, or PP cells. Counting positive cells in single cell preparations, no correlation with gestational age could be found, except for HNK-1 (r_s=-0.511, $p<0.05$). A proportion of chromogranin-A-, synaptophysin-, and HNK-1-positive cells was hormone negative. To test these cells for proliferative activity, tissue fragments and single cell suspensions were incorporated with bromodeoxyuridine. No incorporation was found in chromogranin-A- or synaptophysin-positive cells. In contrast, 5% of A2B5-, and 8% of HNK-1-positive cells before 15 weeks gestation presented with BrdU-positive nuclei. After surface labelling HNK-1 positive cells were isolated by fluorescence activated cell sorting and enriched from 13 $\pm$ 4% to 74 $\pm$ 11%. Concurrently, delta cells were enriched from 0.8 $\pm$ 0.4% to 4.4 $\pm$ 1.8%. In conclusion, human fetal endocrine cell preparations may be enriched by cell sorting with surface markers, which allows the study of their growth and development in culture.

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AUTOFLUORESCENCE-ACTIVATED CELL ANALYSIS AND SORTING OF HUMAN ß-CELL FRACTION BY LIGHT SCATTER FLOW CYTOMETRY.

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A variety of tecniques using rat pancreas have been employed successfully to obtain purified ß-cells. Our aim was to develop a method for the preparation of single, pure and viable human pancreatic ß-cells in numbers sufficient for in vitro analysis. Islet isolation technique according to Gray's method was employed using 8 human pancreata from organ donors. After purification by hand-picking and culture, after different periods of criopreservation, vital islets (> 95%) producing insulin (0.4 ng/islet) were used to obtain endocrine cells. The cells were examined with FACS 440 on the basis of their respective intensities in forward light-scatter and in flavin adenine dinucleotide fluorescence. Islet cells gave different displays on the FACS-dot plot and were characterized by distinct cell populations of α- and ß-cells. The difference in autofluoescence of α- and ß-cells was much more pronounced permitting sorting by FACS-procedure with the achievement of three preparations: 1) insulin-containing ß-cells; 2) glucagon-containing α-cells; 3) coupled ß-cells. The cellular composition purity of the fractions was confirmed by electron and scanning microscopy. Only 2-6% of cells showed signs of damage after sorting. Immunostaining for insulin confirmed the high purity of ß-cells. The latter in culture maintained insulin production ability. Our results may be considered highly interesting not only with regard to human grafts with an excess of ß-cells, but also for other research fields which require purified ß-cells.

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A TECHNIQUE TO OBTAIN FUNCTIONAL, MONODISPERSED HUMAN ISLET CELLS.

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Preparation of single islet cells from rodent and porcine islets is relatively easy, but there are little data on human islets. The aim of this study was to develop techniques for dispersing human islets to obtain single cells. Pancreas was collagenase digested and islets hand-picked under stereomicroscopy. Monodispersion of human islets was attempted using trypsin, collagenases II, IV, V and XI, DNAse and hyaluronidase. Dispersion using EDTA, collagenases, hyaluronidase or a collagenase/trypsin/DNAse mixture gave low yields (median 192 cells/islet, range 23-253) with poor viability (59%, 43-97%). A trypsin/EDTA mixture gave the highest viability (92%, 75-97%) and yield of single cells (868 cells/islet, 549-1193) which secreted insulin (5.3 pg/1000 cells/hour) in 20mM glucose and had a median viability of 68% after overnight culture. Using trypsin/EDTA a total of 48 $x10^6$ islet cells was obtained from 55,300 islets. Passing these through nylon wool gave a suspension comprising >80% single cells, 32% of which stained for cytoplasmic insulin, indicating the ß cell content of the preparation. Cytofluorimetry showed that islet cells express class I but not class II MHC molecules. This study shows that separation of intact, functional, monodispersed human islet cells is readily achieved and on a large scale, providing the optimum targets for studying damaging autoimmune reactions in Type 1 diabetes and potentially a more efficient substrate for transplantation.

PS 12
Ionic Mechanisms in Islets

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SULFONYLUREAS MIMIC THE EFFECT OF GLUCOSE IN PROMOTING Na^+ INFLUX INTO PANCRATIC ß-CELLS

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It has been proposed that hypoglycemic sulfonylureas in addition to closing the ATP-regulated K^+ channels also promote the entry of Na^+ into the pancreatic ß-cells. This hypothesis was evaluated using integrating flame photometry for measuring sodium content of isolated mouse islets after inhibition of the sodium pump with 1 mmol/l ouabain. When added to a medium containing 3 mmol/l glucose, the hypoglycemic sulfonylureas tolbutamide (10-1000 µmol/l), glipizide (10 µmol/l) and glibenclamide (10 µmol/l) mimicked the effect of increasing glucose concentration in promoting uptake of sodium. Whereas 400 µmol/l diazoxide had the opposite effect, the presence of 1 mM of the non-hypoglycemic sulfonamides sulfadiazine and sulfadoxine did not affect the sodium uptake. Neither was there any effect when 100 µmol/l tolbutamide was added to a medium containing 20 mmol/l glucose. Sulfonylurea stimulation of the sodium uptake was also seen in the presence of 4 µmol/l of the Na^+ channel blocker tetrodotoxin. The results support the idea of an important role for a sulfonylurea-induced influx of Na^+ in amplifying the secretory response of the ß-cells additional to the depolarisation obtained with suppression of their K^+ conductance.

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THE SODIUM-CHANNEL ACTIVATOR BDF 9148 INHIBITS GLUCOSE-INDUCED INSULIN RELEASE IN MOUSE ISLETS!

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Na^+-channels are known to be present in islets of Langerhans, though their role in the glucose-mediated insulin release is unclear. To further evaluate this role in isolated mouse islets, we used BDF 9148 (4-[3'-1''-benzhydryl-azetidine-3''-oxy)-2'-hydroxypropoxy]-1H-indole-2-carbonitrile) a new cardiotonic agent which delayes the inactivation of Na^+-channels in other tissues. BDF 9148 inhibited concentration dependent the glucose induced (16.7 mM) insulin release, the effect being most pronounced at 100 µM (361.3 ± 33.1 vs 142.7 ± 14.3 µU/5 islets, $p<0.05$). BDF 9148 (10 µM) also inhibited insulin release evoked by tolbutamide (100 µg/ml) or KCl (20 mM) significantly but was ineffective on insulin release induced by low glucose concentrations (3 to 11.1 mM). BDF 9148 (10 µM) neither affected $^{86}Rb^+$ efflux nor $^{45}Ca^{2+}$ uptake induced by glucose, tolbutamide or KCl. Electrical activity of mouse islets recorded by glass-microelectrode technique remained unchanged in the presence of 10 µM BDF 9148 both, at 3 or 16 mM glucose. Our data show that in the mouse islet the activation of Na^+-channels with BDF 9148 inhibits glucose-stimulated insulin release though this effect does not affect Ca^{2+} uptake and electrical activity suggesting that BDF 9148 acts at a site following the uptake of Ca^{2+}.

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RUNDOWN OF ATP-SENSITIVE K-CURRENTS IN MOUSE PANCREATIC ß-CELLS

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Studies of the ATP-sensitive K^+-channel (K-ATP channel) using the patch-clamp technique are hindered by the rapid decline of K-ATP channel activity on forming an excised-patch or going whole-cell ("rundown"). Rundown is reduced by intracellular Mg-ATP but not by non-hydrolysable ATP analogues, suggesting that phosphorylation maintains channel activity and that rundown is due to dephosphorylation. We have examined this idea using mouse pancreatic ß-cells. In the whole-cell configuration with standard internal solution (107KCl, 2$MgCl_2$, 1$CaCl_2$, 11EGTA, 10HEPES in mM, pH7.2 with KOH) in the pipette, channel activity declines to 50% of maximum with a half time, $t_{1/2}$=4.0±0.3min (n=8). Addition of 0.3mM ATP to the internal solution (0.16mM MgATP) reduced $t_{1/2}$ to 11±3min (n=5). Microcystin (10μM), an inhibitor of protein phosphatase 1, 2A and 2B did not significantly affect rundown ($t_{1/2}$=3.5±0.5min,n=10), however, addition of 10μM protein kinase A inhibitor peptide with 0.3mM ATP reduced rundown significantly ($t_{1/2}$=18±4min,n=5). Rundown was also increased by Mg^{2+}: $t_{1/2}$=16±2min (n=4) with 5nM free Mg^{2+}; $t_{1/2}$=7±1min (n=3) with 650μM Mg^{2+}. These results suggest that rundown is a Mg^{2+}-dependent process and that phosphorylation by protein kinase A is probably not involved in maintaining K-ATP channel activity.

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VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) - EFFECT ON CALCIUM FLUXES OF MOUSE ISLETS

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We have recently shown that VIP stimulates insulin secretion and $^{45}Ca^{2+}$ net uptake in isolated mouse islets even at basal glucose concentrations (3 mM) without affecting ATP-sensitive K^+-channels. Moreover VIP increased electrical activity but only at high glucose concentrations (16.7 mM). To clarify the influence of VIP on Ca^{2+} movements we investigated its effect on $^{45}Ca^{2+}$ uptake (silicone oil method) employing Ca^{2+}-antagonists and $^{45}Ca^{2+}$ efflux (perifusion technique). At 16.7 mM glucose VIP increased $^{45}Ca^{2+}$ uptake from 75.0 ± 4.3 to 101.6 ± 9.8 pmol/10 islets; $p<0.05$. The Ca^{2+}-channel blockers isradipine or cobalt reduced the glucose-induced $^{45}Ca^{2+}$ uptake to almost basal levels (29.08 ± 2.22 resp. 31.75 ± 2.37; $p<0.05$). Addition of VIP antagonised this inhibition to 49.5 ± 6.0 resp. 56.3 ± 7.2; $p<0.05$. Simultaneous measurement of $^{45}Ca^{2+}$ efflux and insulin secretion showed an enhanced insulin release with VIP but surprisingly no changes in $^{45}Ca^{2+}$ efflux. In either a Ca^{2+}-deprived medium or if Na^+ was substituted by choline no effet of VIP was seen. Presence of VIP during the preloading period did not affect the increase in the $^{45}Ca^{2+}$ efflux rate by carbamylcholine. Our data show that VIP-stimulated Ca^{2+} uptake is partly independent from the activation of voltage-dependent Ca^{2+}-channels and that this increase is not associated with appropriate changes in the Ca^{2+} efflux rate.

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DIFFERENT RESPONSIVITY TO GLUCOSE OR GLYBURIDE OF RAT PANCREATIC ISLETS PRE-EXPOSED TO IL-1ß: POSSIBLE INVOLVEMENT OF K^+ AND CA^{2+} CHANNELS.

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In the early phases of IDDM, ß-cells loose the capacity to secrete insulin in response to glucose but not to other secretagogues. Since *in vitro* ß-cell exposure to IL-1ß inhibits the islet responsivity to glucose, we have studied whether a similar IL-1ß inhibition occurs also in response to other stimulators. Moreover, we have also investigated the possible involvement of ion channels in this IL-1ß effect.

Rat pancreatic islets were cultured for 24 h in the presence or absence of 50 U/ml IL-1ß and then stimulated for 1 h at 37°C. Basal Insulin secretion was 117±32 pg/islet/h (m±SE, n=7) in control islets and greatly increased in response to 16.7 mM Glucose (2140±293), 10 mM Arginine + 8 mM Glucose (1679±307) and 10 µM Glyburide (1464±234). When islets were exposed to IL-1ß, insulin release was significantly reduced ($p<0.001$) in response to Glucose (323±80) and Arginine + 8 mM Glucose (167±46) but not in response to Glyburide (1316±185).

In control islets, the increased insulin secretion in response to both 16.7 mM glucose or 10 µM glyburide was associated to the reduction of ^{86}Rb efflux (Δ of decrement -50±1.2 and -49±2.3% respectively, m±SE, n=5). In contrast, in IL-1ß treated islets both glucose and glyburide stimulation only slightly modified ^{86}Rb efflux (Δ -19±1.9 and -5.3±3.1% respectively, n=5, $p<0.001$).

Furthermore, in control islets basal $^{45}Ca^{2+}$ uptake (2.6±0.4 pM/islet/20 min, m±SE, n=6) increased to 16.8±3.2 after glucose and to 10.7±2.1 after glyburide. In IL-1ß treated islets basal Ca^{2+} uptake was 4.6±0.6 and significantly reduced after glucose (7.1±1.1, $p<0.01$) but not after glyburide (12.8±2.5).

The present data demonstrate that rat pancreatic islets treated with IL-1ß for 24 h loose their responsivity to glucose and aminoacid-stimulation, but not to glyburide. The ability of glyburide to stimulate IL-1ß treated islets is probably due to its capacity to directly influence the Ca^{2+} uptake.

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HUMAN PANCREATIC B-CELLS POSSESS TWO TYPES OF CA-CHANNEL

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Ca^{2+}-influx through voltage-gated Ca^{2+}-channels is the key signal for the stimulation of insulin secretion from pancreatic ß-cells. Using the patch-clamp technique we have investigated single Ca^{2+}-channel currents in human ß-cells. Human ß-cells, isolated with permission from pancreatic islets of normal cadaver organ donors, were bathed in a high-K^+ solution to zero the membrane potential. Single-channel Ca^{2+}-currents were recorded from cell-attached patches using pipettes containing 100mM $BaCl_2$. With holding potentials of -90mV two types of single-channel Ca^{2+}-currents were elicited by depolarizing pulses. The first (T-type) activated at potentials positive to -70mV, inactivated during the pulse, had a slope-conductance of 14±0.5pS (n=15) and was insensitive to the dihydropyridine agonist, BAY-K-8644. The second (L-type) was activated by potentials positive to -30mV, showed little inactivation, was larger in size, (slope-conductance: 21±0.8pS, n=9), and had channel openings which were greatly prolonged by BAY-K-8644. These properties are characteristic of T- and L-type Ca^{2+}-channels.

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EXOCYTOSIS INDUCED BY A SINGLE ACTION POTENTIAL IN INSULIN-SECRETING B-CELLS.

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Using the whole-cell configuration of the patch-clamp method, microfluorimetry and a circuit-analysis technique to measure changes in membrane capacitance ($C_m \propto$membrane area), we have monitored the correlation between Ca^{2+}-currents, cytoplasmic Ca^{2+}-concentration ($[Ca^{2+}]_i$) and changes in insulin release in mouse B-cells. A single action potential is occasionally sufficient to induce exocytosis. Larger responses were obtained by 4 or 8 action potentials. Ca^{2+}-currents, ($[Ca^{2+}]_i$) and exocytosis exhibits the same voltage-dependence with maximum responses at +20 mV. The Ca^{2+}-currents, exocytosis and $[Ca^{2+}]_i$ could be blocked by 5 mM extracellular Co^{2+}. When 2 mM EGTA was included in the pipette solution, the capacitance- and $[Ca^{2+}]_i$-response to a voltage-clamp pulse disappeared. In some cells, a single action potential produced a change in $[Ca^{2+}]_i$ sufficient to initiate exocytosis. However, in most cells 4 or 8 action potentials were required. This suggests that intermittent electrical activity consisting of action potentials generated at a high frequency represents a more effective way of initiating the exocytotic machinery, than continuous electrical activity consisting of action potentials fired at a lower frequency. This may represent a functional explanation to the fact that the B-cell produces bursts of action potentials.

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PITUITARY ADENYLATE CYCLASE ACTIVATING PEPTIDE INCREASES INSULIN AND CYTOSOLIC Ca^{++} IN RAT ISLET β-CELLS.

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Pituitary adenylate-cyclase activating peptide (PACAP) was suggested to function as a neurotransmitter, vasodilator and hypophysiotrophic factor. The purpose of this study is to investigate the effects of PACAP on insulin release and the mechanisms to influence the secretion. We examined insulin secretion from incubated and perifused rat islets and measured cytosolic Ca^{++} in single rat β-cells by microfluorometry using fura-2. PACAP increased insulin secretion from incubated islets dose-dependently (0.93, 1.04 and 1.37pmole/50islets/hr with 10^{-10}, 10^{-9} and 10^{-8}M PACAP respectively, $p<0.01$ vs control) in the presence of 8.3mM glucose but failed in the presence of 2.8mM glucose. PACAP also increased cAMP content by 71% in islets. The most remarkable increase in insulin secretion was observed for the initial 10 min upon PACAP stimulation. PACAP also produced a transient increase in cytosolic Ca^{++} in β-cells only in the presence of an elevated(8.3mM) glucose. 10^{-6}M Nitrendipine, a specific blocker of L-type Ca^{++} channel, inhibited completely the PACAP-induced increase in both insulin release from perifused islets and cytosolic Ca^{++} in single β-cells. These data suggest that PACAP may be a potenciator of insulin release in pancreatic islets and that the potenciating effect appears to be mediated by the rise in cytosolic Ca^{++}.

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MECHANISMS OF THE CONTROL OF INSULIN RELEASE BY GLUCOSE IN ABSENCE OF CHANGES IN B CELL MEMBRANE POTENTIAL

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Glucose stimulation of insulin release depends on a rise of cytoplasmic Ca^{2+} in B cells, which results from an influx of Ca^{2+} through channels activated by membrane depolarization. Recent evidence, however, suggests that glucose can control release by an additional mechanism that can be evidenced when the membrane potential of B cells is clamped at a depolarized level by a medium containing diazoxide and high K^+. Incubated and perifused mouse islets were used to identify this mechanism. The increase in insulin release that glucose causes under these conditions is not due to a further rise in cytoplasmic Ca^{2+}. It cannot be ascribed to acceleration of phosphoinositide metabolism, or to activation of protein kinases A or C. Thus, glucose did not increase inositol phosphate levels and hardly affected cAMP levels. Moreover, increasing inositol phosphates by vasopressin or cAMP by forskolin, and activating PKC by phorbol esters did not mimic the action of glucose on release. On the other hand, the effects of glucose and other agents on insulin release correlated with their ability to raise the ATP/ADP ratio. We suggest that changes in ATP availability contribute to the control of insulin release that glucose can exert independently from changes in B-cell membrane potential and cytoplasmic Ca^{2+}.

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DUAL EFFECTS OF Na/K PUMP INHIBITION ON CYTOPLASMIC Ca^{2+} OSCILLATIONS IN PANCREATIC ß-CELLS.

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The effects of inhibiting the Na/K pump on the cytoplasmic Ca^{2+} concentration $[Ca^{2+}]_i$ in mouse ß-cells was studied using dual wavelengh fluorometry and the indicator fura-2. The activity of the ATP-regulated K^+ channel was determined with the patch-clamp technique and intracellular sodium with micro flame photometry. At 3 mmol/l glucose, addition of ouabain or removal of K^+ resulted in a small gradual increase of intracellular sodium. This action of ouabain was parallelled by closure of the ATP-regulated K^+-channels and a slow elevation of $[Ca^{2+}]_i$. In most ß-cells increase of the glucose concentration to 11-20 mmol/l induced large amplitude oscillations of $[Ca^{2+}]_i$ with a frequency of 0.2-0.5/min. Ouabain had dual actions on these glucose-induced oscillations in promoting their appearance and at higher concentrations, transforming them into a sustained increase of $[Ca^{2+}]_i$. Ouabain (100 μmol/l) reduced the frequency of the glucose-induced oscillations but nevertheless raised time-average $[Ca^{2+}]_i$ by increasing the amplitudes and half-widths of the $[Ca^{2+}]_i$ peaks. When high concentrations of ouabain or removal of K^+ transformed the oscillations into a sustained increase of $[Ca^{2+}]_i$, the level reached exceeded that obtained in response to rise of glucose alone.

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NOVEL PEPTIDE PRODUCTS OF PROSOMATOSTATIN (pro-SS) PROCESSING IN PORCINE PANCREAS AND ANTRUM.
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We previously identified proSS 1-64 as the predominating N-terminal product of proSS in pancreas and gut, but a decapeptide (antrin, proSS 1-10) has been described to be produced in antral D-cells and to be secreted from islets. Using a radioimmunoassay against the N-terminus of proSS we isolated immunoreactive peptides from fresh specimens of both antral mucosa and pancreas. By analytical HPLC the major form was identified to be proSS 1-64, but additional forms were found and characterized by mass spectrometry and sequence analysis: one in antrum corresponding to proSS 1-14 and 2 in pancreas, proSS 1-14 and 3-14. Synthetic proSS 1-10, well detected in the system, was not found in either tissue. By analytical HPLC on effluent from isolated perfused preparation of porcine antrum and pancreas stimulated with luminal HCL or intravascular isoproterenol these peptides were shown also to be secreted. Synthetic replicas of proSS 1-10 and 1-14 infused at 10^{-8} mol/l had no effect on secretion of gastrin, insulin or glucagon from these perfused preparations. Their role if any must be sought outside pancreas/antrum. The processing site (Ser-Leu) for the formation of proSS 1-14 or 3-14 is novel.

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GLUTAMATE STIMULATES GLUCAGON RELEASE BY ACTING ON SPECIFIC RECEPTORS IN RAT PANCREAS.
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L-glutamate, the major excitatory neurotransmitter in brain, exerts its effects through two main receptor subtypes : the N-methyl-D-aspartate (NMDA) and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. Recently we have shown that L-glutamate potentiates glucose-induced insulin secretion via an AMPA receptor subtype. This study was designed to investigate whether this amino acid may also affect pancreatic glucagon secretion and to characterize the receptor subtype involved. In isolated rat pancreas perfused with 2.8 mM glucose, L-glutamate (30-100 µmol/l) induced an immediate, transient and concentration-dependent glucagon response with a maximum of + 300 ± 20 % at 3 min. NMDA (100-1000 µmol/l) was ineffective. In contrast the three non-NMDA receptor agonists AMPA (30-100 µmol/l), quisqualate (3-10 µmol/l) and kainate (30-1000 µmol/l) all elicited a peak-shaped glucagon response. The two partial agonists for the AMPA receptor, quisqualate and AMPA exhibited a similar efficacy to that of glutamate whereas kainate, a full agonist, caused a 4 fold higher maximal glucagon response. The antagonist of AMPA receptor, 6-cyano-7-nitroquinoxaline-2,3-dione (50 µmol/l) totally prevented the glucagon response to glutamate (100 µmol/l). In conclusion, as previously reported on insulin release, the stimulatory effect of L-glutamate on glucagon secretion is mediated by an excitatory amino acid receptor of the AMPA subtype.

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EFFECTS OF ALPHA-2 AND BETA ADRENERGIC AGONISM ON PANCREATIC A CELLS IN RATS.
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Insulin secretion is inhibited by alpha-2 adrenergic agonism and stimulated by beta adrenergic agonism in both experimental animals and humans. In contrast, there is controversy with regards to adrenergic control of pancreatic A cells. Furthermore, it is not clear whether these effects are direct, because intra-islet insulin is a potent inhibitor of glucagon secretion. The present study was designed to determine the effects of alpha-2 and beta adrenergic agonism on pancreatic A cells, using isolated perfused pancreata of normal and streptozocin-induced diabetic (STZ-D) rats. Insulin and glucagon concentrations were measured by RIA. The alpha-2 agonist clonidine at 10^{-7}mol/l significantly ($p<0.05$) stimulated glucagon secretion as compared with the basal levels in both normal (1330±212 vs 420±71ng/l) and STZ-D rats (534±140 vs 100±8ng/l). Also, the beta agonist isoproterenol at 10^{-7}mol/l significantly stimulated glucagon secretion vs basal levels in both normal (915±122 vs 397±37ng/l) and STZ-D rats (171±33 vs 72±12ng/l). Furthermore, these effects were inhibited in the presence of the alpha-2 antagonist yohimbine or the beta antagonist propranolol at concentrations of 10^{-6}mol/l. Insulin secretion was markedly reduced in STZ-D rats. We conclude that both alpha-2 and beta adrenergic agonism may directly stimulate glucagon secretion from pancreatic A cells in rats.

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A ROLE FOR BASIC FIBROBLAST GROWTH FACTOR IN THE DEVELOPMENT OF HUMAN PANCREATIC ISLETS
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We have previously shown that adult rat and human islet cells are resistant to the action of basic fibroblast growth factor (bFGF)-saporin mitotoxin, suggesting the absence of functional bFGF receptors. In contrast, all of the cells present in human fetal pancreatic monolayers were killed after 72-h incubation with bFGF-saporin. To explore the role of bFGF in fetal islet development immunohistochemical and in situ hybridization studies of human fetal pancreases were done at 13 to 24 gestational weeks. Basic FGF immunoreactivity was concentrated at the epithelial-mesenchymal cell boundary, whereas bFGF receptor was expressed in the majority of pancreatic cells. The effects of bFGF on the function of free-floating islet-like cell clusters were studied after 7 days in RPMI 1640/1% human serum (expressed as µU of insulin or cpm per µg of DNA):

	Insulin release	Insulin content	^{3}H-thym. incorp.
Control	78.8±16.4	395±66	2300±215
bFGF 10 ng/ml	25.4±3.8*	318±34	3318±372
bFGF 100 ng/ml	19.7±2.7*	338±26	3897±294*

Data are mean±SEM of 13-15 replicates from 3 experiments.
*, $p<0.001$, vs. contr.

Our results suggest that bFGF may act as a stromal mediator of fetal islet cell growth and differentiation.

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ATTACHMENT IS IMPORTANT IN THE STIMULATION OF ISLET CELL GROWTH.

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The effect of attachment on pancreatic islet cell proliferation was studied using ^{3}H-Thymidine and 5-bromo-2'-deoxyuridine (BrDU) incorporation into neonatal rat islets with the aim of elucidating culture conditions favouring islet growth. Islets were isolated using a non-enzymatic method and sub-cultured to eliminate fibroblast contamination. In medium containing 11.1 mmol/l glucose (baseline) ^{3}H-Thymidine incorporation (12,475 cpm/µg DNA ±2777(SEM)) in attached islets was significantly higher than in free-floating islets, (5,074±758, p<0.001). ^{3}H-Thymidine incorporation in attached islets remained significantly higher than in free-floating islets at all glucose concentrations, at 26.1 mmol/l (the highest concentration tested) being 22,129±7,938cpm/µg DNA vs. 8,473±954 (p<0.01). There was no difference in ^{3}H-Thymidine or BrDU incorporation in the presence or absence of basic fibroblast growth factor (FGF) between attached and free-floating islets, although there was a three-fold increase when "fibroblast-contaminated" islets were plated as controls. ^{3}H-Thymidine incorporation was significantly higher at 21.1 mmol/l glucose in comparison with baseline in both attached (43,043±9,203, p<0.007) and free-floating (11,789±1,610, p<0001) islets. In conclusion, a fibroblast free islet culture was used to document the stimulatory effect of islet attachment on DNA synthesis, which was greater than the stimulatory effect of glucose in the tested range.

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Glucokinase and GLUT-2 glucose transporter gene expression in neonate pancreatic islets and RINm5F insulinoma cells

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Neonate pancreatic islets and RINm5F insulinoma cells show a defective insulin secretory response to glucose stimulation. Glucokinase and GLUT-2 high K_m glucose transporter gene expression were studied in pancreas from 2 day old neonatal and 16 day old rats after weaning to a high carbohydrate diet. The 2.8 kb glucokinase mRNA transcript was expressed already in pancreas from 2 day old neonatal rats, whereas distinct expression of the 4.4 kb glucokinase-related transcript occurred only in the pancreas from 16 day old rats. GLUT-2 glucose transporter was expressed already in the pancreas from 2 day old neonatal rats, albeit to a lesser extent than in the pancreas from 16 day old rats. Northern Blot Analysis of glucokinase gene expression in RINm5F insulinoma cells revealed a 2.8 kb and a 4.4 kb transcript, while GLUT-2 glucose transporter mRNA was undetectable. Sodium-butyrate treatment led to a cell arrest. This treatment increased the 2.8 kb glucokinase mRNA levels by 74 %, but decreased the GLUT-1 and did not improve GLUT-2 glucose transporter gene expression. Thus the immature insulin secretory response to glucose in neonate pancreas and RINm5F cells may be related to a deficient glucokinase and GLUT-2 glucose transporter gene expression.

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INTERCELLULAR DIFFERENCES IN B CELL RESPONSIVENESS TO GLUCOSE ARE NOT CAUSED BY DIFFERENCES IN GLUCOSE TRANSPORTER GENE EXPRESSION

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Expression levels of the glucose transporter GLUT2 have been proposed to determine functional responsiveness of pancreatic B cells. This concept was tested by comparing GLUT expression in LOW and HIGH rat B cell subpopulations which differ in their metabolic activity and threshold sensitivity for glucose-induced biological functions. Rates of 3-O-methyl glucose uptake were comparable in LOW and HIGH B cells (15-20 pmol/min/10^3 B cells at 37°C - 7.5 mM substrate) and exceeded maximal rates of glucose utilisation by two orders of magnitude. Immunostaining with GLUT2 antisera was homogenous and ubiquitous in LOW and HIGH B cells; no GLUT1 or GLUT4 immunoreactivity was detected. Northern blot analysis of LOW and HIGH B cells resulted in similar relative abundance of GLUT2 mRNA over ß-actin mRNA. Low levels of GLUT1 mRNA were detected in both subpopulations after prolonged exposure times. PCR amplification of GLUT cDNA fragments with isoform-specific and degenerate primer sets was followed by dot-blot hybridisation and sequence analysis. LOW and HIGH B cells had the same pattern of GLUT expression : predominance of GLUT2, low amounts of GLUT1 and no GLUT4 or other GLUT-like isoform. It is concluded that the previously described functional and metabolic heterogeneity of pancreatic B cells cannot be explained by quantitative or qualitative heterogeneity in GLUT expression.

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HUMAN PANCREATIC ISLET FUNCTION AT THE ONSET OF TYPE 1 DIABETES MELLITUS.

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We report data concerning pre-proinsulin mRNA and insulin content, as well as insulin secretory behaviour, of human pancreatic islets at the onset of Type 1 diabetes mellitus. Islets were isolated from the pancreas of a ICA positive 18-years old woman donor who died because of diabetic ketoacidotic coma. She was free of symptoms until two days before, when polydipsia and polyuria were initiated. Islets were obtained by an automatic digestion technique. Insulin response to glucose (5.5 and 16.7 mM), glucose+forskolin (16.7 mM + 0.005 mM) and insulin content were analyzed. Total RNA was isolated by the method of Chirgwin. Results were compared with those obtained processing control pancreas by the same methodology. Insulin response to increasing glucose concentration, as well as glucose+forskolin in the media, was fully abolished in diabetic islets, as oppossed to control islets. Insulin content was reduced, but not absent, in diabetic islets (395.0±3.5 µU/islet), when compared to control islets (935.0±51.7). Northern blot disclosed a severe reduction in the content of pre-proinsulin mRNA. Immunohistochemical study of pancreatic sections reveals that only 12.2±4.8% of islets contains insulin positive cells. Our results demonstrate a decrease in insulin synthesis and secretion at the onset of Type 1 diabetes mellitus.

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INSULIN GRANULE ELECTRONDENSITIES: AN INDEX FOR THE METABOLIC STATUS OF RAT B-CELLS.

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The aim of our study was to determine whether the percent of pale over total insulin granules correlates with the functional state of islet B-cells. It is based on the recent observation of different percentages in B-cell subpopulations which were separated according to their (un)responsiveness to 7.5mM glucose. Cells responsive to 7.5mM glucose contained a higher percent of pale granules (12%) than the unresponsive ones (4%, $p<0.05$), whereas their total number of granules per cytoplasmic area was similar. Percentages were now determined in B-cells cultured for 10 days at 6mM, 10mM or 20mM glucose. The functions of 6mM-cultured cells were markedly less sensitive to glucose than those of 10mM- or 20mM-cultured cells. The percent of pale over total granules was significantly lower in B-cell preparations with the lower glucose sensitivity (3 % in 6mM-cultured cells versus 16% in 10mM- and 26% in 20mM-cultured cells, $p<0.001$-0.03). The 6mM- and 10mM-cultured cells exhibited comparable total granule counts per cytoplasmic area, whereas the 20mM-cultured cells contained 45 % less granules than the 6mM group ($p<0.05$). These findings suggest that the percent of pale over total insulin granules can be taken as an index for the glucose sensitivity of rat B-cells.

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LACTATE PRODUCTION BY ISLETS OF RATS AND OB/OB-MICE MEASURED WITH A NEW METHOD.

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A new sensitive method for direct assay of lactate was employed for determination of lactate production in isolated islets. Medium lactate was allowed to react with α-p-dibromoacetophenone, the resultant ester separated isocratically (acetonitrile:water,3:7) by reverse phase HPLC (retention time 9 min) and quantitated by its light absorbance.The amount of lactate produced was correlated to the DNA content of the incubated islets and expressed as pmoles of glucose-equivalents/μgDNAxhour. It increased with the glucose concentration in rat islets: 506 ± 76 (23) at 0mmol D-glucose/l (control),788 ± 49 (29) at 0.2mmol /l ($p < 0.01$),1742 ± 111(22) at 1mmol/l ($p < 0.001$),2220 ± 161 (19) at 3mmol /l ($p<0.001$) and 2492 ± 293 (6) at 20mmol/l ($p<0.001$). Comparable values were obtained from microdissected ob/ob-mouse islets: 410 ± 69 (9) at 0mmol/l, 1598 ± 195 (9) at 3mmol/l and 1685 ±141 (9) at 20mmol/l glucose. The lactate production from glucose was not affected by Antimycin A (10^{-5} mol/l) but it was reduced by iodoacetamide (1mmol/l).Except for mannose, other hexoses showed lower conversion rates to lactate as compared to D-glucose at 20mmol/l in rat islets: D-fructose [1636 ± 172 (13) vs. 2876 ± 188 (12), $p<0.001$] and D-galactose [1024 ± 94(13) vs.2876 ± 188 (12),$p < 0.001$]. In conclusion,islet lactate production is almost maximal at 3mmol/l D-glucose and it is significantly lower with D-hexoses which are poor secretagogues.

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EFFECT OF INSULIN TREATMENT ON PANCREAS FUNCTION AND ISLET-CELL PROLIFERATION IN FEMALE RATS.

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The effect of experimental hyperinsulinaemia on endocrine pancreas function and on islet-cell proliferation was investigated in cyclic Wistar rats. Osmotic minipumps releasing 4.8 IU insulin per day were implanted on day 0 and were removed on day 6. Six rats were subjected to ivgtt's (1 g glucose/kg bw) on day 0 (before implantation), on day 6 (before pump removal) and on days 7,8,9,10,13 and 17. Islet-cell proliferation was measured by Bromodeoxyuridine (24 hr infusion) on day 0 (n=6) and days 6,9,13 and 17 (all n=5). Insulin treatment resulted in hypoglycaemia (1.9±0.6 mmol/l), hyperinsulinaemia (114±15 mU/l; normal 28±1 mU/l) and in 50% reduction in islet-cell proliferation. On day 7 rats showed hyperglycaemia (11.7±1.5 mmol/l), hypoinsulinaemia (21±2 mU/l), a reduced insulin response and a reduced glucose tolerance. Basal glucose and insulin levels were normal again on day 8, but insulin response and glucose tolerance were still impaired. On day 9 islet-cell proliferation was 5-fold increased as compared with day 0. On day 13 endocrine pancreas function and islet-cell proliferation, had returned to normal values. It was concluded that hyperinsulinaemia inducing hypoglycaemia causes reversible suppression of endocrine pancreas function and islet-cell proliferation.

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PROLONGED EXPOSURE OF HUMAN PANCREATIC ISLETS TO HIGH GLUCOSE CONCENTRATIONS IN VITRO IMPAIRS THE B-CELL FUNCTION

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The aim of the present study was to clarify whether prolonged in vitro exposure of human pancreatic islets to high glucose concentrations impairs the function of these cells. For this purpose islets were isolated from 14 human pancreata, and cultured for 7 days in the presence of either 5.6, 11 or 28 mM glucose. Culture in the presence of 11 or 28 mM glucose left the islet DNA content unchanged and induced no signs of morphological damage. However, islets cultured at 11 or 28 mM glucose showed a 45 % or 60 % decrease in insulin content, as compared to islets cultured at 5.6 mM glucose ($P<0.01$). When such islets were submitted to a 60 min stimulation with a low (1.7 mM) followed by a high (16.7 mM) concentration of glucose, the islets cultured at 5.6 mM glucose showed a higher insulin response to glucose than those of the two other groups ($P<0.01$). The rates of glucose oxidation, (pro)insulin biosynthesis and total protein biosynthesis were similar in islets cultured at 5.6 or 11 mM glucose, but they were decreased by 30-50 % in islets cultured at 28 mM glucose ($P<0.05$). A subsequent 2-day culture at 5.6 mM glucose reverted some of the effects of high glucose on islet insulin content and insulin release. These combined results suggest that a lasting exposure to high glucose concentrations impairs the function of human pancreatic islets.

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LACK OF GLUCOTOXICITY IN MURINE ß-CELLS EXPOSED ONE WEEK TO HIGH GLUCOSE CONCENTRATION IN VITRO

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The objective of the present work was to study the effects of glucose on the in vitro function of islet ß-cells from two mouse strains with different in vivo glucose sensitivity of their ß-cells. Isolated islets of each strain (C57BL/Ks and C57BL/6) were kept for one week in culture at either 5.6, 11 or 56 mM glucose. Islets from both strains cultured at 56 mM showed a significantly increased glucose-stimulated insulin release (ng/10 islets·60 min) compared to corresponding control islets (11 mM); BL/Ks: 29.9 ± 5.4 vs 10.7 ± 2.3, ($p<0.01$), BL/6: 45.5 ± 11.7 vs 18.8 ± 4.2, ($p<0.01$). Rates of (pro)insulin biosynthesis (kDPM/10 islets·90 min) were decreased in BL/Ks islets at both 5 mM and 56 mM: 35.6 ± 11.4 and 38.1 ± 2.8 vs 63.2 ± 8.6, ($p<0.05$). In BL/6 islets there was no decrease at 56 mM, but a marked decrease at 5.6 mM: 13.1 ± 2.8 and 48.2 ± 7.6 vs 52.2 ± 9.4, ($p<0.01$). There were no differences in DNA content (µg DNA/10 islets) in islets cultured at different glucose concentrations: control islets BL/Ks: 0.13 ± 0.04, control islets BL/6; 0.27 ± 0.03. Islet insulin content (ng/ng DNA) was decreased at 56 mM in both strains; BL/Ks: 457 ± 183 vs 1105 ± 445, BL/6: 1204 ± 193 vs 2093 ± 238, ($p<0.05$). The present study indicates that irrespective of genetic background murine ß-cells can adapt to very high glucose concentrations in vitro without any obvious signs of so called glucotoxicity.

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IMPROVEMENT OF FUNCTIONAL BEHAVIOUR OF HUMAN ISLETS AFTER CHRONIC EXPOSURE TO HIGH GLUCOSE LEVELS.

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Chronic exposure to high glucose media sensitizes human islets to glucose stimulus. In order to clarify this phenomenon we have studied insulin response to other secretagogues, glucose metabolism (D-5-^{3}H-glucose utilization and D-6-^{14}C-glucose oxidation), insulin content and Northern blot analysis of insulin mRNA in human islets cultured at 5.5 and 16.7 mM glucose for one and seven days. Insulin responses to glucose after 7 days culture at 16.7 mM glucose to 5.5 and 16.7 mM glucose stimulus was 5-fold increased compared with 1 day culture. Insulin secretion to 10 mM leucine + 10 mM glutamine and 16.7 mM glucose + 5 µM forskolin, after 1 day culture (5.5 and 16.7 mM glucose) was (14.43±1.98 ; 17.28±1.95 and 73.97±3.46 ; 79.40±2.80 µU/islet per 90 min), respectively. Insulin response increased to (24.38±6.10 ; 40,61±9.15) and (207.87±16.16 ; 274.36±10.88) after 7 days at 5.5 and 16.7 mM glucose culture. Glucose utlization and oxidation increased significantly after 7 days culture in both glucose conditions. Moreover, the ratio D-6-^{14}C-glucose/D-5-^{3}H-glucose, was much higher after 7 days culture (0.16, stimulus 5.5 mM glucose ; 0.22, stimulus 16.7 mM glucose) than values obtained after 1 day culture (0.03 and 0.05). Insulin content and mRNA insulin expression were also increased after 7 days culture at high glucose concentration. These findings demonstrates that chronic exposure of islets to high glucose media improves their overall functional behaviour.

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GALANTIDE II IS A STABLE GALANIN ANTAGONIST.

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The neuropeptide galanin occurs in pancreatic adrenergic nerves and inhibits insulin secretion. The peptide has been suggested to be an adrenergic mediator of stress-induced inhibition of insulin release. To verify its physiological function, galanin receptor antagonists are required. We recently demonstrated that the 20 amino acid chimeric peptide, galantide, is a galanin antagonist. However, galantide contains a methionine moiety, and is therefore easily oxidized and unstable. In this study, we examined whether a galantide analogue not containing methionine is a galanin antagonist. This peptide, galantide II, is also chimeric: galanin(1-13) coupled to bradykinin(2-9). Galantide II had no effect alone on glucose (11.1 mM)-stimulated insulin secretion in isolated mouse islets, but potently counteracted the inhibitory action of galanin (100 nM). The lowest effective dose of galantide II was 10 pM. In contrast galantide II did not counteract the inhibitory actions of clonidine (1 µM) or somatostatin (1 µM). Furthermore, galantide II displaced 125-I-monoiodo-[26-Tyr]-galanin from membranes of RINm5F cells. The displacement curve fitted to a two-site model in which 60% binds with K_1 (0.1±0.01 nM) and 40% with K_2 (3±0.5 nM). **In conclusion,** galantide II is a specific galanin antagonist and might distinguish different classes of galanin receptors. Galantide II is non-methionine containing and therefore more stable than galantide.

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INSULIN RELEASE UPON PERIFUSION AND XENOTRANSPLANTATION OF CRYOPRESERVED PORCINE ISLETS.

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We previously described a method for successfully cryopreserving porcine islets (PI). Hereby we report on insulin release (IR) from cryopreserved, perifused PI, and xenotransplantation of cryopreserved PI (CPI) into C57B/B6 mice with streptozotocin-induced diabetes and temporarily immunosuppressed with aL3T4 antibody. Basal (3.3 mmol/l glucose -G-) IR from CPI and non-cryopreserved PI (NCPI) from the same pancreases was 69±18 and 43±10 pmol/200 islet equivalents-IE-/min (NS). Peak IR from CPI in response to 16.7 mmol/l G and 16.7 mmol/l G plus 10 mmol/l theophylline (T) was 157±48 and 479±140 pmol/200 IE/min, significantly ($p<0.05$) higher compared to IR from NCPI (respectively 85±28 and 221±102 pmol/200 IE/min). Total IR at 16.7 mmol/l G and 16.7 mmol/l G plus T was significantly ($p<0.02$) higher from CPI (3756±746 and 7505±2075 pmol/200 IE/min) than from NCPI (1412±375 and 2161±371 pmol/200 IE/min). IR returned to basal values upon perifusion with 3.3 mmol/l G again. Normoglycemia was restored in mice within 7 days after transplantation of 1500-2000 CPI under the kidney capsule. Mean survival time of CPI xenografts was 41±7 days, slightly longer than with overnight cultured NCPI (36±4 days) and similar to that with 7 days cultured NCPI (43±3 days). Thus: IR is well maintained from perifused CPI, with the reasons still unknown of the increased release after cryopreservation; reversal of diabetes is feasible with CPI, with the exact role of cryopreservation to be still defined in reducing PI immunogenicity.

PS 14
β-cell Damage

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THE RECEPTORS AND MECHANISMS INVOLVED IN THE EFFECTS OF VASOPRESSIN AND OXYTOCIN IN PANCREATIC B CELLS

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Recent studies have suggested that arginine vasopressin (AVP) and oxytocin (OT) increase insulin release by stimulating phosphoinositide metabolism in B cells. We used mouse islets to investigate this issue and identify the receptor(s) involved in the effects of AVP and OT. Both agents caused dose-dependent (10^{-10} to 10^{-5} mol/l) increases in insulin release and inositol phosphate (IPs) levels. Their potencies were similar and their maximal effects were not additive. Synthetic agonists selective for AVP (V_1) and OT receptors in other tissues were equipotent with the natural agonists on insulin release and IPs. Antagonists allegedly selective for V_1 and OT receptors inhibited both types of effects with a potency that varied with the nature of the agonist. Both AVP and OT were about ten-fold more potent on insulin release (EC_{50} ~ 2 nmol/l) than on IPs (EC_{50} ~ 25 nmol/l). Moreover, the antagonists more readily inhibited the effects of all agonists on IPs than on insulin release. It is suggested that AVP and OT act on a single type of receptor that is distinct from classical V_1 or OT receptors but is closer to the latter. The acceleration of phosphoinositide turnover might not be the sole mechanism involved in the stimulation of insulin release by AVP and OT.

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TNF-α PERTURBS CELL ORGANIZATION OF PSEUDOISLETS.

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Dispersed rat islet cells can reaggregate in culture forming pseudoislets with cellular architecture reminiscent of native islets. We have assessed the effect of the cytokine tumor necrosis factor (TNF-α) on pseudoislet formation. Sorted rat islet B- and non-B-cells were labeled respectively with the carbocyanine fluorescent dyes DiI and DiO, mixed together, and allowed to reaggregate in culture, with or without non-cytotoxic doses (100U/ml) of TNF-α. In control pseudoislets segregation between centrally located fluorescent red (DiI) B-cells and peripheral green (DiO) non-B-cells was observed after 5 days (confocal microscopy). In aggregates formed in the presence of TNF-α such segregation was grossly perturbed. This effect was reversed after removal of the cytokine. Short-term (45 min) homotypic aggregation of sorted B-, but not non-B-cells, was also affected by a 20-h pre-exposure to the cytokine with an increase (from 28.5 ± 2.5% to 43.2 ± 2.1%; n=6, p<0.001) in calcium-independent adhesion. We have shown previously that B-/non-B-cell segregation in pseudoislets is due at least in part to higher expression of calcium-independent adhesion molecules on non-B-cells. The increase in calcium-independent adhesion of TNF-α-treated B-cells diminishes this difference, possibly explaining the random cell type organization of TNF-α-treated pseudoislets. The data suggest a novel pathway by which cytokines could affect islet structure/function.

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EXPRESSION OF DNA DAMAGE-INDUCIBLE GENES IN RAT PANCREATIC ISLETS

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Pancreatic B-cells are able to repair themselves following sublethal injuries in vitro. However, the repair mechanisms activated in these cells remains unknown. The aim of the present study was to determine whether two DNA damage-inducible genes (DDI), gadd 45 and gadd 153, are expressed in rat pancreatic islets following exposure to different alkylating agents. Rat pancreatic islets were exposed in vitro to 0.55 mM streptozotocin (SZ) or 1 mM methyl methanesulfonate (MMS) for 30 min. Exposure to SZ or MMS reduced the insulin release over the next 4 h by 40-50 %. The baseline expression of gadd 45 and gadd 153 was increased by 2-3-fold 2 and 4 h following SZ exposure, as evaluated by Northern blot analysis, while MMS induced a 3-fold increase in gadd 45 and a 5-6-fold increase in gadd 153 (n = 4). Islet exposure to MMS or SZ did not modify the expression of actin mRNA. These data show that DDI are induced in pancreatic islets following exposure to alkylating agents, and that DDI expression is more marked in MMS-treated islets. Since islets are able to recover completely their function following MMS exposure, but not after SZ exposure, it will be of interest to further characterize the role of these DDI genes in the repair of islet DNA damage.

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DNA STRAND BREAKS IN ISOLATED RAT ISLET CELLS AS AN EARLY EFFECT OF NITRIC OXIDE RADICALS.

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We have recently shown, that macrophage-mediated islet cell cytotoxicity is L-arginine-dependent, pointing to an important toxic role for nitric oxide (NO). Recent reports demonstrate in in vitro models DNA-damaging or -altering effects of NO. We therefore determined, whether we can find DNA-damage in isolated rat islet cells after incubation with various chemical NO-generators.
Cells were incubated with Nitroprusside (NP) or S-nitroso-N-acetyl-D,L-penecillamine (SNAP) at various concentrations and times. Viability of cells was assessed by trypan blue exclusion. Using a modified in situ nick translation method DNA-strand breaks were shown at single cell level: After cell fixation on glass slides DNA polymerase I incorporates biotin-dUTP into the DNA beginning at the break. Biotin is detected in a streptavidin-coupled peroxidase reaction. Brown nuclei are counted under the microscope.
As a result strand breaks were demonstrable after 3 hours of incubation and preceeded lysis for about 3 hours. Positive control was TNF induced apoptosis in L929, negative control was SNAP on L929 cells known to be TNF-sensitive and NO-resistant.
The relevance of this observation was backed by the demonstration of massive DNA-damage in situ in pancreas sections of high dose SZ-treated mice.

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THE EFFECT ON INSULIN SECRETION OF AGENTS WHICH ALTER NITRIC OXIDE AND CYCLIC GMP.

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Treatment of islets of Langerhans with IL-1ß is associated with nitric oxide generation, raised cyclic GMP and a glucose-induced insulin secretory response which is either stimulated (pM doses IL-1ß) or inhibited (nM dose). We have attempted to mimic IL-1's effects using a compound which generates nitric oxide and increases cGMP (3-morpholinosydnonimine; SIN). We also used the guanylate cyclase inhibitor LY83583 to decrease intracellular cGMP. In all cases 20mM glucose-induced insulin secretory responses, cGMP *and* cAMP production in rat islets were measured. Incubation of freshly isolated islets with SIN (100μM) for 30 minutes decreased insulin secretion (494 +/- 72 vrs control 713 +/- 69 fmol/islet/30 min; P=0.03). SIN also increased cGMP (6.6 vrs control 3.0 fmol/μg islet protein/30 min; P= 0.019; n=6-11) and decreased cAMP (14.8 vrs control 22.7 fmol/μg protein/30 min; n=6-11). LY83583 (10μM) inhibited insulin secretion (487+/-65 vrs control 966 +/-93 fmol/islet/30 min; P<0.001) decreased cGMP (2.73 vrs control 4.02 fmolμg protein/30 min; n=5; P=0.01), while having no effect on cAMP. In addition we found that culturing islets with a cGMP analogue (10-100μM 8-bromo-cGMP) stimulated insulin secretion. These results suggest that, while islet cGMP and secreted insulin are directly correlated during stimulation of insulin secretion, it is likely that the nitric oxide-mediated inhibitory effects of IL-1ß and SIN are not *directly* induced by raised cGMP.

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ROLE OF cGMP AND cAMP IN THE INHIBITORY EFFECTS OF INTERLEUKIN-1ß ON INSULIN SECRETION

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We have previously associated the inhibitory effect of interleukin-1ß (IL-1ß) on glucose-induced insulin secretion in rat islets of Langerhans with the increased production of nitric oxide *in vitro*. This study investigates the effect of IL-1ß on cyclic nucleotide production and whether this is dependent on nitric oxide. Rat islets were pre-cultured for 48h then treated for 12h with IL-1ß (100pM) in RPMI medium supplemented with arginine (1mM) or the arginine analogue - N-w-nitro-L-arginine methyl ester (NAME) - which blocks nitric oxide production. Cyclic nucleotides were extracted from groups of 15-40 islets; values were expressed as fmol nucleotide/μg islet protein. In the *presence* of isobutylmethylxanthine (IBMX), islet cGMP levels were significantly increased versus control (8.2±1.1 from 2.2±0.3 fmol/cGMP/μg protein/12h, p<0.001, n=12) and cAMP levels remained unchanged. Islet cGMP levels in the *absence* of IBMX were similarly increased (fourfold), however cAMP was significantly *reduced* from (13.3±0.7 to 9.3±0.8 fmol cAMP/μg protein/12h, p=0.006, n=8). IL-1ß's lowering of cAMP was not observed in the presence of NAME, which decreased IL-1ß's ability to generate cGMP (by 70%). Our findings suggest that IL-1ß has no direct effect on adenylate cyclase activity and are consistent with cGMP-dependent activation of phosphodiesterase(s) which accelerate breakdown of cAMP and which may contribute to IL-1ß's inhibition of insulin secretion.

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DIFFERENT SENSITIVITY TO CYTOKINES OF HUMAN ISLETS FROM VENTRAL AND DORSAL ORIGIN

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Islets both from the ventral and dorsal portion of the human pancreas are being used for clinical islet transplantation. There exist functional differences between both kinds of islets in diverse animal species. In humans only immunohistochemical differences have been documented at present. On the other hand, cytokines have been shown to inhibit insulin release and to be cytotoxic, and they have been postulated as important effectors in islet rejection. The present study was designed to compare the hormone secretory response and lipid synthesis by freshly isolated human islets from ventral with those of dorsal origin, as well as their response to different cytokines, in order to find out differences that could suggest a different efficiency for transplant and even a different susceptibility to rejection. Multiple organ donor pancreata, stored for less than 6 hours in UW solution, were used for islet isolation. Insulin, as well as somatostatin, secretion by dorsal islets was different from that of ventral islets in presence of both 5 mmol/l (0.08±0.01 dorsal, vs 0.04±0.01 ventral, ng/islet x 120 min, n=8, p<0.05) and 20 mmol/l (0.29±0.04 dorsal, vs 0.12±0.03 ventral, ng/islet x 120 min, n=8, p<0.01) glucose. Tumor-necrosis factor (TNFα), IL-1 and IL-6 inhibited insulin (0.29±0.04 control, vs 0.13±0.03 TNFα, 0.11±0.03 IL-1, vs 0.12±0.02 IL-6, ng/islet x 120 min, n=8, p<0.01 in all cases) and somatostatin response of dorsal islets to glucose 20 mmol/l without affecting TRH response. Hormone response of ventral islets was not affected by cytokines. The suppression of insulin response induced by cytokines in dorsal islets were correlated with reduction in 20 mmol/l D-(U-^{14}C)-glucose incorporation into both phosphatidyl-choline and phosphatidic acid. Our results demonstrate that secretory response of ventral and dorsal islets show a different sensitivity to cytokines. Differences on "de novo" lipid synthesis suggest that cytokine action could be linked to an impairment in this metabolic route.

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EFFECT OF TUMOR NECROSIS FACTOR ON HORMONE SECRETION BY FETAL CULTURED ISLETS.

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The objective of this study was to investigate whether tumor necrosis factor (TNFα) modifies hormone release in fetal islets and to test the hypothesis that PGE_2 mediates the effect of TNFα. Islets were isolated from fetal rats after partial digestion of the pancreas with collagenase. After overnight culture, the islets were separated from the exocrine tissue and cultured for 2 days before TNFα or PGE_2 were added. The islets were then cultured for 18 hours in an atmosphere of 95% air/ 5% CO_2 at 37ºC. At the end of the culture period the islets were washed with RPMI-1640 medium to remove the additives and incubated in Krebs-Ringer bicarbonate (0 or 20 mmmol/l glucose) and hormone content of both the incubation medium and the islets was determined. In some cases, the medium was replaced whith RPMI-1640 medium containing no additives and cultured for 8 days before the experiment was performed. In all experiments, control islets were washed and handled in a manner identical to treated islets. TNFα decreased insulin (36.5±3.9 vs 25.6±2.0 ng/islet, n=24; p<0.01) and somatostatin (12.0±2.0 vs 7.8±1.4 pg/islet, n=10; p<0.05) content of the islets. TNFα also inhibited insulin (0.38±0.06 vs 0.18±0.02 ng/islet x h, n=15; p<0.005) and somatostatin (0.29±0.01 vs 0.14±0.01 pg/islet x h, n=7; p<0.01) release in response to 20 mmol/l glucose. PGE_2 had a similar effect on insulin content of the islets (37.5±2.4 vs 27.2±4.7 ng/islet, n=9) but it increased glucose-induced insulin release (0.37±0.07 vs 0.54±0.1 ng/islet x h, n=9) and this effect was apparent even in the presence of TNFα. After 8 days-culture, neither TNFα nor PGE_2 modified insulin or somatostatin release. These results show that TNFα inhibits secretory response to glucose by fetal islets; this effect was not mediated by PGE_2 that induces changes in islet function which appear to be different from those observed in islets exposed to TNFα.

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STUDIES ON GLUTAMIC ACID DECARBOXYLASE IN ISLETS.

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Glutamic acid decarboxylase (GAD) has been identified a major autoantigen in diabetes. Two forms of the enzyme, GAD 65 and GAD 67, encoded by different genes, exist.

We have examined the two forms of the enzyme by transient expressions in COS cells and by incubations of rat islets in different glucose concentrations. GAD was analyzed by immunoprecipitation of [^{35}S]-labelled lysats using patient sera, by Western blotting using a monoclonal (GAD-6) specific for GAD 65 and a rabbit serum (K2) directed against GAD 67.

We observe that upon increasing glucose concentrations GAD 65 increases selectively on Commassie staining to appear as a prominent protein. GAD 65 is recognized in Western blots by GAD-6 as a doublet. The slowly migrating component is weak in islets from freshly prepared islets and increases upon exposure to glucose. Transient expression of GAD 65 in COS cells gives rise to a single protein, which might suggest that the slow component reflects a splicing phenomenon. Testing 20 sera from patients with IDDM GAD 65 but not GAD 67 was immunoprecipitated.

The present data underline GAD 65 as the islet autoantigenic form of GAD associated with IDDM and emphasize glucose as a stimulus for its synthesis.

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DIFFERENTIAL ISLET CELL EXPRESSION OF TWO FORMS OF GLUTAMATE DECARBOXYLASE, BOTH BEING AUTOANTIGENS IN DIABETES.

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Two different forms of glutamic acid decarboxylase (GAD_{64} and GAD_{67}) appear to be two independent targets for autoantibodies in insulin-dependent diabetes mellitus. Both enzymes are expressed in rat pancreatic islets as well as in rat brain. We have studied the expression of GAD_{64} and GAD_{67} in rat and human islets and in islet tumor cell cultures. The study confirmed that, on rat pancreatic sections, the expression of GAD_{64} and GAD_{67} was co-localized to the islet β-cells. We also made several interesting observations: firstly, sera from certain diabetic patients recognize only GAD_{67}, even though we did not detect this form in human islets; secondly, in monolayers of rat islet- or islet tumor cells, the expression of GAD_{67} appeared also in non-β-cells; thirdly, in rat islet tumor cell lines at various differential stages, GAD_{64} expression was found only in cells at a high level of differentiation as exemplified by insulin production, whereas GAD_{67} was expressed also in the more immature phenotypes along with other hormones such as glucagon and CCK. Thus, the two isoenzymes followed different patterns of expression. These findings have several interesting implications for the pathogenesis of insulin-dependent diabetes.

PS 15
Epidemiology in Type 1 Diabetes

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PROTECTIVE EFFECT OF NICOTINAMIDE ON PRESERVATION OF MOUSE ISLET FUNCTION AND MORPHOLOGY

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The aim of this study is to assess the in vitro effect of NA on islets cultured in presence of Interleukin-1ß(IL-1ß). Swiss/Webster mouse islets were maintained in culture for 20 days in Dulbecco's medium containing 10%FBS and 5.5mmol glucose w/wo recombinant human IL-1ß (1.0 U/ml) and w/wo NA (50mmol). Islet survival was assessed and accumulated insulin and islet insulin content were measured. After 20 days there was no significant difference between control and IL-1ß treated islets. In both, islet disintegration and intensive fibroblast growth were observed. However 50mmol NA preserved the islet structure and completely inhibited fibroblast growth.

	Control	NA	IL-1ß	NA+IL-1ß
survival				
intact islets (%)	11.7± 3.3	43.3±3.4	6.7±2.3	76.7±6.0
(n=4)		**p=0.003**		**p=0.001**
Accumulated insulin				
(pmol/islet/24 h)	0.09± 0.05	0.75±0.15	0.017±0.005	3.67±0.97
		p=0.007		**p=0.013**
Islet insulin content				
(pmol/islet)	2.02±0.55	8.83±0.32	3.34±0.15	11.99±0.27
		p=0.001		**p<0.001**

In conclusion, high concentrations of nicotinamide are not toxic to islets and can preserve islet structure. NA stimulates insulin release and synthesis up to 20 days and has a strong inhibitory effect on fibroblast growth. The effect of NA on survival and function of mouse islets is amplified by addition of low dose IL-1ß.

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RISK OF DIABETES IN PARENTS AND OFFSPRING OF DRUG TREATED FINNISH DIABETIC PATIENTS.

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In Finland the annual incidence of diabetes in children is the highest in the world (36/100000 under the age of 14 years). The aim of this nationwide study is to investigate incidence of diabetes in parents and offspring of patients with diabetes. In Finland patients with diabetes regularly treated with drugs, belong to the Finnish Diabetes Drug Registry. From this registry we identified all patients with diabetes diagnosed since 1965 which were less than 30 years of age at diagnosis. Through the Finnish Population Registry we identified all offspring and parents of those patients. We then looked which of the offspring and parents belong to the Drug Registry, 1990 inclusive. 1890 diabetic women had 2536 offspring of which 47 (1,85%) had diabetes. 2152 men had 3364 offspring of which 114 (3,39%) had diabetes. The difference was significant ($p<0,001$, chi squared test). Among parents of 13846 patients with diabetes, 353 mothers (1,27%) and 568 fathers (2,05%) had diabetes before the child's birth ($p<0,001$). 19 children with diabetes had both parents with diabetes. In conclusion, risk of diabetes in Finland is bigger in offspring of men with diabetes and diabetes occurs more commonly in fathers than in mothers of patients with diabetes.

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EPIDEMIOLOGY OF TYPE 1 AND TYPE 2 DIABETES IN THE YOUNG POPULATION OF MOSCOW .

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Incidence (I) and prevalence (P) of IDDM and NIDDM in city Moscow (total population 8.8 x 10^6) was studied. Case report forms of all patients under the age of 40y were taken into account. The analysis was made according to age intervals of 5y. It was found that I of IDDM increased significantly in the age group 5-9y versus the age group 0-4 (8x10^5 cases/year vs. 2.4x10 cases/year). After the age of 10y the I of IDDM stayed approximately at same level (6-8.5x10^5cases/year) up to the age of 40. The IDDM P rose steadily up to the age of 40 reaching 140x10^5in men and 90x10^5in women in age group 35-39. The analysis of IDDM I in the age group 0-15y in 1980-89 comparing the 1970-79 interval have shown no significant differences (5.6 vs. 5.4x10^5). In the age group 20-24y I of NIDDM is lower than of IDDM (0.6x10^5in males and 0.9x10^5in females). In the age group 25-35y the I is nearly the same for NIDDM and IDDM , but after 35y the I is higher for NIDDM (12x10^5in males and 16x10^5 in females). The NIDDM P in the age group 35-39y is about 50x10^5in males and 102x10^5 in females.

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INCREASING INCIDENCE OF TYPE I DIABETES MELLITUS IN THE NETHERLANDS.

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In past years the incidence of type I diabetes mellitus has increased in a number of countries. To study the trend of the incidence in the Netherlands in 1991 a questionnaire was sent to all paediatricians and internists. The questionnaire was the same as for a previous study for the years 1978-1980. In the present study data were requested on patients aged 0-19 years who started with insulin in 1988-1990. All paediatricians and 87% of the internists responded. They reported 1169 patients in total. As a secondary source of validation the same questionnaire was sent to members of the Dutch Diabetes Association, of whom 799 responded.

The ascertainment adjusted average annual incidence for the 0-19 years old was 13,21/100.000 with a 95% confidence interval (CI) of 12,59-13,83 and for the 0-14 years old, 12,41 (CI 12,00-12,82). For 1978-1980 the incidence for the 0-19 years old was 10,95 (CI 10,31-11,56) and for the 0-14 years old 11,10 (CI 10,47-11,69)

Conclusion: The incidence of type I diabetes mellitus has increased during the past 10 years in the Netherlands too.

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THE YORKSHIRE REGIONAL CHILDHOOD DIABETES REGISTRY

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A registry for cases of childhood insulin dependent diabetes has been established in Yorkshire in Northern England. It collected cases retrospectively from 1978 to 1990, and prospectively thereafer. Cases were first identified through local paediatricians and diabetologists, and details were abstracted from hospital records. Registration was verified from general practitioners' records and hospital admissions computer records. From 1978 to 1990, 1171 children aged 16 or under were diagnosed with diabetes whilst resident in the region. The total population at the 1981 census in the region was 863,522. The overall incidence rate was 10.41 per 100,000 children per year, 10.80 in males, and 10.01 in females. The male to female ratio was 1.08:1 [95% CI= 0.96-1.21). The second (pubertal) peak was earlier and higher in females, 13.96 at ages 9-11, than in males, 12.27 at ages 12-14 [t=2.11, df=11676.9, p=0.03]. There was an increasing incidence of diabetes over the 12 years [Mantel-Haenszel X^2 for trend = 56.10, df=1, p<0.0001) with peaks every fourth year.

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INCIDENCE OF CHILDHOOD TYPE 1 (INSULIN-DEPENDENT) DIABETES IN COIMBRA, PORTUGAL,1987-91

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Little information is available concerning the incidence of type 1 diabetes in the portuguese population. 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. Population at risk 84,640 (total population 430,533). Included were cases diagnosed between 1987-91, with age at onset less than 15 years and residence in the district of Coimbra at diagnosis. As the primary source of cases we used: hospital admissions, diabetologists and general practitioners. A school survey was used for validation of case ascertainment. The degree of ascertainment by the primary source was 100%. Age specific incidence rates (/100,000/year) were as follows: 6.7 (95%CI:1.3-12.1) in 1987, 4.6 (0.1-9.2) in 1988, 8.3 (2.2-14.5) in 1989, 8.5 (2.2-14.7) in 1990 and 7.4 (1.5-13.3) in 1991. The age group 10-14 years showed the highest incidence rate, 9.9. There was no sex difference. These rates are similar to those reported for other mediterranean countries (e.g. France, Italy), lower than those reported for Spain but higher than in portuguese-heritage populations in Brazil.

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SEX DIFFERENCES IN SEASONALITY AT ONSET OF TYPE 1 (INSULIN-DEPENDENT) DIABETES MELLITUS IN CATALONIA.

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The aim of this study was to evaluate factors associated with seasonal trends of clinical onset of Type 1 diabetes. A prospective Type 1 (insulin-dependent) diabetes incidence study has been carried out in Catalonia during the period 1987-1990, including cases with onset less than 30 years of age and residents in Catalonia at diagnosis. Population at risk (0-29 y) is 2.690.394 inhabitants. 1154 cases were identified (ascertainment degree 90.1%). Seasonality was assessed by Edward's Test. Factors analized in relation with temporal trends were age, sex, metabolic status at diagnosis (ketoacidosis, ketosis or hyperglycemia) and place of residence. A seasonal onset pattern was observed, with higher incidence in December-February during the five-years study period ($p<0.05$). This pattern was maintained when cases were analyzed for age groups, metabolic status at diagnosis and place of residence. According to sex, seasonal fluctuations were only observed in males, either in the whole group or in the different age groups ($p<0.001$). Females only followed a seasonal pattern when ketoacidotic cases were excluded ($p<0.05$). We conclude that clinical onset of type 1 (insulin-dependent) diabetes mellitus in Catalonia follows a seasonal pattern only in males, and it does not deppend on age, clinical severity at onset or area of residence.

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THE INCIDENCE OF CHILDHOOD DIABETES MELLITUS TYPE 1 IN SLOVAKIA

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The incidence of diabetes mellitus Type 1 was prospectively evaluated in Slovak children up to 14 years of age during the period 1985-91. The population consisted of 1,338.025 children -683.105 boys and 654.920 girls.During this period 949 newly diagnosed diabetics-495 boys and 454 girls- were registered. All the diabetics were independently identified from two sources:from the pediatricians who have estimated the diagnosis and from the hospitals where the patients were treated.Estimated ascertainment completness was over 95%. Mean incidence rate in the 6 years was 7/100.000,with marked age differentiation.The mean prevalence of diabetes was 35/100.000. Highest age-specific incidence rates (per 100.000) were in the following age groups: In the 3-4 age group it was 9, in the 7-8 age group it was 10 and in the pubertal age group /13-14 years/ it was 14.Seasonal manifestation of diabetes was found with a maximum in autumn and winter.No direct relation was found between coxsackie-virus infection passed before diabetes manifestation /in 55-66%/ and the seasonal diabetes incidence. We conclude that the incidence of diabetes in Slovakia in the latest years revealed increasing rates, namely in the youngest childrens groups.

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INCIDENCE OF DIABETES MELLITUS TYPE I IN BULGARIEN CHILDREN

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The incidence of Type I Diabetes Mellitus was prospectively evaluated by newly diagnosed patients aged from 0 to 14 years in the Clinic of Endocrinology and Diabetes Mellitus during the period of 1987-1991. The overall incidence rate was 6.7 per 100000 per year. The evaluated incidence rate is higher compared with that of the previous decade (6.2 per 100000). The incidence rate increased from 6.2 to 6.9 per 100000 for 1987 and 1988 respectively. A steadily increasing gradient with age to peak incidence in the age group 10 - 13 years was observed. Children up to 2 years aged exibited the lowest rate (2.4). In the autumn and winter significantly more cases were diagnosed than in summer season. In conclusion our study supports up to date investigations and hypothesis that north-south gradient may be an explanation of the geographical variations in the incidence of diabetes observed in Europe.

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EPIDEMIOLOGY OF TYPE I (INSULIN-DEPENDENT) DIABETES MELLITUS IN A PREVIOUSLY UNDESCRIBED EUROPID POPULATION - A STUDY OF 1303 CASES.

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Study objective : analysis of epidemologic characteristics of type 1 (insulin - dependent) diabetes. Two independent registration systems were utilised for case ascertainment. Inclusion criteria were (i) age at diagnosis ≤ 29 years; (ii) insulin dependence; (iii) diabetes development between Jan. 1st 1960 to Dec. 31st 1984. 1303 (681 male, 622 female) satisfied the above criteria. Males were significantly older at diagnosis (16.5 ± 7.5 yrs vs. females14.6 ± 7.2 yrs, $p < 0.001$). 56% males vs. 41% females were > 14 years and 38% males (27% females) ≥ 20 yrs. The overall male : female ratio 1.01 : 1.0 reflected male preponderance (1.6 : 1.0) in the third decade. 751 (415 male, 336 female) were studied for seasonal variation. Significant seasonality (winter 30.5 % vs. summer 19.3%, $p <0.001$) was observed overall, and was related to age at high incidence of type 1 (insulin - dependent) diabetes - seasonality was significant for both sexes aged 7 - 15 years ($p < 0.02$) and older males ($p <0.05$) but not for cases ≤ 6 years or females > 15 years. 94% of 608 newly diagnosed cases had classical symptoms. 71% presented within 4 weeks of symptom onset. Symptom duration increased with age ($r = 0.200$, $p < 0.001$) but did not differ with gender (5.7 ± 8.9 vs. 5.1 ± 7.3 weeks) despite older male age (16.7 ± 7.7 vs. 14.7 ± 7.5 yrs, $p < 0.001$). This is the first description the epidemiology of type 1 (insulin - dependent) diabetes in our Europid population. Aetiology may differ with gender and age. Males are older at diagnosis, appear less susceptible than females to factors responsible for a peripubertal rise in incidence of type 1 (insulin - dependent) diabetes, and may be more susceptible to the influence of seasonally active agents.

Abstract 505a see page A 218

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Epidemiology in Type 2 Diabetes

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DIABETES INCIDENCE AMONG ELDERLY FINNISH MEN

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We report diabetes incidence and related risk factors among elderly men of the Finnish cohorts of Seven Countries Study. Oral glucose tolerance tests were performed to all responders without anti-diabetic medication in connection with 25- and 30-year follow-up surveys. Men with diabetes (WHO criteria) at baseline and those with unknown diabetes status at the 30-year survey were excluded from analysis. The final sample consisted of 395 men aged 65-84 years. 171 men died during the follow-up. Age-adjusted incodence of diabetes was 13/1000 person years. Men who developed diabetes had higher baseline fasting (6.5 vs 5.6 mmol/l, $p<0.05$) and 2-h post-challenge (11.5 vs 8.6 mmol/l, $p<0.001$) blood glucose levels, body mass index (26.8 vs 25.7 kg/m^2, $p<0.05$) and prevalence of impaired glucose tolerance (IGT) (75 % vs 50 %, $p<0.05$) and obesity (58 % vs 36 %, $p<0.05$) and lower plasma total cholesterol level (5.8 vs 6.2 mmol/l, $p<0.05$) compared with those remaining non-diabetic. In logistic regression analysis diabetes risk was increased in IGT men (odds ratio 2.99, 95 % CI 1.15-7.78) and decreased among those in highest cholesterol tertile (odds ratio 0.32, 95 % CI 0.17-0.58). Other factors had no independent predictive value.

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BIMODALITY OF BLOOD GLUCOSE DISTRIBUTION IS GENETICALLY DETERMINED

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Bimodality of blood glucose (BG) distribution described for some rare populations at high risk for Type 2 diabetes has been assumed to have a genetic origin. It has remained unclear what these genetic factors are and whether the bimodality also exists in European populations. The aim of the present study was to examine BG distributions among the representative cohort of 172 Finnish elderly men with or without a genetic susceptibility to diabetes. The basic assumption was that the genetic susceptibility to diabetes in elderly is associated with the same high risk HLA haplotypes defined through the nationwide study of childhood diabetes in Finland. The median of fasting BG was 5.2 and 5.6 mmol/l ($p<.05$) and that of 2-hour post-load BG 5.7 and 10.7 mmol/l ($p<.001$) in men with diabetes associated and non-diabetes associated haplotypes, respectively. The 2-hour BG distribution in men with diabetes associated haplotypes was normal but drastically shifted to the right as compared with fasting BG distribution, whereas in men with non-diabetes associated haplotypes the distributions for fasting and 2-hour BG were similar. Our findings confirm that the bimodality is a genetic trait and determined by specific HLA haplotypes.

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THE EFFECT OF AGE ON FASTING PLASMA GLUCOSE. AN EPIDEMIOLOGICAL APPROACH.

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To evaluate the effect of age on fasting plasma glucose(FPG) we studied 612 "normal" subjects representing 5% of the population of age 20-79 of a suburb. Fasting blood was taken for the measurement of FPG. Subjects with FPG<95 were considered normal while 241 with FPG>94 were called for OGTT. 88 refused and the remaining 524 formed Group A. From these 13 were known diabetics, 52 had diabetic OGTT and 22 had IGT. These 87 subjects were removed from A and the remaining 437 normals formed Group B. The data was analyzed by age, ie. 20-39, 40-59 and 60-79. FPG in A increased with age, $4.5\pm.1$vs$5.2\pm.2$vs$6.0\pm.3$mmol/l $p<.01$ while in B did not, $4.4\pm.1$vs$4.6\pm.1$vs$4.6\pm.1$mmol/l, $p>.05$. BMI increased with age both in A, $25.3\pm.5$vs$27.6\pm.3$vs$28.1\pm.4$, $p<.01$ and B, $25.1\pm.5$vs$27.5\pm.3$vs$28.2\pm.4$, $p<.01$. In A, FPG was correlated with age, males $r=.2130$, $p<.01$, females $r=.2570$, $p<.001$, correlation which disappeared in B, males $r=.0470$, females $r=.0770$, $p>.05$. No correlation of FPG with BMI was found in A or B. Conclusion The progressive increase of FPG with age, irrespective of BMI, disappears if subjects with abnormal glucose tolerance are excluded, suggesting that it is rather due to higher prevalence of diabetes in older age than to a direct effect of age on FPG in all subjects.

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EPIDEMIC TYPE 2 (NON-INSULIN-DEPENDENT) DIABETES AND BIMODAL PLASMA GLUCOSE DISTRIBUTION IN WANIGELA PEOPLE OF PAPUA NEW GUINEA

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The prevalence of type 2 (non-insulin-dependent) diabetes and the distribution of fasting and 2-hour (post 75-gram oral glucose load) plasma glucose were studied in 750 (response rate 73.7%) Melanesian Wanigela adults aged 25 years and above living in Koki, an urban settlement in Port Moresby. Plasma glucose was measured on site using a YSI glucose analyser. The prevalence of type 2 (non-insulin-dependent diabetes) was 31.7% in men and 34.2% in women, and an additional 19.5% of men and 21.7% of women had impaired glucose tolerance. Even in the youngest age-group (25-34 years) the overall prevalence of diabetes was 21.9% and of impaired glucose tolerance was 15.8%. The age-standardized prevalence of diabetes increased clearly across tertiles of both body mass index and waist:hip ratio, and was higher in subjects with lower physical activity scores. In all age groups (25-34, 35-44, 45-54 and 55+ years) both fasting and 2-hour plasma glucose concentrations were bimodal, a mixture of 2 log-normal distributions. Unimodal models were rejected in all 8 groupings ($p<0.001$ in 6 and $p<0.05$ in 2). The urbanized Wanigela people of Koki Settlement have one of the world's highest frequencies of glucose intolerance.

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NON-INSULIN DEPENDENT DIABETIC WOMEN HAVE MORE CHILDREN THAN NON-DIABETIC.

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The aim of the study was to compare the number of offspring between persons with non-insulin dependent diabetes and non-diabetics. In 1981-82 all persons with known diabetes aged 60-74 years in Fredericia, Denmark, were identified. Of 236 persons 228 (127 women and 101 men) consented to an extensive examination including an interview on the number of children. At the same time a control population was collected, consisting of randomly selected non-diabetic persons of the same age and sex as the probands. In the diabetic persons plasma C-peptide was measured after glucagon stimulation. A value $\geq$ 0.60 pmol/ml was considered to indicate NIDDM, leaving 117 women and 93 men. Complete information on the number of childbirths was obtained in 113 NIDDM women who had 322 children, mean 2.85. 126 non-diabetic women had 275 children, mean 2.18 ($p<0.00001$). 18 of the probands' children died before the age of 12 months, 11 of the non-diabetic women's ($p=0.37$). 82 NIDDM men had a total of 166 children, mean 2.02, 84 non-diabetic men had 192 children, mean 2.29 ($p=0.12$). The number of childbirths may be a risk factor for the development of NIDDM, or the NIDDM genotype may offer a selective advantage, thus explaining the high prevalence of NIDDM and counterbalancing the possible low fertility in NIDDM men.

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THE PREVALENCE OF DIABETES MELLITUS IN TWO SOUTH AFRICAN BLACK POPULATIONS.

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An urban (Mangaung) and a partly rural (Qwaqwa) black population in the province of the Orange Free State was studied to determine the prevalence of diabetes. From Mangaung 761 subjects (284 male and 457 female) and from Qwaqwa 853 subjects (279 male and 574 female) 25 years and older participated in the study. After an overnight fast a 75g OGTT was performed and the results were interpreted by WHO criteria. All results were age and sex adjusted. The prevalence of diabetes was 6% and 4.8% and the prevalence of IGT was 12.2% and 10.7% for Mangaung and Qwaqwa respectively. A BMI of 25+ was associated with abnormal glucose tolerance in the Mangaung sample (relative risk 1.9, 95% CI 1.4; 2.5) whereas the relationship was not statistically significant in the Qwaqwa sample (relative risk 1.3, 95% CI 0.93; 1.7). Upper segment body fat distribution was also associated with abnormal glucose tolerance (relative risk 2.8 (95% CI 2.0; 3.8) and 2.3 (95% CI 1.7; 3.2) for the Mangaung and Qwaqwa populations respectively). In both populations more treated than untreated hypertensives were diabetic (19% vs 9.4% and 16.8% vs 5% for Mangaung and Qwaqwa respectively). These results indicate that urban-rural differences in the prevalence of diabetes in the populations studied are slight.

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DIABETES MELLITUS IN ITALY: A PREVALENCE STUDY IN TUSCANY.

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The prevalence of diabetes mellitus (DM) in Pisa (Tuscany-Italy), 189.085 inhabitants, was evaluated using 4 independent data sources.The main source, represented by computerized prescriptions for antidiabetic agents collected throughout a 4 mounth period was validated using 3 minor sources: a)the list of diabetic patients who take material for self-care from the National Health Service; b) clinical records of diabetic patients obtained from a random sample of family doctors; c) clinical records of diabetic patients attending to our out-patient clinic. The principal source provided 3806 patients to whom 697 patients were added from the minor sources, thus identifying a total number of 4503 diabetics. DM prevalence assessed from the main source resulted 2,01%, but when corrected for the minor sources resulted 2,4%. Males (m.) (mean age 63±14 yrs) were 43,3% and females (f.) (mean age 66±13 yrs) 55,7%. The prevalence of DM was 2,21% in m. and 2,54% in f.. 141 patients had Type 1 Diabetes (3,2% of identified diabetics, prevalence 0,074% inhabitants):50% m. and 50% f., age 30±13 yrs, diagnosis of DM at 19±7 yrs., duration 16±14 yrs., BMI 24±4 Kg/m^2, insulin dose 45±18 IU/daily. 4362 patients had Type 2 Diabetes (96,8% of the total number of diabetics, prevalence 2,36%) m. 47,5% and f. 52,5% .Their characteristics were: age 66±8 yrs., diagnosis of DM at 53,7±11,5 yrs, DM duration 12,4±9 yrs, BMI 27±4 Kg/m^2; 10,5% of type 2 Diabetics were treated with diet,, 65% with oral hypoglicemic agents (OHA), 23% with insulin and 1% with insulin plus OHA in1%.

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5 YEAR STUDY OF A RURAL MELANESIAN POPULATION AND THE EFFECTS OF WESTERNIZATION.

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We previously reported increased diabetes and cardiovascular risk factors in an Urban over 40 (yrs) population of Fijian Melanesians compared to an isolated traditional living rural population (**group A**). We restudied the rural population at 5 years (**group B**) during which a new road and shop had led to increased westernization.
Group A 87 male(M),109 female(F) mean age 53.7 ±1 (M),52.0 ±1 (F) yrs; weight 72.4 ±1.1 (M),65.7 ±1.1(F) kg; fasting blood glucose (FBG) 3.6 ±0.1 (M),3.9 ±0.1 (F) mmol/l; 2Hr post 75g GTT (PBG) 4.3 ±0.1 (M),4.7 ±0.2 (F) mmol/l; Fasting cholesterol 3.8 ±0.1 (M),4.0 ±0.1 (F) mmol/l; apolipoprotein A1 1.01 ±0.02 (M),0.93 ±0.02 (F) g/l; apolipoprotein B 0.80 ±0.03 (M),0.90 ±0.02 (F) g/l, albumin 33.4 ±0.4 (M),33.8 ±0.3 (F) g/l; systolic bp 122 ±1.2 (M),123 ±1.5 (F) mmHg; diastolic bp 80 ± 0.9 (M),78.7 ±1.0 (F) mmHg.
Group B (* = Significant increase vs group A $p<0.01$) 68 (M),63 (F) mean age 53.3 ±1.5 (M),55.9 ±1.2 (F) yrs; weight 73.9 ±1.0 (M),67.8 ±1.4 (F) kg; (FBG) 5.4 ±0.2* (M),5.3 ±0.2* (F) mmol/l; (PBG) 6.5 ±0.4* (M),7.9 ±0.3* mmol/l; Fasting cholesterol 5.2 ±0.2* (M),5.5 ±0.2* (F) mmol/l; apolipoprotein A1 1.42 ±0.03* (M),1.36 ±0.03* (F)g/l; apolipoprotein B 1.14 ±0.03* (M),1.31 ±0.04* (F) g/l, albumin 37.4 ±0.5* (M),38.6 ±0.5* (F) g/l; systolic bp 123 ±1.7 (M),124 ±1.9 (F) mmHg; diastolic bp 85.3 ±0.9* (M),86.5 ±0.9* (F)mmHg.
Westernization and not obesity is the risk factor associated with diabetes and cardiovascular disease.

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CARDIOVASCULAR RISK FACTORS IN TYPE 2 DIABETES WITH MICROALBUMINURIA.

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Microalbuminuria is related to an increased risk for cardiovascular complications in type 2 diabetic patients. To gain insight this relationship we evaluated the blood pressure, serum lipids and fibrinogen levels in relation to urinary albumin excretion rate (AER) in 65 non-insulin dependent diabetes mellitus with normoalbuminuria (AER < 20µg/min) and 42 type 2 diabetic patients with microalbuminuria (AER 20-200 µg/min) matched for age, sex, body mass index and glycohemoglobin levels. During two years cardiovascular events (sudden death or miocardial infarction (MI) in both groups were recorded. A positive linear correlation between fibrinogen and AER levels was found ($r=0.47$; $p<0.01$). Microalbuminuric patients had significantly higher plasma fibrinogen levels than the normoalbuminuric group (5.3±1.02 vs 3.9±0.7 g/L; $p<0.01$). No signifficant differences in blood pressure, total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides were observed between the two groups. Two years later 11 (10.3%) of these patients had MI; 7 (16.6%) from the microalbuminuric cohort and 4 (6.1%) from the normoalbuminuric group ($p<0.05$). 2 deaths after MI happened in microalbuminuric group. No patient with AER < 20 ug/min died of cardiovascular disease. These data suggest that the presence of microalbuminuria is an independent cardiovascular risk-factor in type 2 diabetes.

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MICROALBUMINURIA AND ATHEROSCLEROTIC RISK FACTORS IN TYPE 2 (NON-INSULIN-DEPENDENT) DIABETICS.

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Supranormal urinary albumin excretion (microalbuminuria) predicts development of overt nephropathy and is associated with higher cardiovascular mortality in both type 1 and type 2 diabetes. In a cross-sectional study, we analyzed the relationship between microalbuminuria and atherosclerotic risk factors in 304 (160 males, 144 females) unselected type 2 (non-insulin dependent) diabetic subjects [age: 63±10 yrs; known duration of diabetes: 10.9±8.8 yrs; age at diabetes diagnosis: 52±11 yrs; BMI 27±4 (range 19-39); SBP: 150±23 mmHg; DBP: 86±11 mmHg - m±sd]. In an "early morning" urine sample, albumin (nephelometry) and creatinine were assayed. By albumin/creatinine ratio (A/C, mg/mmol), we subdivided patients in normoalbuminurics (N; A/C <2.0; n. 152, 50%), microalbuminurics (m; A/C 2-20 mg/mmol; n. 128, 42%) and macroalbuminurics (M; A/C >20 mg/mmol; n. 24, 8%). Among the three groups there were no differences in mean age, age at diagnosis and duration of diabetes, fasting plasma glucose, urinary glucose, serum triglyceride and HDL-cholesterol levels. Differences were observed for:

	Normo-albuminurics	Micro-albuminurics	Macro-albuminurics	*Anova, p<*
SBP (mmHg)	146±20	153±24 (a)	168±26 (b,c)	*0.001*
DBP (mmHg)	84±10	88±11 (a)	89±9	*0.005*
HbA1c (%)	6.9±1.4	7.4±1.5 (d)	7.2±1.4	*0.05*
Serum creatinine (mg/dl)	1.01±0.21	1.06±0.95	1.23±0.41 (b,c)	*0.005*
Total cholesterol (mg/dl)	198±43	218±45 (d)	207±29	*0.005*
LDL-cholesterol (mg/dl)	131±38	145±42 (d)	131±28	*0.05*

a - m vs N, p<0.01 *b - M vs N, p<0.01* *c - M vs m, p<0.01*
d - m vs N, p<0.05

Further, differences were seen for the occurrence of hypertension, defined as blood pressure ≥160/95 mmHg and/or drug treatment (N: 51%, m: 65%, M: 78%; p<0.001) and obesity, stated as BMI >30 (N: 15%, m: 26%, M: 32%; p<0.05), not for smooking habits. Finally, both ischaemic heart disease (15% vs 30%) and claudicatio intermittens (7% vs 18%) were more frequent in "m' than in "N" subjects (p<0.05). In conclusion, microalbuminuria tends to aggregate with risk factors for atherosclerotic vascular disease, e.g. increased prevalence of hypertension and obesity, elevated total and LDL-cholesterol levels. These abnormalities may in part to explain the excess of cardiovascular morbidity and mortality in type 2 diabetics with increased albuminuria.

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EXAGGERATED SERUM INSULIN RESPONSE TO AN ORAL GLUCOSE LOAD IN NORMOGLYCAEMIC CHINESE SUBJECTS RESIDENT IN THE UNITED KINGDOM

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An increased risk of ischaemic heart disease (IHD), diabetes and hyperinsulinaemia has been reported in Asian Indian immigrants in the UK, and in Chinese immigrants in Mauritius, but little is known of insulin responses and IHD risk in Chinese immigrants to the UK. We have therefore examined the serum insulin response to a 75 gram oral glucose load in those 534 Europid and 111 Chinese adults screened with normal glucose tolerance (fasting and two hour venous plasma glucose <7.8 mmol/l) and no symptoms of coronary heart disease. The 2 ethnic groups were of similar age (range 20-70 years), gender distribution, and body mass index, and blood presure was comparable. Fasting serum insulin (total immunoreactive insulin) was similar in the Chinese and Europid groups (8.3±1.0 (mean±SE) Vs 7.1± 0.3 mU/l, respectively). In contrast, both 1 and 2 hour post-glucose load serum insulin concentrations were increased in the Chinese subjects (94.9±8.6 Vs 58.7±1.4 and 51.2±4.5 Vs 27.6±0.9 mU/l, $p<0.0001$ and <0.001, respectively), implying insulin insensitivity. The contribution of proinsulin related molecules is presently unknown. This type of exaggerated pancreatic response to oral glucose has previously been reported in Asian Indians where beta cell hypersecretion and peripheral insulin insensitivity are characteristic. The previously noted increased risk of progression to diabetes mellitus in Asian Indians and in the Mauritian Chinese might also extend to the UK Chinese community.

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10 YEAR MORTALITY IN DIABETICS AGED 40-65 AT DIAGNOSIS: INTENSIVE DIETARY MANAGEMENT.

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The 10-year outcome has been prospectively observed in 432 newly-diagnosed symptomatic diabetic patients presenting between 1970 and 1980. All were managed by intensive dietary therapy, with 3-monthly medical and dietetic assessments. The average diet was energy restricted - 1500 cal daily, 50% carbohydrate, 38% fat, 12% protein. For 158 patients whose mean fasting plasma glucose exceeded 12.0 mmol/l and whose weight remained above standard, an oral hypoglycaemic was given: for 73 patients whose weight fell below standard, insulin was started. The person years at risk method showed that the 105 deaths observed compared with 82.7 deaths expected from Northern Ireland rates, giving a ratio of 1.3 (95%CI 1.0, 1.5). Cause-specific ratios were: ischaemic heart disease 2.1 (1.6, 2.7), other circulatory disease 0.8 (0.4, 1.4), neoplasms 0.8 (0.5, 1.3) and other causes 0.7 (0.4, 1.2). All-cause ratios for males 1.1 (0.8, 1.4) and females 1.6 (1.2, 2.2) differed significantly (P=0.05). The ratios while managed on diet only 1.2 (0.9, 1.5), while on oral hypoglycaemics 1.5 (1.0, 2.3) and after starting insulin 1.7 (0.9, 2.9) did not differ significantly (P=0.30). The excess risk of ischaemic heart disease became apparent after the first two years of follow-up. In this group of patients excess mortality is confined to ischaemic heart disease and largely to females.

PS 17
Genetics

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HLA-DRB1*0405 HAPLOTYPE IS MOST STRONGLY ASSOCIATED WITH IDDM IN ALGERIANS

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In order to determine genetic markers associated with Type 1 diabetes in the Algerian population, we identified HLA-DRB1, DQA1 and DQB1 alleles in the families of 50 diabetic probands and in 46 unrelated controls by extensive PCR-non radioactive sequence specific oligonucleotide typing. DRB1*0301-DQw2 haplotypes were significantly increased among patients (45% vs 13% in controls, RR=5.5, $p<10^{-6}$). Unlike in other Caucasian populations, the DRB1*0405-DQw8 haplotype was among six DR4 subtypes the only one found at increased frequency in the Algerian patients (25% vs 1% in controls, RR=30.3, $p<10^{-3}$). Of 23 DR3-DQw2/DR4-DQw8 heterozygous patients, 17 (74%) possesed DRB1*0405 compared with 0 of 2 controls. This genotype, the only one significantly associated with diabetes in Algerians (34% in patients vs 0% in controls, RR=49, $p<10^{-4}$) was not previously described. It suggests the specific involvement of DRB1*0405 in the susceptibility to diabetes in this population, possibly due to Asp 57-negative DRβ chain.

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IS THE HIGH INCIDENCE OF DIABETES IN YOUNG CHILDREN IN FINLAND DETERMINED BY GENETIC FACTORS?

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The incidence of diabetes in Finland is already very high among 1-4 year old children (31/100,000) while in the age group 5-14 years it is 40/100,000. In a population-based study 161 children were diagnosed under the age of 4 years and 533 between 5 and 14 years. The frequencies of the two most common haplotypes which confer susceptibility to Type 1 diabetes in middle and northern European populations the A2,Cw3,Bw62,DR4,DQw8 and the A1,Cw7,B8,DR3,DQw2 haplotype were similar in the two age-groups. A2,Cw3,Bw62,DR4,DQw8 was 9.3% in the 0-4 year age group and 10.5% in the 5-14 year age-group. A1,Cw7,B8,DR3,DQw2 was 8.4% and 9.6%, respectively. In contrast, the A2,Cw1,Bw56,DR4,DQw8 susceptibility haplotype which has so far only been found in Finland was more frequent in the younger age group (8.1% versus 4.6%, p=0.0104). Also the A3,Cw4,B35,DR4,DQw8 haplotype was twice as frequent in the younger age-group (4.3% versus 1.7%, p=0.0027). None of the other haplotypes differed significantly, with age nor did the frequency of DR3,DR4 heterozygosity.

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RISK OF TYPE I DIABETES IN SIBLINGS ACCORDING TO GENETIC AND IMMUNOLOGICAL MARKERS. A 8-YEAR PROSPECTIVE STUDY.

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To determine the predictive values of genetic markers for development of Type I diabetes in relatives of diabetic children, 536 siblings aged 2-29 years were consecutively recruited since 1983 for prospective follow-up. Risk of developing diabetes was estimated by actuarial methods according to shared HLA-haplotypes, DR antigens, C4 allotypes and islet-cell antibodies (ICA). Survival curves were compared by logrank test. Fifteen siblings developed diabetes during the survey (actuarial risk 4% by age 22 years). Risk was significantly higher for DR3,4 (12%) than DR3 or DR4 positive siblings (4% and 3%, respectively, $p<10^{-5}$), and for HLA-identical (7%) than haploidentical siblings (4.4%, $p<0.01$). C4BQ0-positive siblings had higher risk (11%) than C4BQ0-negatives (3%, $p<0.01$). The predictive value of genetic markers alone was poor (DR3,4 = 12%, identical = 7%, C4BQ0 = 9%) compared with ICA ≥ 5 JDFU (41%). Combined analysis showed higher predictive values for $ICA^+DR3,4^+$ siblings (58%; risk after 8 years = 70%, by age 22 years = 84%), compared with $ICA^+DR3,4^-$ siblings (20%; risk after 8 years = 37%, by age 22 years = 20%, $p<0.005$). In conclusion, HLA markers improve the prediction of diabetes among siblings with moderate ICA levels by identifying a subgroup with increased risk and more rapid progression to diabetes.

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A GENE IN THE HLA CLASS I REGION CONTRIBUTES TO SUSCEPTIBILITY TO TYPE 1 DIABETES

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In Finland the HLA haplotype A2, Bw56, Cw1, DR4, DQA1*0301, DQB1*0302 has the highest absolute risk for type 1 diabetes. Haplotypes of Bw56, Cw1, DR4 containing HLA-A genes other than A2 are not increased in diabetes indicating that HLA-A2 contributes to disease susceptibility. To confirm that differences exist only at the HLA-A locus, HLA-A, -B, and -C genes from the high risk A2 haplotype were sequenced and the HLA-DR locus was oligotyped on both the high risk A2, and low risk non-A2 haplotypes. HLA class I genes were PCR amplified from cDNA and cloned into M13. HLA-DR4 genes were PCR amplified and hybridised with allele specific oligonucleotides. HLA-A and -C were identified as A*0201 and Cw*0101. The HLA-B gene was identical to the Bw56 sequence from an A1, A3; B8, Bw56; Cw1 haplotype (WH Hildebrand, personal communication). Fifteen of 16 subjects oligotyped were DRB1*0401. Except at HLA-A, the A2 and non-A2, Bw56, Cw1, DR4 haplotypes are identical. Therefore, HLA-A*0201, or a gene close to it, may contribute to susceptibility of type 1 diabetes on this haplotype. Comparison of HLA-A sequences on high risk and low risk haplotypes reveals amino acid differences at positions involved in peptide presentation.

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HLA-DQA1 AND -DQB1 POLYMORPHISM IN SWEDISH CHILDREN WITH TYPE I (INSULIN DEPENDENT) DIABETES

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Type I (insulin dependent) diabetes has been shown to be associated with the HLA-DQ locus. In the present study the polymorphism of HLA-DQA1 and DQB1 genes were analysed in 476 consequtively diagnosed Type I (insulin dependent) diabetic Swedish children (0-14 years) and in 372 unrelated population based controls. Amplification by the polymerase chain reaction and hybridization with sequence specific oligonucleotide probes for DQA1 and DQB1 was done in all individuals. The DQA1*01 alleles (DQA1*0101, DQA1*0102, DQA1*0103) were reduced ($p<0.001$) in patients (31%) compared to controls (58%). DQA1*0301 and DQA1*0501 were more frequent ($p<0.001$) in patients (80% and 53%) than controls (42% and 34%) DQB1*0302 and DQB1*0201 were also increased ($p<0.001$) in patients (75% and 56%) compared to controls (30% and 29%). DQA1*0501-DQB1*0201 was present in 49% of patients and 22% controls ($p<0.001$) and DQA1*0301-DQB1*0302 in 75% patients and 30% controls ($p<0.001$). It is confirmed that DQA1*01 alleles confer protection and DQA1*0301 and DQA1*0501 susceptibility but more than one DQA1-DQB1 combination contributes to disease susceptibility.

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HLA CLASS II GENETIC HETEROGENEITY OF TYPE 1 DIABETES MELLITUS ACCORDING TO THE AGE OF ONSET

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The association of Insulin dependent diabetes mellitus (IDDM) with certain alleles at the DRB or DQB locus is well documented in pediatric patients. Whether a similar pattern of association applies to adult onset IDDM is not clear although the disease occurs after the age of 20 in 50% of cases. HLA class II loci have been studied for 24 DRB1, 8 DQA1 and 12 DQB1 alleles in 402 type I diabetics and 405 healthy controls (all Caucasian) using oligonucleotide typing after gene amplification by the polymerase chain reaction. The alleles DR3, DR4, DQB1*0201, DQB1*0302, DQA1*0301 and DQA1*0501, were indeed enriched in diabetics and the highest relative risk was observed in patients carrying both the DR3-DQB1*0201 and the DRB1*0402 or DRB1*0405-DQB1*0302 haplotypes. However none of these alleles, or some specific residues, could account by itself for susceptibility to IDDM, suggesting a role for cis or trans interactions between the different class II loci. Furthermore, major differences in HLA class II gene profiles appeared according to the age of onset of the disease. Patients with onset after 15 (n=290) showed a significantly higher percentage of non DR3/non DR4 genotypes than did the childhood onset subjects (n=112) :18.3 % vs 2.7 %, $p<0.001$ and a lower percentage of DR3/4 genotypes :17.9 % vs 37.5 %, $p<0.001$. These non DR3/non DR4 patients, although presenting clinically as genuine insulindependent type 1 patients, showed a lower frequency of islet cell antibodies at onset : 20% vs 62%, and a significantly milder initial insulin deprivation as reflected by differences in weight loss before treatment. These subjects probably represent a particular subset of IDDM patients in whom frequency is increased with age : 2.7 % below 15 years, 13 % between 15 and 30, and 26 % after 30. These data confirm the genetic heterogeneity of IDDM as previously suspected by serologic methods. They prompt for caution in extrapolating to adult patients the genetic concepts derived from childhood IDDM.

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MHC FACTORS AT NON DR/DQ LOCI ARE LIKELY TO BE INVOLVED IN DR4-ASSOCIATED SUSCEPTIBILITY TO TYPE 1 DIABETES IN A CHINESE POPULATION.

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Approximately 60% of genetic susceptibility to Type 1 diabetes is HLA encoded. At least two distinct genes are involved, one associated with DR4 and one with DR3. In contrast with other ethnic groups DR4 is not significantly associated with Type 1 diabetes in the Southern Chinese. This may be due to differences in the DR4 haplotypes in this race. A potential site for such a difference is at the DRB1 locus. This was investigated using an allele specific polymerase chain reaction (PCR) to amplify the DR4 alleles of 10 DR4-positive Type 1 diabetic subjects and 21 DR4-positive control subjects of Southern Chinese ethnic origin, and gene probing to distinguish the DR4 subtypes. No DR4 subtype was significantly associated with diabetes in this race. The DR4 subtypes, Dw4 and Dw10, which are associated with the disease in Caucasians were absent from this population. A Chinese DR4 haplotype encoding DR4 (Dw15)-DQA1*0301-DQB1*0401 was reduced in diabetic subjects [2/10 (20%) vs 12/21 (57.1%), not significant]. This directly contrasts with Japanese subjects where this apparently identical haplotype is strongly associated with Type 1 diabetes, suggesting that MHC factors at non DR/DQ loci are involved in DR4-associated disease susceptibility.

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Polymorphism in non-coding regions of the HLA-DQA genes may contribute to DR3 associated susceptibility to Type I (insulin-dependent) diabetes.

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HLA-DR3 is positively associated with type I diabetes in many races. It also occurs in over 30% non-diabetic controls and thus its disease association must result from linkage with another susceptibility gene. The DR3 associated DQA1 and DQB1 alleles (DQA1*0501 and DQB1*0201 respectively) are identical in diabetic and control subjects and thus are not primary susceptibility determinants. It has been reported that a DQA BglII RFLP pattern with a 7.2kb band is present on all DR3 positive diabetic haplotypes but in only 50% of DR3 positive controls, suggesting that variation in non-coding sequences of the DQA genes may determine disease susceptibility. We tested this hypothesis on DNA from 42 caucasian DR3 positive diabetic and 28 DR3 positive control subjects. Genomic DNA was digested with BglII and probed with a DQA probe. The 7.2kb fragment was present in 21/42 (50%) diabetic and 7/28 (25%) control subjects ($p<0.05$). Although this difference was not as great as in the smaller previous study, we conclude that the 7.2kb fragment differentiates between diabetic and control DR3 haplotypes suggesting that polymorphism in non-coding or promoter regions of the DQA genes may contribute to DR3 associated disease susceptibility.

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ANALYSIS OF INSULIN GENE REGION-ENCODED SUSCEPTIBILITY TO TYPE 1 DIABETES IN A UK DATA SET.

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We examined insulin gene region-encoded (INS) susceptibility to type 1 diabetes in multiplex families, sporadic diabetics and unaffected controls. A multiplex family is one with at least two diabetic siblings (one diagnosed <17 years) and living parents. Sporadic diabetics were unrelated, diagnosed <17 years without affected siblings. All subjects were caucasian with grandparents born within the British Isles. There was an association between the common (+) INS allele and disease ($P<10^{-6}$). The overall relative risk for +/+INS homozygosity (RR-INS) was 2.5 (Confidence Intervals 1.7-3.6, $P<10^{-6}$) compared to 8.8 (5.1-15.1, $P<10^{-6}$) for HLA-DR4/4,4/X versus -DRX/X. When all diabetics were classified by HLA-DR genotype, there was no difference in RR-INS between HLA-DR3/4 (2.3, 1.4-3.8), HLA-DR3/3,-DR3/X (2.9,1.6-5.3) and HLA-DR4/4,-DR4/X (2.6,1.5-4.4) individuals. For probands, RR-INS was 3.2 (1.9-5.5;$P<10^{-5}$) compared with 2.2 (1.5-3.3;$P<0.0005$) for sporadics. Studies in 1973-4 and 1988 show that the incidence of type 1 diabetes in the UK has doubled. Taking the mid-point, 83% of sporadics diagnosed before 1981 were +/+INS homozygous compared with 71% diagnosed since then ($P<0.05$). The INS region confers susceptibility to type 1 diabetes independent of HLA type. The effect of INS appears to depend upon year-of-onset, suggesting an interaction with environmental factors.

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T-CELL RECEPTOR POLYMORPHISMS IN TYPE 1 DIABETES AND SUSCEPTIBILITY TO COMPLICATIONS.

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We and others have previously shown that the T-cell receptor (TCR) constant-ß (Cß) chain locus is associated with susceptibility to type I diabetes, although other studies have failed to show this. We have extended our study by investigating a further 125 individuals with type I diabetes and confirmed the increased frequency of the 10;9.2 TCR-Cß/BgI-II genotype in our patient population (56.0% vs 42.3% in 78 normal controls). Combining the two studies gave 199 patients and 204 controls (heterozygote frequency 56.8% vs 42.2%, $P<0.005$). Further analysis of the combined studies has shown that this increase in the 10;9.2 kb TCR-Cß genotype is associated with the absence of any microvascular complications after 20 years of diabetes (DC group, n=61) compared to those patients with the diabetic complications proteinuria, overt neuropathy and moderate or severe retinopathy (FH group, n=44), (62.3% vs. 31.8% respectively, $Pc<0.005$). The failure of some investigators to confirm the association between TCR-Cß and type I diabetes may be due to heterogeneity in the patient populations being studied. Finally these results suggest that the TCR ß chain genes or adjacent loci may be important in susceptibility to diabetic complications, rather than the disease itself.

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DR4 OLIGOSUBTYPES AND INSULIN-DEPENDENT DIABETES MELLITUS (IDDM) HETEROGENEITY.

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The genetic susceptibility to insulin-dependent diabetes mellitus (IDDM) is determined, in part by a gene or genes encoded within the major histocompatibility complex (MHC). The strongest associations involve DR3-DQw2 and DR4-DQw3 haplotypes in Caucasian populations. However IDDM is a heterogenous disease if we consider age at onset on the disease or association (type Ib-IDDM) or not (type 1a-IDDM) with other auto-immune diseases. We performed a genetic study to define all DR4 subtypes associated to IDDM regarding this IDDM heterogeneity. This study was performed by Polymerase Chain Reaction (PCR) with DR4 specific and DQB specific amplifications and oligotyping (PCR-SSO) using different DR4 and DQB oligoprobes. Allelic frequencies of DR4 variants were compared within 3 groups of DR4 subjects : type Ia-IDDM (n=112), type Ib-IDDM (n=24), and healthy and ethnically matched controls (n=153). The comparison between the 2 groups of IDDM clearly showed an increased frequency of Dw15-DQw8 subtype in type Ia vs type Ib (21.8 % vs 10.7 % respectively) (controls 6.2 %). Conversely, Dw14-DQw8 was largely increased in type Ib-IDDM (35.7%) vs type Ia-IDDM (13.4%) ($p < 0.02$) or controls (20.1%). Taking into account the age at onset of the disease, a surprising increase of Dw15 (DQw8) subtype (26.4%) was noted in patients aged less than 20 years at the onset of the disease (vs 15.4% of patients aged more than 20 years). Moreover Dw4 was increased in the group of patients with age more than 20 years (62.7%) vs group of patients with age less than 20 years (50%). In conclusion, through this large genetic study, we demonstrated that different DR4 subtypes were preferentially associated to some particular IDDM forms. Thus, Dw14-DQw8 or Dw15-DQw8 could be associated to type Ib-IDDM or to early form of IDDM respectively. This unequal distribution of these 2 DR4-DQw8 haplotypes regarding the genetic susceptibility to 2 types (1a and 1b) of IDDM, strongly suggests an important role of some particular epitopes of DRB1*04 molecules in IDDM susceptibility.

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THE ROLE OF HLA DQA1 AND DQB1 GENES IN PROTECTION FROM TYPE I (INSULIN-DEPENDENT) DIABETES MELLITUS.

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Protection from Type I (insulin-dependent) diabetes may be determined by aspartate at position 57 of the HLA-DQB molecule. We typed the DRB1, DQA1 and DQB1 genes from 156 caucasian diabetic and 119 control subjects using RFLP and sequence specific oligonucleotide analysis to identify protective alleles. DR2 was protective (diabetic (D) 0.6% v control (C) 24%, preventative fraction (PF) 0.24, $Pc<10^{-6}$) but DRw6 conferred stronger protection (D 2% v C 30%, PF 0.28, $Pc <10^{-6}$). DR7 was also protective (D 9% v C 25%, PF 0.18, $Pc <0.005$). The most protective DQA1 allele was DQA1*0102 which occurred on both DR2 and DRw6 haplotypes (D 2% v C 43%, PF 0.41, $Pc <10^{-6}$), DQA1*0103, which occurred on 35% DRw6 haplotypes, (D 2% v C 10%, PF 0.08, $Pc <0.035$) and the DR7-associated DQA1*0201 (D 8% v C 25%, PF 0.18, $Pc <0.004$) were also protective. DQB1*0602 and DQB1*0603 occurred on DR2 and DRw6 haplotypes respectively and were protective (DQB1*0602: D 1% v C 17%, PF 0.17, $Pc <10^{-5}$; DQB1*0603: D 0% v C 10%, PF 0.10, $Pc <10^{-3}$). The DRw6-associated DQB1*0604, which is an Asp-57-negative allele, and the DR7-associated DQB1*0303 were also protective (DQB1*0604: D 2% v C 16%, PF 0.14, $Pc <10^{-3}$; DQB1*0303: D 3% v C 20%, PF 0.17, $Pc <5x10^{-4}$). We conclude that DQA1*0102 is associated with greater protection from disease than any DQB1 allele and that DQB-Asp 57 is not a primary protective determinant.

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TNFB GENE POLYMORPHISMS AS DETECTED BY POLYMERASE CHAIN REACTION IN PATIENTS WITH TYPE 1 DIABETES MELLITUS AND GRAVES' DISEASE

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Organ specific autoimmune diseases display an association with factors with the HLA-system encoded by genes on the short arm of chromosome 6. Both type 1 diabetes and Graves' disease patients show an increase of the specificity HLA DR3 and the extended haplotype A1B8DR3. Individuals with this haplotype have a rarer polymorphism of the tumor necrosis factor beta (TNFB) gene (5.5kb NcoI fragment) as detected by restriction fragment length polymorphism (RFLP) analysis. They also produce different levels of cytokines such as TNFB upon stimulation compared to individuals with other haplotypes. We therefore investigated whether patients with type 1 diabetes and with Graves' disease differ from normals in terms of genetic variation at the TNFB locus, which has been shown to reside in an intron of TNFB. We designed specific flanking primers of this region spanning a 740bp exon1/intron3 TNFB fragment and performed a polymerase chain reaction on genomic DNA of 194 patients with type 1 diabetes, 174 patients with Graves' disease and 173 controls. Subsequent NcoI digestion and agarose gel electrophoresis revealed the alleles TNFB*1 (740bp) and TNFB*2 (555bp+185bp). Heterozygosity for TNFB*2/*1 was significantly increased both in 107 of 194 (55%) type 1 diabetes patients ($p<6x10^{-5}$), 96 of 174 (55%) patients with Graves' disease ($p<0.02$) in comparison with 72 of 173 (42%) controls. Homozygosity for TNFB*2 was found to be reduced in patients with type 1 diabetes (52, 27%) and with Graves' disease (60, 34%) compared with controls (84, 49%), whereas homozygosity for TNFB*1 was only increased in patients with type 1 diabetes (35, 18%) compared with Graves'disease patients (10%) and controls (10%). These results confirm our earlier data obtained with RFLP analysis and endorse the association of TNFB gene variation with type 1 diabetes and Graves' disease. This genetic variation could be functionally relevant via differential production of lymphotoxin (TNFB) in activated T-lymphocytes infiltrating the target tissue.

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BOTH DQA1*0201 AND DQB1*0303 CONTRIBUTE TO DR7 ASSOCIATED PROTECTION FROM TYPE I (INSULIN-DEPENDENT) DIABETES MELLITUS IN BRITISH CAUCASIAN SUBJECTS.

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The aim of this study was to define further DR7 associated protection from Type I diabetes in a large British Caucasian population. Previous work by this group has shown that DR7 protects against Type I diabetes in this population. We determined the DQA1 and DQB1 alleles in 150 Type 1 diabetic subjects and 110 controls. The DR7-DQB1*0303 haplotype was found to be protective and the DR7-DQB1*0201 haplotype was neutral. Both haplotypes carry DQA1*0201 which was negatively associated with the disease and occurred only on DR7 haplotypes (Diabetic (D) 7.6% V Control (C) 24.8%, P_c <0.004). DQB1*0201 was positively associated with the disease (D 74.5% V C 39.4%, P_c $<10^{-6}$) and occurred predominantly on DR3 haplotypes. DQB1*0303 was negatively associated with the disease (D 3.37% V C 20.2%, P_c $<5x10^{-4}$), this allele also occurred on the neutral DR9 haplotype. DQA1*0201 occurred on all but three DR7 diabetic haplotypes in this population and may explain the protective effect of DR7. The neutral disease association of DR7-DQA1*0201-DQB1*0201 may result from the opposing effects of the DQA1 and DQB1 alleles on this haplotype. In contrast the protective effect of DQB1*0303 on DR7-DQA1*0201-DQB1*0303 haplotypes maintains the negative disease association. In conclusion both DQA1*0201 and DQB1*0303 contribute to DR7 associated disease protection.

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COMBINED ANALYSIS OF BOTH DQA1 AND DQB1 GENE POLYMORPHISMS IS REQUIRED TO EVALUATE THE RISK FOR TYPE 1 DIABETES

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Susceptibility to type 1 diabetes has been shown to correlate with the absence of aspartic acid in position 57 of the DQ beta chain (NONASP57) and with the presence of arginine in position 52 of the DQ alfa chain (ARG52). We analysed DQA1 and DQB1 gene polymorphisms in a group of patients from Central Italy (n=60) and in randomly selected controls (n=93). Analysis was carried out by Polymerase Chain Reaction amplification of DNA encoding the first polymorphic domain of DQB1 and DQA1 genes. DQB1 gene polymorphism was evaluated by Dot Blot analysis. DQA1 typing was performed using a procedure based on heteroduplexes analysis of DNA molecules formed by annealing of mismatched allele strands. 63.3% of patients and 23.7% of controls were NONASP/NONASP (RR=5,6), 33.3% of patients were NONASP/ASP vs 41.9% of controls (RR=0.7) and 3.3% of patients were ASP/ASP vs 34.4% of controls (RR=0.06). For DQA1 52.7% of patients and 20.4% of controls were ARG/ARG (RR=4.42), 40% of patients vs 6.2% of controls were ARG/NONARG (RR=0.4) and 7.3% of patients vs 19.3% of controls were NONARG/NONARG (RR=0.33) Individuals homozygous for both susceptibility markers showed the highest relative risk (RR=57). We conclude that analysis of both genetic markers is required to evaluate the risk for type 1 diabetes.

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DIFFERENT EFFECT OF FASTING AND HYPOGLYCEMIA ON GLUT-2 AND GLUCOKINASE GENES IN LIVER AND PANCREAS.

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Effect of 3 day fasting (F), 5 day insulin-induced hypoglycemia (H) on the expression GLUT-2 and glucokinase (GK) genes in pancreas and liver was tested. The mRNA levels of GT2 and GK in pancreas were determined by ribonuclease protection assay, while in liver by dot blot hybridization. The RNA/DNA ratio decreased in (-64%, $p<0.001$) in F group (n=5), therefore all data were calculated per µgDNA. There was a decline in plasma glucose (-30%, $p<0.05$), Proinsulin mRNA (-75%, $p<0.01$), Amylin mRNA (-80%, $p<0.01$), islet GLUT-2 mRNA (-73%, $p<0.01$), islet-GK mRNA (-44%, $p<0,05$) and liver-GK mRNA (-80%, $p<0.01$) levels. In H.group (n=5) Proinsulin (-71%, $p<0.05$), Amylin (-7%, $p<0.01$), islet-GLUT2 (44%, $p<0.05$), islet-GK (-50%, $p<0.05$) mRNA levels were decreased and liver-GK mRNA increased (+61%, $p<0.05$). Correlation between Proinsulin and Amylin mRNAs ($r=0,974$, $p<0.001$) suggests common transcriptional regulation for these genes. These data extend the observation that GLUT2 and GK genes are regulated in different fashion in islet and liver.

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THE CONTRBUTION OF GLUCOKINASE TO TYPE 2 (NON-INSULIN-DEPENDENT) DIABETES - A POPULATION ASSOCIATION STUDY.

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Type 2 (non-insulin-dependent) diabetes is tightly linked to the glucokinase locus in 45-95% of pedigrees with Maturity Onset Diabetes of the Young (MODY), suggesting that defects in the glucokinase gene are important in MODY. The contribution of the glucokinase gene to Type 2 (non-insulin-dependent) diabetes (diagnosis >40 years) is uncertain. To assess this, we performed a population association study. We studied the polymorphic dinucleotide repeat 10 Kb 3' to the glucokinase gene. Five alleles were detected by amplification using the polymerase chain reaction and resolution by 6% polyacrylamide gels. In 50 unrelated Caucasian non-diabetic controls (fasting plasma glucose <5.5 mmol/l, age 55.5 +/- 11.2 years; mean +/- s.d.) the allelic frequencies were 0.66, 0.11, 0.22, 0.01, and 0.00 (z, z+2, z+4, z+6, z+8) In 100 unrelated Type 2 (non-insulin-dependent) diabetics (age 57.8 +/- 8.7 years; mean +/- s.d.) the allelic frequencies were 0.655, 0.075, 0.265, 0.00, and 0.005. There were no significant differences in the allelic frequencies between the two subject groups (X^2=3.985, 4 d.f. p=0.408; Fishers exact test p=0.407). Our finding suggests that Type 2 (non-insulin-dependent) diabetes does not frequently arise from a common mutation in the glucokinase gene.

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ALDOSE REDUCTASE (AR2) GENE EXPRESSION IN CULTURED HUMAN RETINAL PIGMENT EPITHELIAL CELLS (RPE).

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Modulation of AR2 in the kidney is part of adaptation to osmotic stress. Expression of AR2 in non-renal tissues under hyperosmolar **(HO)** and hyperglycemic **(HG)** conditions is less well understood. This study determined the expression of AR2 in response to HO (300 mM glucose = 595 mOsm/kg) and HG stress (20 mM glucose = 310 mOsm/kg). Four different RPE cells were exposed for up to 7 days to isotonic (5 mM glucose = 295 mOsm/kg), HO, and HG media, then AR2 mRNA, sorbitol and myo-inositol levels (mmol/mg protein) were measured. RPE cell lines 0308, 125, & 45 had a 33-55 fold induction of AR2 by HO but did not change in HG conditions. AR2 mRNA levels declined approximately 30% after 7 days exposure to HO conditions . Production of S (from undetectable to 164±14.9) and depletion of MI (from 32.43±.0.7 to 8.29±0.45) followed increases in AR2 mRNA. RPE cell line 91 had a high constitutive expression of AR2 which precipitated a rapid and profound production of S (from undetectable to 468.4±39.2) and reciprocal depletion of MI (from 44.1±1.7 to 1.67±0.3). HO but not HG stress increases AR2 expression in most RPE lines. High constitutive expression of AR2 may confer increased susceptibility to AR2-linked diabetic complications.

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Mutation in mitochondrial $tRNA^{Leu(UUR)}$ gene in a kindred of maternally transmitted non-insulin dependent diabetes mellitus and sensorineural hearing loss.

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Non-insulin dependent diabetes mellitus (NIDDM) is a heterogeneous disorder characterized by impaired glucose homeostasis. NIDDM has clearly an inherited component, but the nature of the genetic defects remains unknown. We have identified a large pedigree in which NIDDM, in combination with a sensorineural hearing loss, is maternally inherited. The maternal inheritance and the observed decrease in respiratory chain enzyme activities in skeletal muscle indicate a defect in the mitochondrial DNA. We analyzed mitochondrial DNA of family members by RFLP and DNA-sequence analysis. A heteroplasmic A to G transition mutation at nucleotide 3,243 was identified, being an evolutionary conserved position in the mitochondrial gene coding for $tRNA^{Leu(UUR)}$. This mutation correlated with the disease in this family and was absent in a large panel of controls. The mutation affects RNA processing and translation efficiency in the mitochondrion. It is expected that an impaired mitochondrial activity leads to an increased glycolytic flux and, via the Cori cycle, to enhanced gluconeogenesis in the liver. Clinical studies are in progress to elucidate the basis of the impaired glucose homeostasis. We conclude that mitochondrial defects are responsible for the genesis of some types of diabetes mellitus.

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CONCORDANCE RATE OF TYPE 2 (NON-INSULIN-DEPENDENT) DIABTES AMONG MONOZYGOTIC AND DIZYGOTIC TWINS

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Concordance rate of Type 2 (non-insulin-dependent) diabtes in monozygotic twins (MZT) is reported to be about 80-90%. However, the blood glucose levels to define Type 2 diabetes are rather arbitrary. The concordance rate for diabetes may vary depending upon the diagnostic criteria. We collected informations on 100 twin pairs with Type 2 diabetes. Concordance rate by doctors' report was 77%(60/78) and 41%(9/22) in MZT and dizygotic twins (DZT). The probands of discordant MZT pairs had greater body mass index (BMI) than probands of concordant pairs at the time of survey (24.3±3.6 vs 21.3±2.8, $p<0.001$), and the maximal BMI in the past was 27.3±4.4 vs 24.9±3.0 ($p<0.01$) respectively. Type 2 diabetes was subdivided into 3 categories: (A) those requiring drug treatment, (B) fasting plasma glucose exceeding 7.8 mmol/l, and (C) diabetes defined by WHO 75g GTT criteria. According to criteria A, B and C, the concordance rate among MZT was 61%(34/56), 66%(45/68) and 85%(66/78), and the concordance among DZT was 47%(7/15), 41%(9/22) and 54%(12/22) respectively. The concordance rate becomes higher when milder diabetes is included, particularly among MZT. These data suggest that glucose intolerance is primarily determined by heredity, but the development of diabetes may be affected by environmental factors such as obesity.

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TYPE 2 (NON-INSULIN-DEPENDENT) DIABETES MELLITUS IN SOUTH INDIANS IS A POLYGENIC DISEASE

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Though a dominant model of inheritance is probable in populations at extreme risk of type 2 (non-insulin-dependent) diabetes mellitus, the mode of inheritance in populations at lower risk is unclear. Forty-one Dravidian pedigrees (281 subjects) were ascertained through an affected offspring (blind to family history) having both parents and at least one sibling available for glucose tolerance testing. A majority of parents proved diabetic (58/82): in all but two families, at least one parent was diabetic. The number of diabetic parents within a family was not associated with earlier age-of-onset amongst probands, but was associated with increasing proband obesity (BMI mean(SD) 21.6(2.0)kg/m^2, 24.3(3.4), 26.9(4.0) : none, one or both parents diabetic, p=0.02). Segregation analysis was performed using POINTER : IGT was considered non-affected. Best fit for single locus models was obtained for co-dominance (d=0.49, gene frequency=0.20) (likelihood ratio vs. dominant model 86, vs. recessive, 1.5x10^4): a closer, more parsimonious fit was obtained from a polygenic model (heritability(H)=0.97 : likelihood ratio vs. co-dominance 5.2x10^3, p<0.001). Attempts to fit a mixed model failed to improve the polygenic model. These preliminary results suggest that type 2 (non-insulin-dependent) diabetes mellitus in Dravidians is a polygenic disease but do not indicate the extent of genetic heterogeneity.

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AN ASSOCIATION EXISTS OF APOD POLYMORPHISM AND TYPE 2' DIABETES IN SOUTH INDIANS AND NAURUANS

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The apolipoprotein (ApoD) gene is located to chromosome 3 near the islet-cell glucose transporter gene. Genetic mutations in this chromosomal region could link both Type 2 diabetes and ischaemic heart disease. The purpose of this study was to look for an association between ApoD gene polymorphism and Type 2 diabetes in three ethnic groups from Nauru (controls n=53; diabetics n=56), South India (controls n=79; diabetics n=77) and Finland (controls n=31; IGT n=26; diabetics n=60). DNA was digested with Taq 1 and studied by Southern blot hybridisation technology and an ApoD gene probe; two alleles were identified sized 2.2 and 2.7 kb. No association was found between ApoD and Type 2 diabetes in the Finnish population. A reduction of the 2.2 homozygotes in diabetics was found in both Nauruans (3.5% compared to 21% in controls, p=0.01) and South Indians (6.5% compared to 19% in controls, p=0.002). Several explanations exist for these results. Heterogeneity of Type 2 diabetes or within the disease. Alternatively, linkage disequilibrium may exist between the ApoD RFLP and a diabetogenic locus on chromosome 3, which is only maintained in certain ethnic groups. Further investigations are required to explore the association between ApoD gene polymorphism and Type 2 diabetes.

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DIABETES MELLITUS IN CYSTIC FIBROSIS: GENETIC AND IMMUNOLOGICAL MARKERS

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Genetic and immunological markers of Type 1 diabetes mellitus were studied in 34 diabetic and 34 matched non-diabetic patients with cystic fibrosis (CF). Frequency distributions of HLA-DR3, -DR4, and DR3/4, confering susceptibility to Type 1 diabetes, and of HLA-DR2, confering resistance to Type 1 diabetes, were normal in diabetic and non-diabetic CF patients. Unexpectedly, in diabetic CF patients frequencies of tumor necrosis factor beta and heat shock protein 70 alleles, located in the HLA region on chromosome 6, were not different from those in Type 1 diabetic patients, while non-diabetic CF patients and normal subjects shared other patterns. Frequencies of interleukin 1 beta alleles, located on chromosome 2, did not differ between non-diabetic and diabetic CF patients, Type 1 diabetic patients, and normal subjects. Islet cell cytoplasmic antibodies, measured 5 years before, at, and 1 year after diabetes diagnosis in the index case, were detected in 2 of 236 sera (0.8%): once in a prediabetic and once in a non-diabetic CF patient. Thus, diabetes in CF is without HLA-DR association and serological evidence for autoimmune destruction of the pancreatic beta cells. The significance of similar frequencies of tumor necrosis factor beta and heat shock protein 70 alleles in Type 1 diabetic patients and diabetic CF patients remains to be determined.

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REGENERATING (REG) GENE EXPRESSION IN THE PANCREAS OF THE PREDIABETIC BB/S RAT

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High levels of Reg gene expression occur in hyperplastic rat islets in surgically induced diabetes. We have studied Reg gene expression in pancreatic biopsies from 46 BB/S rats (23 diabetes prone - DP; 23 diabetes resistant - DR) in the prediabetic period. Each individual animal was biopsied 4 times at ages 30/40, 50/60, 70/80 and 90/100 days (biopsies B1, B2, B3, B4 respectively). Northern/dot blot analyses were used to semiquantify biopsy levels of Reg, preproinsulin and β-actin mRNA. In DR rats levels of Reg mRNA (mean $\pm$ SD ratio of Reg/β-actin) were 1.14 $\pm$ 0.42 (designated 100%) in B1 followed by a steady decline (86% - B2; 56% - B3) to 50% in B4. DP animals also showed high Reg mRNA levels in B1 (0.92 $\pm$ 0.37 - NS vs DR values) with a similar decline in B2 (81%) and B3 (56%) (NS from DR rats). In B4 Reg mRNA levels in DP rats increased to B1 values ($p<0.001$ vs DR rats). The mean ratio of preproinsulin/β-actin did not change significantly with age in either DP or DR BB/S rats. Findings indicate: i) Decrease in islet cell growth with increasing age is associated with a reduction in Reg gene expression; ii) Increased Reg mRNA levels in DP rats at or around the mean age for onset of diabetes (90/100 days) suggests an association of the Reg gene with an activation of islet cell adaptive repair/regeneration mechanisms in response to autoimmune islet cell attack.

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Clinical Diabetes

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AGENESIS OF THE DORSAL PANCREAS IN A WOMAN WITH DIABETES MELLITUS AND IN BOTH OF HER SONS

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Complete agenesis of the dorsal pancreas has rarely been reported. Except for two female patients (1923 and 1987), all other reported cases were males with insulin-dependent diabetes mellitus. We report complete agenesis of the dorsal pancreas in a female with normal pancreatic function until gestational diabetes mellitus developed during the second pregnancy. This anomaly of agenesis of the dorsal pancreas was suspected by abdominal ultrasound and confirmed by CT and endoscopic retrograde pancreatography (ERP). The head of the pancreas was in normal location and appeared to be enlarged, while the body, tail and uncinate process were completely absent. ERP showed a normal duct in the head of the pancreas, but there was nonvisualization or absence of a minor papilla and the duct system of the corpus and cauda. Dietary restrictions, treatment with acarbose, biguanides and sulfanyl urea derivatives did not adequately correct blood glucose levels. Insulin therapy led to satisfactory levels of blood glucose and HbA1c. Both of our patient's sons also had complete agenesis of the dorsal pancreas as shown by CT but signs and symptoms of diabetes mellitus where not present. This familial occurrence suggests that hereditary factors may play a role in the pathogenesis of this anomaly.

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LONGITUDINAL EVALUATION OF GLUCOSE TOLERANCE AND INSULIN SECRETION IN NON DIABETIC CYSTIC FIBROSIS PATIENTS.

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Cystic fibrosis (CF) patients have a high prevalence of glucose tolerance abnormalities,which have been referred to a progressive ß-cell damage.However no longitudinal data are available on glucose tolerance and ß-cell function in these subjects. We studied 32 CF patients (14 males,mean age 12.8 ±4.6 years,mean body mass index 17.5±2.2),selected on the basis of repeatedly normal fasting blood glucose,who underwent both oral (OGTT) and intravenous (IVGTT) glucose tolerance tests at the beginning of the study and 2 years later. A pathological (impaired) glucose tolerance was observed in 12/32 subjects at the first examination and in 16/32 (14=impaired,2=diabetic tolerance) after 2 years. Insulin secretion during OGTT significantly decreased during the follow-up (insulin area=586±224 vs 813±255 pM/L,2p<0.01),especially in those patients whose glucose tolerance worsened (445±94 vs 760±190 pM/L,2p<0.001).Also first-phase insulin secretion during IVGTT was significantly reduced after 2 years(328 ±150 vs 498±96 pM/L,2p<0.01). Clinical conditions,assessed by Shwachman score,pulmonary function tests,nutritional status and body mass index,did not change during the follow-up also in patients with deteriorating glucose tolerance.These perspective data suggest that a progressive ß-cell impairment precedes and may affect the onset of glucose tolerance abnormalities in CF patients and is not related to a concomitant worsening of clinical conditions.

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EVIDENCE OF IMPAIRED EXOCRINE PANCREATIC FUNCTION IN TYPE 1 DIABETES.

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The aim of this study was to assess the exocrine pancreatic function in type 1 diabetes. Thirty insulin-dependent diabetic patients (age 38.1± 17.8 yr, duration of diabetes 14.1±10.3 yr and body mass index (BMI) 22.8±6.2 kg/m²) were compared to 24 healthy subjects matched for age, sex and BMI. Serum amylase, Lipase, p-isoamylase, glycohemoglobin,basal and stimulated C-peptide were determined. Exocrine pancreatic function was evaluated by non-invasive methods: faecal quimiotripsine and urine pancreolauryl test. A decrease in urine pancreolauryl test (< 30%) was found in 16 (53.3%) from diabetic cohort, suggesting an impairment in exocrine pancreatic function. Only two patients showed exocrine pancreatic dysfunction symptoms. Diabetic patients compared to controls subjects had lower levels in the following parameters:amylase (101.2± 34.5 vs 136.7±36.2UI/L; p<0.01), Lipase (67.4±43.8 vs 126.9±53 UI/L ; p<0.01), p-isoamilase (33.3±13.5 vs 70.5±22.1 UI/L;p<0.01), faecal quimiotripsine (25.1±15.5 vs 41.6±17.6 U/g ;p<0.01) and pancreolauryl test (31.2±16.6 vs 43.5±12.8 %;p < 0.01). There was a positive linear correlation between debut age and Lipase (r=0.55) and p-isoamylase (r=0.38); stimulated C-peptide with p-isoamylase (r=0.67) and Lipase (0.71). These data suggest that a mild exocrine pancreatic hypofunction seems to develop in a high percentage of type 1 diabetes.

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CARBOHYDRATE ANTIGEN CA-50 AS AN INDEX OF LONG-TERM METABOLIC CONTROL IN TYPE 1 AND TYPE 2 DIABETIC PATIENTS.

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The ganglioside carbohydrate antigen CA-50 is a glycoprotein associated with pancreatic carcinoma and other gastrointestinal malignant diseases.Increased levels of carbohydrate antigens have been demonstrated in poorly controlled diabetic patients,suggesting a role for metabolic control in their synthesis from exocrine pancreatic cells.This study concerns 26 Type 2,39 Type 1 diabetics and 18 controls. Serum CA-50(IFMA-Delfia,Pharmacia)was measured together with CA-19/9(IEMA,Roche)and CEA(ICMA-Berilux,Behring).Higher serum CA-50 levels were found in diabetics(33.7±11 U/ml) than in controls(5.11±3.7 U/ml,p<.001).Type 1 patients showed the highest values(38.4±13.6)if compared with Type 2 patients(27.9±9.6 U/ml,p<.01).No changes of CEA were observed among the groups,whereas CA-19/9 was significantly increased in diabetics compared to controls(57.3±20 U/ml,vs, 22.7±9.3 U/ml,p<.001).CA-50 significantly correlated with HbA1c(r=.69,p<.001),fructosamine(r=.80,p<.001),mean 24-h blood glucose(r=.57,p<.001),fasting blood glucose(r=.51,p<.001)and glicosuria(r=.47,p<.001).Moreover higher CA-50 levels were found in poorly controlled patients than in those with good metabolic control(49.5±18 U/ml,vs,18±9 U/ml,p<.001).A reduction of CA-50 concentrations was found only after 60 days(p<.001)in parallel with the decrease of HbA1c levels,and was independent of short-term metabolic changes. CA-50 was more increased in patients with retinopathy and nephropathy,while no differences were found in neuropathic subjects.CA-50 may be suggested as an index of long-term metabolic control and might be implicated,as advanced glycosylated end products,in the development of angiopathy.

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INCREASED RISK FOR ACTIVE CHLAMYDIA INFECTION IN TYPE II DIABETES MELLITUS

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Infectious diseases are more frequent in diabetic than in control patients, but there is no information about active chlamydia infection. A group of 79 consecutive diabetics (60±12 years) with a mean diabetes duration of 12±9 years (1-31) and a non-diabetic control group of 125 patients (66±9 years) from another study (in which preselection was excluded) were investigated for serum chlamydia antibodies (IgG- and IgA-ChlAB) using an immunoperoxidase reaction. HbA1c was determined using HPLC. 46% of the diabetics und 55% of the control population were IgG-ChlAB positive (n.s.). Using IgA-ChlAB to define active chlamydia infection 22% of the diabetic and 14% of the control population were positive (historical controls 10%). Thus active chlamydia infection of all IgG-ChlAB-positive patients amounted to 47% in the diabetic versus 25% in the control population respectively ($p<0.05$). Forming subgroups significance was reached only in females (52% versus 32%, $p<0.05$), whereas the trend was less pronounced in males (36% versus 21%, n.s.). There was no correlation of active chlamydia infection with HbA1c. Conclusions: The study shows increased risk for active chlamydia infection in the type II diabetic, independent from blood glucose control. Further studies will be necessary to define the clinical impact of our findings.

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PROGNOSTIC FACTORS OF FATAL OUTCOME IN DIABETIC PATIENTS WITH BACTERAEMIA.

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We have studied 983 consecutive adults suffering from bacteraemia to determine the influence of specific clinical and laboratory variables on the fatal outcome in diabetic patients (11.3%). Age, sex, underlying disease, alcoholism and drug abuse, hospital and intensive care unit (ICU) acquired infection, source of infection, isolated microorganism, septic shock, type of diabetes, previous antidiabetic treatment, micro and macrovascular chronic complications, and acute metabolic decompensation (AMD) were collected prospectively. Mortality rates were 29% in diabetics and 22% in non-diabetic patients (p=0.09). Only one death was directly attributable to AMD. Variables correlated with a fatal outcome in a univariate analysis were included in a stepwise logistic regression model. Diabetes mellitus was an independent risk factor of fatal outcome (Odds ratio=1.8, Confidence intervals=1.1-2.9). In diabetic patients age (OR 9, CI 1.1-75), septic shock (OR 6.6, CI 1-49), and AMD on admission (OR 4.4, CI 1.1-17) correlated with higher mortality. We conclude diabetes mellitus is associated with a poorer prognosis in patients suffering from bacteraemia and that AMD on admission constitutes an additional risk factor despite the improvement of metabolic parameters.

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Diabetic Eye

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Retinopathy in children and adolescents with insulin dependent diabetes mellitus (IDDM).

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In order to assess the prevalence of retinopathy in children and adolescents with onset of IDDM before the age of 15.0 years we invited all 747 patients born before 1.1 1979 in eight Swedish counties with onset of the disease between 1.7 -77 and 31.12 -86. 557 (75%) were examined with fundus photography. Their age (median (95 % confidential interval)) was 15.0 (14.6-15.2) years and duration of diabetes 5.5 (5.2-5.9) years.

Retinopathy was demonstrated in 81 patients (14.5%), age 9,5 - 24 years. Mild background in 68, IRMA and soft exudates in 11 and proliferative retinopathy in one patient. Retinopathy was significantly correlated to age, duration, pubertal stage, glycosylated hemoglobin and systolic blood pressure during the last 2 years before examination, but not to insulin IE/kg, c-peptide in urine or microalbuminurea.

Even if the risk of developing retinopathy increases with age and pubertal stage, severe retinopathy could be demonstrated in a few very young children. Screening for retinopathy seems important from the age of 10.

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Predictive value of humoral markers of endothelial and basement membrane metabolism for the development of retinopathy in children with diabetes mellitus

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90 children with type I diabetes (age: 14.8 ± 3.8 years, diabetes duration: 5.8 ± 4.3 years, $\bar{x}$ ± SD) without retinopathy at the onset of the study were studied whether humoral parameters of matrix metabolism are of predictive value for the retinal findings at least 3 years later (average observation period: 4.5 ± 0.9 years). Methods: retinal status: fluorescein angiography; serum: laminin P1 (radioimmunoassay, Behring, Germany) and type IV collagen (NC1-fragment, radioimmunoassay, Schuppan, 1986); plasma: fibronectin (nephelometry, Behring) and v. Willebrand-factor (ELISA, Boehringer, Germany). Results: Elevated levels (> 2SD of normal) were found for laminin in 18%, of NC1 in 14% , of fibronectin in 46%, and v. Willebrand-Factor in 54% of the patients. Only Laminin and v. Willebrand-factor showed an association to HbA1 (microcolumn method, Panchem, Germany) (r=0.30 and r=0.33 respectively, both $p < 0.01$).

development of retinopathy	von Willebrand-factor (%)		fibronectin (mg/dl)		type IV collagen (ng/ml)		laminin (U/ml)	
	$\bar{x}$±1SD (n)	elevated levels	$\bar{x}$±1SD (n)	elevated levels	$\bar{x}$±1SD (n)	elevated levels	$\bar{x}$±1SD (n)	elevated levels
none	202±81 (32)	52%	42±15 (32)	50%	8.8±2.0 (29)	7%	1.69±.28 (66)	13%
background	174±75 (14)	50%	36±11 (14)	36%	9.8±2.3 (13)	31%	1.74±.40 (22)	32%

The endothelial parameters fibronectin and v. Willebrand-factor appear to be of no predictive value. In contrast, elevated levels of both basement membrane parameters, laminin and type IV collagen, show an association to the later development of background retinopathy.

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THE EFFECT OF HYPERGLYCAEMIA ON THE AUTOREGULATION OF THE HYPERTENSIVE DIABETIC RETINAL CIRCULATION. V. Patel, S.M. B. Rassam, H.C. Chen, and E.M. Kohner. Department of Medicine, Royal Postgraduate Medical School, London. Hypertension and poor glycaemic control are important risk factors for the pathogenesis and progression of diabetic retinopathy. The effect of hypertension on retinal vascular reactivity to 60% oxygen was studied in 8 hypertensive controls and 7 hypertensive diabetic subjects with background diabetic retinopathy at blood glucose <10mmol and>15mmol. Retinal blood flow was calculated from red cell velocity using bidirectional laser Doppler velocimetry and vessel diameters determined from computerised image analysis of retinal photographs. The oxygen reactivity (% reduction in retinal blood flow, mean ±SEM) in non-diabetics was 31.30±6.58% when hypertensive and 29.66±4.72% when normotensive. As a group the hypertensive diabetics reacted less than when normotensive (15.47±3.22% and 21.91±5.20% respectively, p=0.058). Oxygen reactivity was reduced in both diabetic high glucose groups; 11.35±6.23% (hypertensive, p=0.02)) and 14.68±3.84% (normotensive, p=0.04). Oxygen reactivity was higher in the lower glucose group in both hypertensive (19.58+3.34%, p=0.03)) and normotensive (29.15±5.15%, p=0.03) diabetic when compared to the respective high glucose group . There was no statistically significant difference in reactivity between non-diabetic controls and diabetics at the lower glucose level. In conclusion hypertension and high glucose levels impair retinal vascular autoregulation in diabetic subjects.

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SERUM SIALIC ACID IN TYPE 2 DIABETES AND ITS RELATIONSHIP TO BLOOD PRESSURE AND RETINOPATHY

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As serum sialic acid is thought to be predictive of cardiovascular disease in the general population, we determined whether total and lipid-associated sialic acid (TSA, LASA) are elevated in Type 2 diabetes and whether serum levels are related to glycaemic control, hyperlipidaemia, body weight or the diabetic complications of hypertension and retinopathy. Twenty patients and 20 age and sex-matched non-diabetic controls were studied. Serum TSA was measured with a specific enzymatic method. TSA was increased in the diabetic patients (mean ± SEM 0.74 ± 0.11 vs. 0.60 ± 0.22 g/l, $p<0.01$), as was LASA (0.17 ± 0.03 vs. 0.11 ± 0.04, $p<0.001$). TSA (but not LASA) was correlated with systolic and diastolic blood pressure ($r=0.58$, $p<0.01$; $r=0.58$, $p<0.02$). In the 9 patients with background retinopathy or maculopathy, TSA (but not LASA) was higher than in those without retinopathy (0.81 ± 0.09 vs. 0.69 ± 0.10 g/l, $p<0.01$). TSA and LASA were not significantly related to duration of diabetes, body weight, blood glucose, serum fructosamine, cholesterol or triglyceride. We conclude that serum TSA is elevated in Type 2 diabetes and may be associated with raised blood pressure and retinopathy. It therefore deserves further study as a potential marker or predictor of diabetic complications.

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RETINAL VESSEL CHANGES IN GALACTOSE-FED DOGS: ALDOSE REDUCTASE INHIBITOR EFFECTS

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Vascular changes associated with diabetic retinopathy can be experimentally produced in beagles fed a 30% galactose diet. Chronologically these changes include the appearance of pericyte ghosts, acellular capillaries, microaneurysms, intraretinal hemorrhages, varicose enlargements, occluded vessels and increased areas of nonperfusion, IRMA and apparent new vessel growth. The onset and progression of the formation of pericyte ghosts, acellular capillaries, microaneurysms, and intraretinal hemorrhages have been arrested in a dose-dependent manner by the administration of aldose reductase inhibitors in 36-month prevention studies. To determine if the progression of retinal changes can also be arrested through reduction of galactitol production at early stages of retinal lesion development, an intervention study utilizing young male beagles has also been conducted. In this study dogs were switched to normal diet after 24 months of galactose feeding (a period where pericyte ghosts and acellular capillaries are present) and after 30 months (a period where microaneurysms are present). Investigations of the retinal vessels, isolated by trypsin digestion, from these eyes reveal that no apparent reversal of retinal lesions occurred. Nevertheless, differences in the progression of retinal lesions between the galactose-fed and reversed groups became evident 12-18 months after reversal.

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THE EFFECT OF ORAL ADMINISTRATION OF VITAMIN C ON THE OXIDATIVE MODIFICATION OF DIABETIC LENS β CRYSTALLIN

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This study examined the effect of oral administration of vitamin C, on the oxidative modification of diabetic lens β crystallin, when subjected to a oxidative insult. β crystallin purified by Sephacryl SF300 gel chromatography from control, diabetic (3 week, streptozocin induced), and vitamin C treated diabetic rats were subjected to a graded oxidative insult by free-radicals, generated via the H_2O_2 (0.0-0.5mM)/Cu^{2+}/ ascorbate system. Following this, various oxidative modifications were determined. Changes (across the range of stress) are expressed as a percentage of the control (no added H_2O_2). Diabetes was associated with increased levels of oxidized tryptophan (16→70%), bityrosine formation (115→480%), carbonyl formation (15→923%), advanced glycation endproducts (296→957%) and loss of free sulphydryl groups (15→100%). Vitamin C treatment (200mg/kg/day in drinking water) resulted in, prevention and partial restoration to control levels of tryptophan oxidation (-13→56%), bityrosine formation (110→315%), carbonyl formation (-79→201%), and advanced glycation endproducts (168→697%) and reduced the loss of free sulphydryl groups (20→89%). Our findings show that the diabetic lens β crystallin is more susceptible to oxidative stress, possibly as a result of early glycation and that vitamin C treatment *in vivo* can markedly decreases this susceptibility. Vitamin C may therefore play an important role in the prevention or arrest of the chronic, debilitating complication of diabetic cataract.

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ESSENTIAL HYPERTENSION AND TYPE 1 DIABETES.

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This study was undertaken to characterize Type 1 diabetic patients with essential hypertension with respect to kidney function, renal hormones and endothelial function. After 4 weeks without antihypertensive treatment a cross-sectional study was carried out in the following groups of patients: Group 1- Fourteen healthy controls. Group 2- Thirteen non-diabetic patients with essential hypertension (blood pressure ≥ 140/90 mmHg). Group 3- Eleven Type 1 diabetic patients with hypertension but urinary albumin excretion persistently normal (UAE: 10 (3-18) mg/24 h) both before, during and after discontinuing antihypertensive treatment. Group 4- Fifteen Type 1 diabetic patients with clinical nephropathy (UAE: 611 (192-3837) mg/24h) and hypertension. Systolic and diastolic blood pressure were similar in the three hypertensive groups 147/96, 150/94 and 152/92 mmHg (group 2, 3 and 4, respectively) but elevated compared to controls (117/74 mmHg, $p<0.001$). The diabetic patients with essential hypertension were hyperfiltering in contrast to patients with nephropathy (glomerular filtration rate 114±23 vs 90±21 ml/min/1.73 m^2, $p<0.05$). The following parameters were normal in diabetic patients with essential hypertension and elevated only in patients with diabetic nephropathy as compared to controls: Total body exchangeable sodium (2752±257 vs 3000±247 meq/1.73 m^2, $p<0.05$, group 3 vs group 4), extracellular volume (14.6±1.8 vs 14.8±2.3 l/1.73 m^2, NS), angiotensin converting enzyme (28 (14-46) vs 42 (25-60) U/l, $p<0.01$) and inactive renin (105 (35-211) vs 235 (68-1070) mIU/l $p<0.05$). Our data suggest that two different pathogenetic mechanisms for hypertension occur in Type 1 diabetic patients with and without proteinuria.

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IMPAIRED VASODILATOR RESPONSE TO ATRIAL NATRIURETIC FACTOR IN TYPE 1 DIABETES MELLITUS.

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Atrial Natriuretic factor (ANF) has well-known natriuretic and vasodilator properties in man. Recent studies have demonstrated an attenuated natriuretic response to ANF in diabetes mellitus. We now investigated whether the vasodilator effect of ANF is impaired in diabetes. Methods: In ten patients with type 1 diabetes (no microvascular complications, diabetes duration: 4-12 years, $HbA1_c$: 8.0-10.6%), and in ten healthy volunteers (matched for age, sex and body mass index), the forearm vasodilator response to infusion of ANF (six dose steps: 0.001-0.003-0.01-0.03-0.1-0.3 µg/min/100ml) into the brachial artery was measured by plethysmography. Afterwards the response to intra-arterial infusion of sodium nitroprusside (SNP: 10-30-100 ng/min/100ml) was registered because ANF and SNP share the same second messenger, cyclic GMP. Results: Both ANF and SNP exerted significant decreases in forearm vascular resistance (FVR). However, as shown in the table, the mean percentage ANF-induced decrease in FVR was significantly attenuated in diabetes when compared with controls (*repeated measures ANOVA: $P<0.001$), whereas no differences in groups occurred during SNP infusion.

ΔFVR (%)	ANF1	ANF2	ANF3	ANF4	ANF5	ANF6	SNP1	SNP2	SNP3
Control	-29±5	-43±4	-50±4	-63±3	-67±4	-72±4	-23±4	-38±3	-61±3
Diabetes	-2±7*	-9±3*	-11±6*	-19±7*	-32±6*	-45±4*	-13±8	-27±9	-52±9

Conclusion: the vasodilator response to ANF is specifically impaired in uncomplicated type 1 diabetes mellitus. This observation may be of relevance with respect to the regulation of blood pressure in diabetes.

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GLOMERULAR CHARGE SELECTIVITY IN NORMOALBUMINURIC TYPE 1 DIABETIC PATIENTS

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Microalbuminuria (>30mg/24h) in type 1 (insulin-dependent) diabetes is associated to loss of glomerular charge selectivity. It is not known whether loss of charge selectivity precedes or follows the onset of microalbuminuria. **HYPOTHESIS:** Urinary albumin excretion (UAE) increases continuously from normo- to microalbuminuria, thus if loss of charge selectivity precedes the onset of microalbuminuria, then charge selectivity may be correlated to UAE in short-term normoalbuminuric IDDM patients. **DESIGN:** Urinary IgG/IgG_4 selectivity index (SI), UAE and HbA_{1c}, were measured in type 1 diabetic patients (n=224, diabetes duration 7(3-10)y, age 25(6-40)y), without known onset of microalbuminuria. Mean of 4 consecutive measurements (6 mo interval) were used in the analysis. **RESULTS:** Mean SI was 1.33 (x/÷SD 1.5, lognormal distribution). In multiple regression analysis only gender was correlated to SI (SI males: 1.25 (x/÷1.5), females 1.43 (x/÷1.5), p=0.016). Twelve patients in whom microalbuminuria were diagnosed at entrance had reduced SI (0.90 (x/÷1.6), p=0.030 vs. normoalbuminuric). Consecutive SI measurements were significantly correlated ($p<0.0001$, r=0.3). **CONCLUSION:** The hypothesis was not sustained, thus a statement concerning the time course of loss of charge selectivity in relation to onset of microalbuminuria must anticipate follow up of the cohort. The results confirms the reduction in SI in microalbuminuric patients, and indicates sex dependent differences in SI. A notable subpopulation (20%) of the normoalbuminuric patients had SI below 1, suggesting reduced glomerular charge selectivity in these patients.

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LOSS OF GLOMERULAR CHARGE SELECTIVITY, NOT SIZE SELECTIVITY, ACCOMPANIES ALBUMINURIA IN DIABETIC NEPHROPATHY

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Aim: To study the cause of albuminuria in diabetic nephropathy. Design: A cross sectional study of 51 IDDM patients in 5 groups D 1-5 depending on urinary albumin excretion (mg/24h): D1 normal (n=11), D2 30-100 (n=8), D3 101-300 (n=13), D4 >300 (n=11), D5 >300 + s-creatinin >110 µmol/l (n=11). Methods: Glomerular charge selectivity index (SI) was measured as the ratio of IgG/IgG4 clearance. Mean glomerular pore radius and pore size distribution was estimated from fractional dextran clearances (26-64 Å) as measured by HPLC Results: SI was decreased from (1.87±0.97, mean ±SD) in C and D1 to (0.82±0.42) in D2, and remained depressed (0.79±0.22) in D2-5, p=0.001. No difference in mean pore radius was found between the groups (35.4±1.2 Å). An increase in clearance of dextrans larger than 60 Å was seen only in patients with reduced GFR (<90ml/min/1.73m²) but was otherwise unrelated to albuminuria. Conclusion: Loss of glomerular charge selectivity, not size selectivity, is found during the early stages of diabetic nephropathy. Normal size selectivity across the glomerular capillary filtration barrier was found in patients with markedly increased urinary albumin and IgG clearances indicating the existance of an alternative macromolecular pathway of considerable importance for the development of diabetic nephropathy.

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GLOMERULAR CHARGE SELECTIVITY AND THE INFLUENCE OF IMPROVED BLOOD GLUCOSE CONTROL

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Aims: Compare glomerular charge selectivity-index (SI) in two matched groups of type 1 diabetes patients with micro-(MA) and normoalbuminuria (NA) respectively, and secondly investigate prospectively the influence of metabolic control on SI in diabetic patients with MA. Design: Study 1: Cross-sectional, matched. Study 2: Prospective, randomized clinical trial. Patients: Study 1: 27 patients with MA (albumin excretion $\geq$ 15 µg/min in at least 2 out of 3 overnight urin samples) were matched with NA (n=24) patients (age, diabetes duration, mean 1-yr HbA1c, gender). Measurements: Glomerular charge selectivity (SI) by the ratio of IgG/IgG4 clearance. Intervention: (Study 2) 27 MA patients randomized to either intensive (continuous subcutaneous insulin infusion, CSII) or conventional treatment (CT). Results: MA patients had a significantly reduced SI ($p<0.01$), 1.20 (0.92-1.40) vs 1.68 (1.22-2.21), median and 95% CI compared to the NA patients. In study 2 the HbA1c improved in the CSII-group compared to the CT-group: at 2, 6 and 12 months the difference in mean HbA1c between the groups was 1.1, 1.2 and 1.4 respectively ($p<0.01$). ΔSI, expressed as % change from baseline, increased in the CSII-group compared to the CT-group at 2 and 6 months ($p<0.05$). Conclusion: Adolescents and young adults in an early stage of diabetic nephropathy have reduced glomerular charge selectivity, which possibly may be improved by reducing mean blood glucose level.

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Hyperglycaemia affects renal immunoglobulin-G handling and selectivity index in Type 1 (insulin-dependent) diabetic patients.

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It has been recently documented that the urinary excretion of IgG and, therefore, the selectivity index (IgG/albumin clearance ratio) is elevated in normoalbuminuric diabetic patients compared with control subjects. In the present study the possible effect of hyperglycaemia on renal IgG handling and selectivity index was investigated. First, it was observed that the overnight fractional IgG clearance and the selectivity index were increased in 15 normoalbuminuric Type 1 diabetic patients (1.18(0.54-1.79) x 10^{-6} (median, interquartile ranges) and 0.93(0.78-1.29), respectively) compared with control subjects (0.40(0.32-0.60) x 10^{-6}, $p<0.01$ and 0.69 (0.55-0.72), $p<0.05$, respectively). Second, the selectivity index was measured in these 15 normoalbuminuric patients and in 15 patients with minor elevations in albuminuria (10-200 µg/min) during a fasting day-time clearance period. The selectivity index was higher in 23 patients with glucosuria (1.05(0.67-1.98)) than in 7 patients without glucosuria (0.37(0.29-0.48)), $p<0.01$ and was positively correlated with blood glucose ($R=0.62$, $p<0.001$). Third, fractional IgG clearance and selectivity index were measured during normoglycaemia followed by moderate hyperglycaemia in 7 of these patients. Hyperglycaemia (blood glucose 13.0(10.6-15.6) mmol/l) increased selectivity index from 0.64(0.30-1.08) to 1.81 (1.12-2.38), $p<0.05$. Both fractional IgG clearance ($R=0.58$, $p<0.02$) and selectivity index ($R=0.54$, $p<0.02$) were related to actual glycaemia (21 observation periods)). The present findings necessitate careful consideration of blood glucose when measuring the selectivity index and suggest that hyperglycaemia alters renal IgG handling in Type 1 diabetic patients.

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ABNORMAL URINARY KALLIKREIN IN SHORT-TERM TYPE 1 (INSULIN-DEPENDENT) DIABETES MELLITUS

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Urinary kallikrein excretion after stimulation with i.v. furosemide (0.5 $mg.kg^{-1}$) and during glycemic clamp-induced normo- and hyperglycemia (5 and 12 mmol/l) was evaluated in 16 short-term insulin-dependent diabetics with normal glomerular filtration rate and in 20 weight-, age- and sex-matched healthy controls. Both basal (6.0 ± 1.2 v.s. 10.9 ± 1.7 $mEU.min^{-1}$; $p<0.05$) and furosemid stimulated (12.0 ± 1.6 v.s. 21.3 ± 2.0 $mEU.min^{-1}$; $p<0.01$) urinary kallikrein were significantly lower in diabetics compared to controls. A decreased response to furosemide ($p<0.05$) was found despite the comparable rise of diuresis and natriuresis in both groups. Kallikrein excretion was 10.9 ± 2.03 $mEU.min^{-1}$ during clamp-induced normoglycemia in diabetics (comparable to controls) and it significantly declined during intravenous dextrose-induced hyperglycemia to 5.45 ± 0.88 $mEU.min^{-1}$ ($p<0.01$). We conclude short-term diabetes mellitus without the renal hemodynamic alterations is associated with decreased kallikrein excretion and its functional reserve. This defect is probably directly related to the blood glucose level.

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THE ROLE OF UROKINASE IN THE PATHOGENESIS OF DIABETIC NEPHROPATHY

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The pathogenesis of diabetic nephropathy remains incompletely understood although fibrin deposition within glomeruli indicates that impaired fibrinolysis may be important. Studies in non-diabetic patients indicate that deteriorating renal function is associated with reduced urinary urokinase (u-PA) excretion. To study the role of u-PA in the pathogenesis of diabetic nephropathy, 11 non diabetic controls, 20 Type 1 diabetic patients without proteinuria and 7 with proteinuria and impaired renal function had urine samples collected during the day and overnight. U-PA excretion was significantly greater during the day than in the night in controls (82.8 v 60.1 iU/hr, $p<0.01$) and non proteinuric diabetics (97.7 v 66.3 iU/hr, $P<0.001$). Patients with impaired renal function had significantly reduced u-PA excretion with loss of the diurnal variation (46.6 v 47.4 iU/hr, $p<0.0001$ compared to controls and diabetes without nephropathy). U-PA excretion correlated with creatinine clearance in both groups of diabetic patients (no proteinuria, day $r=0.43$, night $r=0.6$; proteinuria, day $r=0.74$, night $r=0.8$). No correlations existed between albumin and u-PA excretion. The results indicate that loss of renal function is associated with reduced urinary u-PA excretion. The lack of association with urinary albumin excretion indicates that these changes are secondary to the underlying disease and not causative.

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PLASMA PRORENIN AND PROGRESSION OF ALBUMINURIA IN TYPE I DIABETIC PATIENTS: A 2 YEARS FOLLOW-UP.

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Prorenin may be a marker of microvascular complications in type I diabetes.
It is unknown whether plasma prorenin is already elevated in patients with minor elevations in albuminuria and whether an elevated prorenin can predict progression of albuminuria in such patients. We conducted a prospective study in a highly selected group of 25 type I normotensive diabetics with slightly elevated levels of albuminuria: 28 (14-75) ug/min (geometric mean, 95% confidence interval). During two years of follow-up progression of albuminuria was associated with higher baseline blood pressure ($p< 0.001$) and retinopathy ($p< 0.01$) but not with GFR, HbA1c and plasma prorenin at baseline. Both systolic and diastolic blood pressure were significantly higher in the second year in those subjects in whom albuminuria progressed. Plasma prorenin was not different in patients with and without progression of albuminuria: 164 (128-212) mU/l vs. 178 (110-286) mU/l.
Plasma prorenin did not rise in either group during follow-up. Plasma prorenin, however, was strongly associated with both the early phase (micraneurysms only) ($p< 0.001$) and the late phase (excudates and/or proliferative) ($p< 0.001$) of retinopathy.
It appears that elevated plasma prorenin is not a reliable marker of early microalbuminuria. Prorenin, however, is more closely associated with the presence of retinopathy.

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SE-PRORENIN AND URINARY ALBUMIN EXCRETION RATE IN NORMO- AND MICROALBUMINURIC TYPE 2 (NON-INSULIN-DEPENDENT) DIABETES.

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In Type 1 (insulin-dependent) diabetics se-prorenin indicates concomitant nephropathy and is a long-term predictor of urinary albumin excretion rate (UAE). We investigated se-prorenin and UAE in 21 normo and 16 microalbuminuric and, after 40 months, in 26 non-proteinuric Type 2 (non-insulin-dependent) diabetics. Cross-sectional study: No difference in se-prorenin was found between normo- and microalbuminurics (geometric mean$\times/\div$tolerance factor): $140\times/\div 2.08$ and $133\times/\div 2.10$ μU/ml. Se-prorenin correlated with UAE and se-renin in normoalbuminurics ($r=0.43$, $p=0.05$; $r=0.46$, $p=0.04$). In microalbuminurics se-prorenin correlated with diastolic blood pressure and se-renin ($r=-0.51$, $p=0.04$ and $r=0.62$, $p=0.01$). Excluding antihypertensive treated patients did not change the results between the groups. Follow-up study: Initially there was no correlation between se-prorenin and UAE (range: 1.9-183.9 μg/min), but se-prorenin correlated to both follow-up UAE ($r=0.50$, $p<0.01$) and the relative rise in UAE ($r=0.46$, $p=0.02$). Adjustment for different follow-up duration did not change the latter correlation. Excluding antihypertensive treated patients (6 patients) eliminated these correlations. Se-prorenin neither indicates concomitant nephropathy nor predicts long-term increase in urinary albumin excretion rate in normo- and microalbuminuric Type 2 (non-insulin-dependent) diabetic patients, when antihypertensive treatment is taken into consideration.

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RENAL TUBULAR ELECTROLYTE HANDLING AND ALDOSTERONE BIO-ACTIVITY IN CHILDREN WITH TYPE 1 DIABETES

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Tubular dysfunction has been suggested as a characteristic finding for the renal involvement in type 1 diabetes. To evaluate disturbances of renal electrolyte handling measurements of potassium and sodium transport as well as those of plasma concentrations of aldosterone (Aldo), atrial natriuretic peptide (ANP) and urinary excretion of catecholamines were carried out in three groups of diabetic children (I: newly diagnosed patients < 6 months, II: patients with diabetes duration > 5 years, III: diabetic children in ketoacidosis (DKA). Results were expressed as mean $\pm$ SE and compared to those of healthy controls (C). Transtubular potassium gradient (TTKG) was significantly lower in each group of diabetic children compared to controls (I: 3.17 $\pm$ 0.3, II: 2.75 $\pm$ 0.2, III: 4.9 $\pm$ 0.5 vs C: 8.2 $\pm$ 0.7). Plasma Aldo was elevated in diabetic children with longer duration (0.5 $\pm$ 0.09 nmol/L) and in DKA (3.2 $\pm$ 0.3) versus controls (0.3 $\pm$ 0.04). In each group significant positive correlations were found between Aldo and TTKG. Plasma concentration of ANP was high in newly diagnosed diabetic children (25.6 $\pm$ 2.4 fmol/mL vs 16.6 $\pm$ 1.6 in controls) but not in the long duration group (16.3 $\pm$ 0.8). Our conclusion is that TTKG is a valuable measure of mineralocorticoid action on distal and collecting tubules also in pathological condition. The low TTKG is characteristic for the renal involvement in diabetes, which already occurs at a very early stage of the disease.

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RELATIONSHIP BETWEEN C-PEPTIDE, RENAL SODIUM AND DOPAMINE EXCRETION IN TYPE II (NON-INSULIN-DEPENDENT) DIABETES.

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Recent data indicate that C-peptide reduces glomerular filtration rate (GFR) in type I (insulin-dependent) diabetic patients. Furthermore, hyperinsulinemia has been related to an increased renal sodium retention. Moreover it has been evidenced that renal dopamine plays an important role in sodium-water homeostasis. However, renal dopamine production depends on the amount of filtered L-dopa, and hence on GFR. Hyperinsulinemia-induced sodium retention may thus be explained at least partially by an impaired renal dopamine production. Therefore the relationship between basal plasma C-peptide, 24h urinary sodium, dopamine excretion, and creatinine clearance in type II (non-insulin-dependent) diabetic patients (n=45) was studied. A significant negative correlation was found between basal C-peptide and creatinine clearance ($r=-0.44$;$p<0.01$), and between basal C-peptide and urinary dopamine excretion ($r=-0.45$;$p<0.01$). Urinary dopamine appeared to be significantly correlated with urinary sodium excretion ($r=0.37$;$p<0.05$). The correlation between C-peptide and urinary sodium excretion was at the limit of significance ($r=0.26$;ns). These findings suggest that in type II (non-insulin-dependent) diabetes hyperinsulinemia/hyper C-peptidemia is associated with a decreased GFR, and hence decreased formation of renal dopamine. An impairment of the renal dopaminergic system may be one of the factors involved in the pathophysiology of sodium retention in type II (non-insulin-dependent) patients.

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IMPAIRED DOPAMINE/SODIUM RELATIONSHIP IN TYPE 1 (INSULIN-DEPENDENT) DIABETES.

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Considerable evidence suggests that dopamine (DA) intervenes in the sodium (Na) and water homeostasis. To study the role of the renal dopaminergic system in the abnormal sodium handling in diabetes we measured 24h DA excretion (UDA) by HPLC in 61 normotensive type 1 diabetic patients without nephropathy (32.6±1.2 years ; duration of diabetes : 12.5 ± 1.2 years ; mean ± SEM) and in 21 age-matched healthy controls (C). UDA was signicantly lower in D than in C namely 193.3 ± 8.9 µg/24h versus 290.9 ± 11.6 µg/24h respectively ($P<0.001$). A positive correlation was observed between urinary sodium excretion (UNa) and UDA in D ($r=0.28$; $P<0.05$) and in C ($r=0.54$; $P<0.05$) indicating that a comparable UDA in D is associated with a lower UNa. A low-dose DA infusion (3µg/kg/min) for one hour induced a significant increase in urinary flow (UV) and UNa ($P<0.05$) in both C (n=21) and D (n=33). This rise in UV and UNa was more marked in D with a short duration of disease (<5 years ; n=7). With a longer duration of diabetes the response of UV and UNa decreased progressively reaching significant differences after > 10 years of disease (n=19 ; $P< 0.01$). These results suggest that an impaired DA/Na relationship could be implicated in the abnormal sodium homeostasis in type 1 (insulin-dependent) diabetes .

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REDUCTION IN ALBUMINURIA PREDICTS A BENEFICIAL EFFECT ON PROGRESSION IN DIABETIC NEPHROPATHY DURING ANTIHYPERTENSIVE TREATMENT.

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We have evaluated putative predictors of the progression in diabetic nephropathy during long-term antihypertensive treatment. Twenty hypertensive Type 1 (insulin-dependent) diabetic patients with nephropathy were followed for 3 (2-5) years before, and for 3 years during antihypertensive treatment. Fall rate in glomerular filtration rate was 9.5 ± 3.8 ml/min/year (mean ± SD) before and 3.6 ± 3.6 during antihypertensive treatment. Albuminuria was 1442 (150-7564) µg/min in the last year before and 880 (96-3310) µg/min in the first year during treatment. Relative change in fractional albumin clearance (ratio of values obtained during first year of treatment / and last year before) was significantly correlated to fall rate in glomerular filtration rate during the 3 years of treatment ($r=0.41$, $p<0.05$) and to relative change in fall rate in glomerular filtration rate (fall rate during and before treatment were compared) ($r=0.42$, $p<0.05$). No significant correlations were found between fall rate in glomerular filtration rate during the 3 years of treatment and arterial blood pressure, albuminuria or glomerular filtration rate measured the last year before, the first year during treatment or the relative changes in these 3 variables (after-before). In conclusion, a decrease in fractional albumin clearance during antihypertensive treatment predicts an attenuated fall rate in glomerular filtration rate in diabetic nephropathy.

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THE COURSE OF KIDNEY FUNCTION IN TYPE 2 (NON-INSULIN-DEPENDENT) DIABETIC PATIENTS WITH DIABETIC NEPHROPATHY.

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We evaluated the impact of some putative progression promoters on kidney function in albuminuric Type 2 (non-insulin-dependent) diabetic patients with biopsy proven diabetic glomerulosclerosis. 26 patients (1 woman), age 52 ± 9 (mean ± SD) years were followed prospectively for 5.4 (1.0 - 7.0) (median (range)) years. 21 patients received antihypertensive treatment. During the observation period glomerular filtration rate (^{51}Cr-EDTA) decreased from 82 (24-146) to 56 (2-145) ml/min/1.73m^2 ($p<0.001$). Glomerular filtration rate declined 5.5 (-3.5 to 22.0) ml/min per year. Albuminuria increased from 1.0 (0.3-7.2) to 2.2 (0.4-8.0) g/24h ($p<0.001$). Arterial blood pressure remained unchanged: 162/93 ± 23/14 and 161/89 ± 20/9 mm Hg. A significant correlation was found between the rate of decline in glomerular filtration rate and systolic blood pressure ($r=0.71$, $p<0.001$), mean blood pressure ($r=0.56$, $p<0.005$), albuminuria ($r=0.58$, $p<0.005$) and glomerular filtration rate at baseline ($r=-0.49$, $p<0.02$). The rate of decline in glomerular filtration rate did not correlate significantly with dietary protein intake, total-cholesterol, HDL-cholesterol or haemoglobin A_{1c}. Seven patients died; 3 from uraemia and 4 from cardiovascular causes. Two patients required renal replacement therapy at the end of the observation period. Our prospective observational study has revealed that the decline in glomerular filtration rate varies considerably between patients. Increase in arterial blood pressure to a hypertensive level is an early feature of diabetic nephropathy. Elevated arterial blood pressure accelerates the progression of diabetic nephropathy in Type 2 (non-insulin-dependent) diabetic patients.

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Long-term renal effects of protein-restricted diet in Type 1 (insulin-dependent) diabetic patients without clinical nephropathy and hypertension.

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In a two-year prospective randomised study the renal effects of a low protein diet (0.6 g/kg/day) were investigated in Type 1 diabetic patients with minor elevations on albuminuria (overnight albuminuria between 10 and 200 μg/min) and without hypertension. In the low-protein diet group (LPD, n=14), protein intake, estimated by urinary urea excretion, decreased from 1.05±0.32 to 0.79±0.16 g/kg/day ($p<0.005$) but only seven patients consumed <0.8 g protein/kg/day. In the usual-protein diet group (UPD, n=16) protein intake was unaltered ($p<0.001$ from LPD). Baseline albuminuria and renal haemodynamics were similar in the groups. Mean arterial pressure (MAP) was slightly higher in the LPD group. After correction for MAP and diabetes duration albuminuria decreased by 26 (95% confidence interval, 13 to 36)% in the LPD group ($p<0.001$) and did not significantly change by 5 (95% confidence interval, -24 to 46)% in the UPD group ($p<0.005$ from LPD group). Multiple regression analysis showed that the actual decrease in protein intake inhibited ($p<0.005$), whereas prevailing MAP accelerated albuminuria ($p<0.001$). Low-protein intake independently reduced effective renal plasma flow (ERPF) ($p<0.01$) and glomerular filtration rate (indirectly via ERPF, $p<0.001$) after one year. Minor changes in renal haemodynamics occured between the first and second year of study. In conclusion, long-term dietary protein restriction beneficially reduces albuminuria and renal haemodynamics in Type 1 diabetic patients with early signs of renal involvement, but systemic blood pressure counteracts these effects even in the absence of hypertension. Suboptimal compliance limits dietary efficacy.

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ALDOSE REDUCTASE INHIBITOR ZOPOLRESTAT REDUCES HYPERFILTRATION AND ALBUMINURIA IN DIABETIC RATS.

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Elevated glomerular filtration rate (GFR) and urinary albumin excretion (UAE) are early markers linked with subsequent diabetic nephropathy. We examined the effect of a structurally distinct aldose reductase inhibitor, zopolrestat (Z), on GFR and UAE in rats with short-term streptozocin(STZ)-induced diabetes. STZ-diabetic rats received either no treatment (group D), or 100 mg/kg/day, p.o., Z (group D+Z), for ~1 or ~4 weeks. Controls were non-STZ-injected (group N). GFRs were measured after ~1 week (^{3}H-inulin clearance, Inactin anesthesia). Twenty-four-hour UAE was determined after ~4 weeks (antibody-based assay). Results are for groups D, N, and D+Z, respectively. All STZ-treated rats had significantly elevated (~3X) plasma glucoses: e.g., at ~1 week, 318±67(11) vs. 126±16(12) vs. 346±54(7) mg/dl (mean±SD(n)). Hyperfiltration (+46%, $P<0.05$ D vs. N) was reduced 73% by Z ($P<0.05$, D+Z vs. D): 16.4±3.0(11) vs. 11.2±1.7(12) vs. 12.6±3.2(7) ml/min/kg. In separate groups of rats, UAE at ~4 weeks was elevated ~5X ($P<0.05$, D vs. N), and was reduced 77% by Z ($P<0.05$, D+Z vs. D): 2.50(1.56-4.01)(4) vs. 0.50(0.32-.74)(4) vs. 0.97(0.67-1.48)(4) mg/24 hour (mean(range)(n)). Erythrocyte sorbitol, elevated ~5X ($P<0.05$), was normalized by Z: 19.4±6.2(6) vs. 4.2±2.3(5) vs. 3.4±0.6(4) nmoles/ml. These data support the notion that adequate inhibition of polyol pathway flux substantially reduces elevated GFR and UAE in short-term experimental diabetes.

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RENAL PROTECTIVE EFFECTS OF CAPTOPRIL AND METOPROLOL IN DIABETIC NEPHROPATHY

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We assessed whether long-term (6.6 years) inhibition of angiotensin converting enzyme can reduce the rate of decline in kidney function more than reducing blood pressure with other antihypertensive treatment. We performed a non-randomised before-after trial of matched hypertensive Type 1 (insulin-dependent) diabetics (mean age 33) with nephropathy treated with captopril (n=18) or metoprolol (n=19), usually combined with frusemide. Baseline values were identical in captopril and metoprolol treated groups, respectively: mean blood pressure 111 (SE 2) mm Hg v 114 (2) mm Hg, geometric mean albuminuria 982 (antilog SE 1.2) µg/min v 1266 (1.0) µg/min; and mean glomerular filtration rate (GFR) 98 (SE 5) ml/min/1.73 m^2 v 88(4) ml/min/1.73 m^2. GFR declined a mean of 4.5 (0.6) ml/min/year in the group given captopril and 4.1 (0.7) ml/min/year in the metoprolol group. The mean blood pressure during the study was 102 (2) mm Hg in both groups. Albuminuria decreased to the same extent with captopril and metoprolol, respectively: 380 (1.0) µg/min v 320 (0.8) µg/min. Our study suggests that captopril and metoprolol are equally effective in reducing albuminuria and the rate of decline in GFR in hypertensive patients with diabetic nephropathy and normal kidney function.

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PLACEBO CONTROLLED STUDY OF LISINOPRIL IN NORMOTENSIVE DIABETIC PATIENTS WITH MICROALBUMINURIA.

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Normotensive diabetics with albumin excretion rate (AER) >20µg min^{-1} but < 200µg min^{-1} were randomised after 2 weeks run-in to receive lisinopril (L, n=12) or placebo (Pl,n=15) for 48 weeks. The groups were well matched for age [L,48.3±13.0 yr and Pl,49.1±16.0 yr], weight [L,80.8±16.1 kg and Pl,76.6±17.4 kg] and height [L,1.64±0.07 m and Pl,1.69±0.07 m]. Mean changes in AER at 48 weeks were -30.1µg min^{-1} (L) and +37.5µg min^{-1}(Pl), [treatment difference 67.6µg min^{-1},95% CI-115.0 to -20.2, $P<0.01$]. Glomerular filtration rate [GRF,ml min^{-1}] fell [126.9±46.7 to 100.8±27.4(L), 110.8±34.0 to 103.1±38.3 (P)]. Renal plasma flow [RPF,ml min^{-1}] fell 323.1±120.4 to 304.7±115.1(L), and rose 312.6±135.8 to 349.6±189.4 (P). Renal resistances [RR mmHg ml^{-1}min^{-1}] did not alter [0.34±0.13(L), 0.37±0.18 (P1)]. Filtration fractions [FF] fell [0.4±0.09 to 0.35±0.10(L), 0.38±0.11 to 0.33±0.11(P1)]. Plasma creatinine [µmol l^{-1}] did not alter in either group [94±8 to 100±8.0(L), and 97±19 to 108±26(P)]. Treatment differences for GFR, RPF, RR, FF, and creatine were not significant. Changes in random glucose [mmol l^{-1}] were [-3.3(L), +3.9(P)with a treatment difference 7.2, 95%CI -13.3 to -1.2, $P<0.02$]. There was no significant inter-group differences in glycated haemoblobin. Mean systolic blood pressure fell 13.5 mmHg (L) but only 1.4 mmHg (P), $P = 0.1$. There was no change in diastolic blood pressure in either group. In this study angiotensin converting enzyme inhibition with lisinopril decreased AER in incipient diabetic nephropathy without an obvious benefit over placebo on renal haemodynamics.

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COMPARATIVE STUDY ON RENAL EFFECTS OF NITRENDIPINE VS. ENALAPRIL IN MICROALBUMINURIC PATIENTS WITH TYPE 1 DIABETES MELLITUS

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To compare the effects of nitrendipine and of enalapril on variables of glomerular and tubular function in type 1 diabetes mellitus, a randomised comparative study was carried out in 20 microalbuminuric patients (6 females, 14 males, age, 30 - 58 years, diabetes duration, 3 - 41 years, Hb_{A1c}, 5.5 - 10 %). Patients were treated for 1 year with 20 mg/day nitrendipine (group 1, 10 patients) or 10 mg/day enalapril (group 2, 10 patients). Median urinary albumin excretion decreased maximally from 42 ± 12 to 24 ± 22 mg/24h in group 1 and from 51 ± 40 to 19 ± 7 mg/24h in group 2 ($p < 0.01$). 67 % of the measurements in group 1 and 75 % of the measurement in group 2 were decreased in comparison to before treatment (both $p < 0.01$). Blood pressure fell from 132/89 to 112/76 mmHg in group 1 ($p < 0.01$) and from 133/88 to 118/79 mmHg in group 2 ($p < 0.05$). Kidney size decreased from 184 ± 7 to 146 ± 7 ml in group 1 ($p < 0.01$) and from 207 ± 12 to 174 ± 12 ml in group 2 ($p < 0.05$). Glomerular filtration rate (GFR) rose from 107 ± 15 to 148 ± 19 ml/min in group 1 but fell from 103 ± 10 to 87 ± 10 ml/min in group 2 ($p < 0.05$ vs. group 1). In both groups, the excretion of alpha-1-microglobulin was decreased ($p < 0.05$). Thus, there were no differences between the beneficial effects of nitrendipine and enalapril on microalbuminuria and the excretion of a renal tubular marker protein. The different effects on GFR deserve further long-term evaluation.

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NEW RENAL TEST FOR EVALUATION OF EFFECTIVE ENALAPRIL USE IN TYPE I DIABETIC PATIENTS.

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Enalapril (EN) is widely used in Type I diabetic (IDDM) pts due to its protective effect on progression of diabetic nephropathy (DN), though not in all cases its efficacy is proved by decrease of urinary albumin excretion (UAE). The aim of our study was to reveal the criteria of effective use of EN treatment in IDDM pts at different stages of DN. Renal functions were assessed on the basis of baseline glomerular filtration rate (GFR, creatinine clearance, ml/min), UAE (nephelometry method, mg/day), and new renal test evaluating renal functional reserve (RFR, i.e. renal capacity to elevate GFR in response to acute protein load, %). According to RFR all pts were divided into two groups: gr.1(n=10) with normal RFR (GFR increased up to +36% 2 hr. after protein load) and gr.2(n=15) with no RFR (GFR decreased by 24% after the load). Gr.1 included pts with normo- and microalbuminuria (UAE< 300), while gr.2 - with normo-, micro- and macroalbuminuria. In gr.2 EN treatment (5-10 mg/day) for 1 mo caused GFR and UAE fall irrespective of baseline UAE, and restoration of RFR up to +15%. However in gr.1 EN led to slight increase of UAE (from 30 to 45), increase in GFR (from 116 to 160, $p<0,01$) and paradoxical reduction of RFR (by -5%). We suggest that EN-treatment is strongly recomemded only for those IDDM pts, who have no RFR, but contraindicated for pts with normal RFR (irrespective of baseline UAE).

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NEUROELECTROPHYSIOLOGICAL ALTERATIONS IN EXPERIMENTAL DIABETES MELLITUS.

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Neuroelectrophysiological studies have demonstrated that Central Nervous System (CNS) and Peripheral Nervous System (PNS) damage are present in diabetes and occur at an early stage of the disease. To evaluate the natural hystory of diabetic neuropathy using electrophysiological determinations and to compare PNS to CNS alterations, diabetes was induced in 56 male Sprague-Dawley rats by a single i.p. streptozotocin injection (60 mg/Kg b.wt.). Forty normal rats made up the control group. VEP-Visual Evoked Potentials, BAEP-Brainstem Auditory Evoked Potentials, SEP-Somatosensory Evoked Potentials were recorded at 1.5, 3, 6, 9, and 12 months after diabetes induction. VEP-P1 wave latency was significantly increased in diabetic rats at 3 ($p<0.05$), 6 ($p<0.02$), 9 and 12 months ($p<0.01$). Wave BAEP latency was significantly elevated in diabetic rats as soon as 3 months from diabetes induction ($p< 0.001$ for all the waves). CNS conduction decrease (T6-cortex) was demonstrated in SEP recordings of diabetic rats at 3 ($p<0.02$), 6 ($p<0.001$) and 9-12 months ($p<0.01$). PNS conduction showed similar behaviour with a significant decrease in diabetic rats at 3,6,9,12 months ($p<0.001$). Our results document that electrophysiological abnormalities appear at an early stage in experimental diabetes mellitus, persist thereafter and may be demonstrated at CNS and PNS level.

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PHOSPHORYLATION OF Na,K-ATPase α-SUBUNIT IN RAT INTACT SCIATIC NERVES: EFFECTS OF PROTEIN KINASE MODULATORS

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Na,K-ATPase activity is impaired in diabetic animals. Phorbol ester treatment of sciatic nerves acutely restores Na,K-ATPase activity in diabetic rats, but has no effect on nerves of controls. The mechanisms by which Na,K-ATPase activity is modulated by protein kinases (PK) including PKC remain to be elucidated. We have studied the phosphorylation state of Na,K-ATPase α-subunit in sciatic nerves in response to PK modulators. Nerves were removed from anaesthesized rats, desheathed and labeled with $^{32}PO_4$ (1mCi/ml) for 1h in Krebs buffer. The nerves were then exposed to phorbol-myristate-acetate (PMA, 10μM), staurosporine (100nM), forskolin (10μM) or to changes in ambient [Ca^{2+}] for 15min. Cytosolic and membrane fractions were then prepared. Membrane fractions were shown by immunoblotting both to contain >90% of the total nerve Na,K-ATPase α-subunit and to comprise the site of PMA-induced translocation of PKC. This fraction was submitted to immunoprecipitation with an anti-alpha antibody and the specific phosphorylation of the α-subunit was revealed by autoradiography after SDS-PAGE. The results demonstrate a calcium-dependent basal phosphorylation state of the α-subunit which is increased by PMA and decreased by staurosporine and forskolin. This suggests that the Na,K-ATPase is tonically phosphorylated in intact nerves, in part due to direct phosphorylation by PKC while PKA activation promotes dephosphorylation.

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The role of cAMP in regulation of diabetic nerve Na/K-ATPase activity

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We reported that prostaglandin E1 analogue OP1206•αCD(OP) increased nerve Na/K-ATPase(ATPase) activity without affecting myo-inositol content in diabetic rats. In order to clarify the mechanism by which OP increases ATPase activity, in vitro studies were performed. Endoneurial materials from sciatic nerve of Sprague-Dawley rats with 6wk streptozocin-induced diabetes were incubated with OP. OP increased nerve ATPase activity in a dose-dependent manner(basal; 1.39μmol Pi/mg protein/hour, OP 10ng/ml; 2.11). Sixty-three percent of maximal effect appeared after one minute. In addition, OP increased nerve cAMP content in the same manner and basal cAMP content determined with microwave irradiation was significantly reduced in diabetic rats(1.67pmol/mg wet weight, control; 1.99). To convince the role of cAMP in increasing ATPase activity, endoneurial materials were incubated with dibutyryl cAMP or phosphodiesterase inhibitor aminophylline. The enzyme activity was significantly increased with these compounds. Protein kinase inhibitor H8 inhibited the effect of OP on ATPase activity completely. These results suggest that cAMP increases diabetic nerve ATPase activity via phosphorylation by cAMP-dependent protein kinase rather than new enzyme production and that cAMP might be one of the candidates that regulate ATPase activity of diabetic nerve.

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IMPAIRED RESPONSE TO NERVE INJURY IN EXPERIMENTAL DIABETES

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This study describes the effect of diabetes on the ability of peripheral nerve to respond to injury by increasing vasoactive intestinal polypeptide (VIP) levels. Unilateral sciatic nerve crush in control and 3 week diabetic (streptozotocin, 50 mg/kg i.p.) was followed 1 week later by placement of two ligatures around the injured nerve to measure axonal transport. Twelve hours later, the L4 and L5 dorsal root ganglia and nerve segments were removed for VIP radioimmunoassay. Similar pieces were also taken from control and diabetic rats whose nerve had been ligated without a preceding crush. VIP was significantly ($p<0.01$) increased in ganglia and nerve from crushed controls compared to uncrushed controls (uncrushed ganglia = 20.5 ± 0.9 vs crushed ganglia = 49.0 ± 6.3 pg/pooled L4 and L5: uncrushed nerve = 27.2 ± 3.0 vs crushed nerve = 70.9 ± 12.7 pg/5mm: mean ± sem). Injury also increased ganglion and nerve VIP in diabetic rats (uncrushed ganglia = 15.2 ± 2.2 vs crushed ganglia = 30.9 ± 3.2 pg/pooled L4 and L5 , $p<0.05$: uncrushed nerve = 21.3 ± 3.8 vs crushed nerve = 38.1 ± 6.9 pg/5mm) but to levels lower than crushed controls (both $p<0.01$). Nerve injury increased ($p<0.05$) the proximal accumulation of VIP only in controls (uncrushed = 87.9 ± 5.4 vs crushed = 151.2 ± 26.2 pg/12 hr) without altering VIP transport velocity or mobile fraction. Attenuated responses to injury leading to restricted axonal transport may contribute to impaired nerve regeneration in diabetes.

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ANTIOXIDANT TREATMENT PREVENTS PERIPHERAL NERVE DYSFUNCTION IN DIABETIC RATS.

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To test the hypothesis that oxidative stress contributes to the aetiology of diabetic neuropathy, streptozotocin-induced (40 mg/kg i.p.) diabetic rats were treated with a 2% dietary supplement of butylated hydroxytoluene (BHT) for 2 months. Untreated diabetic and non-diabetic controls were also employed. Sciatic nerve motor conduction velocity (MCV) and saphenous nerve sensory conduction velocity (SCV) were determined *in vivo*. Nerves were removed to a chamber, and resistance to hypoxic conduction failure was determined *in vitro* while they were gassed with N_2. MCV to gastrocnemius muscle was 22.5% decreased with diabetes ($p<0.001$). This was 87% prevented by BHT treatment ($p<0.001$). A 26.7% MCV decrease to tibialis anterior was 84% prevented by BHT ($p<0.001$). SCV was reduced by 12% with diabetes ($p<0.001$), but remained within the non-diabetic range ($p<0.001$) with BHT. The duration of hypoxia producing an 80% reduction in sciatic compound action potential amplitude (T_{80}) was 21.8±0.7 min (mean±SEM) in non-diabetic controls. With diabetes, this increased to 33.9±0.7 min ($p<0.001$). In the BHT-treated group, T_{80} was 23.9±1.7 min, not significantly different from controls, but normalized compared to the diabetic group ($p<0.001$). We conclude that BHT protects against nerve dysfunction in diabetic rats, and that oxidative stress could make an important contribution to the aetiology of diabetic neuropathy.

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GLUTATHIONE FOR THE PREVENTION AND TREATMENT OF EXPERIMENTAL DIABETIC NEUROPATHY IN RATS.

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Parameters of oxidative stress are increased in experimental diabetic neuropathy (EDN). Glutathione (GSH) is an intracellular scavenger for free radicals. We studied the effect of GSH treatment on EDN in streptozotocin (STZ) rats. The study period was 10 weeks. Forty rats were divided into four groups: CON(n=10) consisted of non-diabetic, age-matched controls; 30 rats were made diabetic with STZ 40 mg/kg BW. The diabetic rats were divided into: PLA(10) treated with placebo, GSH0,(9) with GSH 200 mg/kg bodyweight iv. from week 0 and GSH4(11) with GSH from week 4, when neuropathy had occurred. At week 0, 2, 4, 6, 8 and 10 sensory and motor nerve conduction velocity (SNCV and MNCV) were measured. Multivariate analysis of variance with repeated measures was used for analysis. Results: CON: mean SNCV±SEM increased from 47.5±0.5 to 59.2±0.3 m/s and MNCV from 47.8±0.8 to 57.1±0.3 from week 0 to 10; for PLA: 47.5±0.3 to 49.6±0.2 and 47.9±0.4 to 49.7±0.3; for GSH0: 48.2±0.6 to 52.7±0.2 and 47.3±0.6 to 52.6±0.4; for GSH4: 47.8±0.3 to 50.4±0.5 and 47.1±0.2 to 50.5±0.6. The difference between mean SNCV and MNCV of PLA and GSH0 was significant ($p<0.001$). The differences between PLA and GSH4 were not significant, apart from mean MNCV from week 4 to 6($p<0.005$). In conclusion GSH partially prevents diabetic neuropathy when started at onset, but is ineffective in existing EDN. This suggests that oxidative stress is noxious at an early stage of diabetic neuropathy.

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EFFECTS OF AMINOGUANIDINE ON NERVE FUNCTION AND POLYOLS IN STREPTOZOTOCIN-DIABETIC RATS.

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Advanced glycosylation end-products (AGEs) may cause diabetic complications. Recently, beneficial effects of aminoguanidine, which prevents AGE formation, have been demonstrated on nerve function. However, recent evidence also suggests that aminoguanidine may be an aldose reductase inhibitor. Thus, it is unclear whether its action depended on AGE or polyol pathway inhibition. To test this, streptozotocin-diabetic rats were treated with aminoguanidine (1 g/kg in drinking water) for 2 months or were untreated. Sciatic motor (MCV) and saphenous sensory (SCV) conduction velocity were measured *in vivo*. Sciatic resistance to hypoxic conduction failure was measured *in vitro*. Sciatic polyols were estimated by GLC. Control MCV of 66.9±1.5 m/s (mean±SEM) was reduced to 52.9±0.9 m/s by diabetes ($p<0.001$), and normalized by aminoguanidine (64.7±1.8 m/s, $p<0.001$). SCV decreased from 57.5±0.6 m/s to 52.5±1.2 m/s with diabetes ($p<0.05$), and was also normalized by treatment (57.9±1.3 m/s, $p<01$). Aminoguanidine did not significantly affect the 48% increase ($p<0.001$) in hypoxic resistance with diabetes. Control sorbitol and fructose were 27±3 and 113±13, µg/g wet weight respectively. They were elevated by diabetes (sorbitol 324±36, fructose 1030±68 µg/g wet weight, $p<0.001$). This was completely unaffected by treatment (sorbitol 316±16, fructose 1044±125 µg/g wet weight). We conclude that aminoguanidine prevents diabetic MCV and SCV deficits and does not act as an aldose reductase inhibitor *in vivo*.

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INNER ESTER DERIVATIVES OF GANGLIOSIDES IMPROVE VESICO-VESICAL REFLEX DEFICITS IN EXPERIMENTAL DIABETES

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We evaluated diabetic bladder function by "in vivo" cystometry in 3 month alloxan (ALX)- and streptozotocin (STZ)-diabetic rats. When compared to age-matched controls, ALX- and STZ-diabetic animals showed a significantly increased bladder weight (+135% in both groups) and urinary output (+860 and +915% respectively, $p<0.01$), associated with enhanced threshold volume (TV) for micturition (+311 and +343%, $p<0.01$). Furthermore, a reduced rate of isovolumetric contractions (RC), evoked by over-distension of the vesical wall during cystometry was detected (-63 and -54%, $p<0.05$). Cystometrogram analysis revealed a significant correlation between RC and inter-contraction time interval ($r=-0.7976$, $p<0.001$) All together these results further support the notion that an impaired afferent input underlies the altered vesical spinal reflex pathway. We also assessed the effect of AGF1 (inner ester derivatives of gangliosides) on diabetic bladder function. ALX-diabetic rats were treated for 2 months with 15, 20 and 30 mg/kg intraperitoneally twice a week, after one month of diabetes. Control and ALX-diabetic rats showed the following values of RC (expressed as contractions per minute): 0.98 ± 0.16 and 0.36 ± 0.05 ($p<0.01$). AGF1 normalize RC deficits in a dose-dependent manner. RC was 0.56 ± 0.12 (not significant vs. diabetics), 0.73 ± 0.12 ($p<0.05$ vs. diabetics) and 0.83 + 0.09 ($p<0.01$ vs. diabetics) in the 15, 20 and 30 mg/kg AGF1 treated groups respectively. Results suggest the potential therapeutic use of AGF1 to treat neurogenic bladder in diabetics.

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EFFECT OF A NEW POTENT ALDOSE REDUCTASE INHIBITOR, "TAT" ON DIABETIC NEUROPATHY OF RATS

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A new potent aldose reductase inhibitor, "TAT" (10 or 40 $mg \cdot kg^{-1} \cdot day^{-1}$, p.o.) was administered for 1 month to streptozocin-induced diabetic rats.

A markedly lower value of caudal motor nerve conduction velocity (MNCV) was detected in an untreated diabetic group (DC : 25.3 ± 2.6 $m \cdot sec^{-1}$) as compared with a non-diabetic "TAT"-treated ($40mg \cdot kg^{-1} \cdot day^{-1}$) control group (NCT : 31.7 ± 3.3, $p<0.005$), but an "TAT"-treated diabetic group (DT : 30.3 ± 3.1, $p<0.005$) had significantly higher MNCV. These findings were well correlated with the changes of sciatic nerve blood flow (SNBF) (DC : 4.4 ± 0.7 $ml \cdot min^{-1} \cdot 100g^{-1}$, NCT : 18.0 ± 2.9, DT : 18.5 ± 1.8, $p<0.001$). Moreover, DT group had markedly higher myo-inositol and significantly lower sorbitol and fructose levels in sciatic nerve as compared with group DC.

These findings suggest that "TAT" has a clear inhibitory effect on the development of delayed MNCV in the diabetic rats, which may be not only due to either increased sorbitol or decreased myo-inositol levels in nerve but also reduced nerve blood flow.

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Autonomic Neuropathy

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PREVALENCE OF CARDIAC AUTONOMIC NEUROPATHY IN INSULIN-DEPENDENT DIABETES MELLITUS (IDDM).

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In a random sample of 143 IDDM patients (male 73, age 14.9 to 58 years, duration 1.6 to 51.9 years, HbA1c 5 to 12 %) prevalence of cardiac autonomic neuropathy (CAN) was asssessed by heart rate variation at rest, heart rate variation with deep breathing, 30:15 ratio, valsalva ratio and postural blood pressure drop. CAN was defined by abnormality of at least two tests. CAN was present in 17%. Patients with CAN differed from non-affected by age (37.1 vs 31.4 years, $p<0.05$), duration (22.1 vs 13.7 years, $p<0.01$), HbA1c (8.3 vs 7.5 %, $p<0.05$), systolic blood pressure (130.9 vs 121.4 mmHg, $p<0.005$), body mass index (24.2 vs 22.9 kg/m², $p<0.01$) and waist to hip ratio (0.87 vs 0.82, $p<0.0001$). With multiple loglinear regression 85.3% of the patients were correctly classified by the independent variables waist to hip ratio, duration and HbA1c. CAN was positive related to peripheral neuropathy, $p<0.01$. Using analysis of covariance patients with CAN did not differ from non-affected by electrodiagnostic testing, but had higher vibration perception thresholds, $p<0.01$, cooling, $p<0.01$, and warming detection, $p<0.01$, thresholds. CAN parallels peripheral neuropathy, but in addition shows more severe involvement of small fiber function.

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ASSESSMENT OF CARDIOVASCULAR AUTONOMIC FUNCTION BY STANDING TO LYING AND DEEP-BREATHING TESTS IN DIABETIC PATIENTS

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Aim of the study was to evaluate the effectiveness of a simplified method for detecting cardiovascular autonomic neuropathy (CAN) in diabetic patients.A group of 160 (56 females, 104 males, 102 Type 1, 58 Type 2)unselected diabetic patients ranging in age from 20 to 69 years (43.8+/-13.9ys)was studied.Patients underwent a preliminary four classical tests:lying to standing, cough test, Valsalva manoeuvre and postural hypotension.Can was detected in 45 patients and 105 subjects were classified as normal.Thus a second examination was carried out using the standing to lying (SL2) as sympathetic and Deep-Breathing (DB) as parasympathetic test.Results were examined by means of the Bayes theorema:

	SL2 %	DB %	SL2 + DB %
SENSIBILITY	44.4	86.7	95.6
SPECIFICITY	93.3	96.2	89.5
ACCURACY	78.7	93.3	91.3
POSITIVE PREDICTIVE VALUE	74.1	90.7	79.6
NEGATIVE PREDICTIVE VALUE	79.7	94.9	97.9
EFFICIENCY	72.9	92.9	90.5
POST TEST ODDS	2.9	9.8	3.9
LIKELIHOOD RATIO	6.7	22.8	9.1

Even if diagnosis of CAD can not be established using only two tests,SL and DB can be applied to screen the diabetic population in routine surveillance.

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QT-INTERVAL PROLONGATION REFLECTS THE SEVERITY OF AUTONOMIC NEUROPATHY IN DIABETICS AND IN DIABETIC ALCOHOLICS

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Aim of our study was to evaluate the relationship between autonomic neuropathy /AN/ and corrected QT-interval /QT_CI/. 162 Type 1 diabetics /mean age: 33,2 range:14-57 yrs/, 94 Type 2 diabetics /mean age: 52 range:27-69 yrs/, 1o alcoholic diabetics without liver disease /mean age:39,9 range: 25-49 yrs/ and 22 diabetics with alcoholic cirrhosis /mean age: 47,1 range: 33-66 yrs/ were studied.We evaluated heart rate responses to deep breathing, Valsalva-manoeuvre and stading and blood pressure responses to standing and sustained handgrip.QT_CI was determined with Bazett's formula. 76/162 patients with Type 1, 51/94 with Type 2 diabetes, 7/1o alcoholic diabetics without hepatopathy and 19/22 with cirrhosis had at least two abnormal reflex-tests.Abnormal QT_CI /> 44o msec/ was found significantly more often in patients with AN compared to patients without AN in all groups. Significant linear regression was found between QT_CI prolongation and severity of AN /$p < o,o2$ in cirrhotics, $p < o,oo1$ in the three other groups/.Analysing the relationship between QT_CI and the five cardiovascular parameters separately, the most strong correlation was found between QT_CI and heart-rate response to deep breathing /$p < o,oo1$ in all groups/, indicating that beside the established role of sympathetic imbalance even parasympathetic damage contributes to development of QT-lengthening. Evaluation of the QT_CI could provide a simple additional diagnostic tool to identify diabetics with increased cardiovascular risk. Our datas suggest that alcoholic diabetics are more endangered even in this respect.

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BAROREFLEX SENSITIVITY INDEXES IN DIFFERENT DEGREES OF DIABETIC AUTONOMIC NEUROPATHY: A NEW NON-INVASIVE ASSESSMENT

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Failure of blood pressure regulation is the major effect of Diabetic Autonomic Neuropathy (DAN). It varies with autonomic involvement but its degree is not detectable by the traditional cardiovascular tests. We constructed a new non-invasive computerised system to measure Baroreflex Sensitivity Indexes (BSI) based on integrated measurements of ECGraphic R-R intervals and their related Pulse Blood Pressure (PBP) variations during standardised Valsalva Maneuver (VM). We used a Photopletismograph, Telecardiograph, PC signal interface, and our specially designed Software. The angular coefficients of R-R/PBP ratio vs time on phase-2 (b2) and on phase-4 (b4) of VM were used for BSI as expressions of peripheral sympathetic and parasympathetic activity respectively. We compared 50 controls and 50 diabetics: 30 without DAN, 8 with mild DAN, 12 with DAN. b2 and b4 did not differ between controls and patients without DAN.

b2 differed greatly in controls (-20.9(2.5); M(SEM)) and patients without DAN (-23.7 (4.3)) vs patients with DAN (22.3(6); $p < 0.001$, $p < 0.005$ respectively), but not vs patients with mild DAN (-15.4(6)).

b4 differred in controls (67.6(6.7)) and patients without DAN (42.4(5.4)) vs patients with DAN (-42.1(15.2); $p < 0.001$, $p < 0.005$) and vs patients with mild DAN (128(2.8); $p < 0.005$, $p < 0.05$).

b2 and b4, despite widely ranging values, showed high individual correlation coefficients, always above 0.90 ($p < 0.01$).

We conclude that our system can reliably detect impairments in blood pressure regulation and in peripheral vascular motility resulting from autonomic dysfunction.

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CLINICAL EVALUATION OF A COMPUTERIZED SYSTEM FOR THE DIAGNOSIS OF CARDIOVASCULAR AUTONOMIC NEUROPATHY

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We evaluated the performance of a computer working with a software prepared by the RR Medical Electronics (Sheffield) for the diagnosis of Cardiovascular Autonomic Neuropathy (CVAN) in Diabetes Mellitus (CVAFT.C), vs the usual EKG battery (CVAFT.E). We used the O'Brian battery of tests because the reference values are age-stratified. We accepted as evidence of CVAN the presence of at minimum one test definitely abnormal. We studied 38 type II diabetic patients (20 F, 18 M), age 59 ± 10 years, disease duration 18 ± 2 years, BMI 29.5 ± 4.8 who underwent at the same time both sets of tests, in 2 separate occasions, at an interval of 30 ± 15 days. We obtained 42% positive diagnoses with the EKG, vs 16% with the CVAFT.C. There was a high discordance rate within all the EKG tests (mean 75%), with a mean 30% having influence on diagnosis. Contrariwise the CVAFT.C had only a 10% variation on repeated tests, with only 4% of the Deep Breathing discordances having influence on the final diagnosis. All the patients positive for CVAFT.C were also positive for CVAFT.E. Time required for the CVAFT.E was 15 ± 3' (excluded time for calculations and reporting) vs 10 ± 2' for CVAFT.C. The system works effectively, reducing the need for repeated tests.

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CARDIOVASCULAR REFLEX TESTS AND POWER SPECTRAL ANALYSIS OF HEART RATE VARIATIONS IN THE DIAGNOSIS OF AUTONOMIC NEUROPATHY: A COMPARISON

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20 controls and 53 diabetics suffering different degrees of Autonomic Neuropathy (AN) were studied by Power Spectral Analysis (PSA) of heart rate variations on resting and by traditional parameters obtained from a battery of Cardiovascular Reflex Tests (CRT): Valsalva Manoeuvre (VM), Deep Breathing (DB), Lying-to-Standing (LS), Sustained-Handgrip (SHG), Postural-Blood-Pressure-Fall -on-Standing (PBPS).

PSA was calculated by autoregressive modelling which gives good quantification of two main bands, which correspond to sympathetic and parasympathetic activity respectively. CRT were automatically calculated with currently used indexes. PSA showed a good level of agreement ($p < 0.01$) with most CRT (DB, LS, VM) but proved better able than CRT to quantify sympathetic impairments between diabetics with severe and mild AN ($p < 0.02$ for PSA vs p = ns for CRT) and between those without AN and with mild AN ($p < 0.01$ vs p = ns). Furthermore PSA showed a good relationship ($p < 0.001$) between high frequency and low frequency band in normal but not in diabetics with AN suggesting a sympathetic-parasympathetic imbalance in these latter. On the other hand, in 5 control subjects studied three time a day for three days, CRT showed lower individual and interindividual variation coefficients in DB (18%), VM (9%), LS (5%) than PSA (mean total 22%, mean hourly 16%). We conclude that PSA is better tools for the detection of sympathetic activity and for the determination of parasympathetic-sympathetic balance, while most CRT have less variability. Hence the integrated measurements of PSA and CRT are the most useful tool in the diagnosis of Cardiovascular AN in diabetics.

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SCINTIGRAPHIC ASSESSMENT OF CARDIAC AUTONOMIC NEUROPATHY - RELATIONSHIP TO CARDIAC FUNCTION

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This study determines the interdependence between subclinical diabetic cardiomyopathy (DCMP) and cardiac autonomic neuropathy (CAN), as assessed by both, myocardial meta-iodobenzylguanidine (mIBG) scintigraphy and conventional cardiovascular reflex tests (RT). **Methods:** In 17 patients (pts.) with type 1 diabetes (age 44±10 yrs.) without overt cardiac disease left ventricular (LV) function was evaluated by Tc99m-ery radionuclide ventriculography (RNV) at rest and during bicycle exercise. Silent ischemia was excluded by Tl-201 myocardial perfusion scintigraphy. Adrenergic cardiac innervation was scintigraphically evaluated by planar and tomographic imaging at 10 min., 2, 4, and 24 hrs. after i.v. 350 MBq I-123 mIBG (non-metabolized norepinephrin analogue). Homogeneity of mIBG uptake and washout was determined. CAN was also assessed by RT (heart rate variability at rest and with deep breathing, Ewing, Valsalva). **Results:** 11 pts. showed evidence of DCMP as reflected by an abnormal systolic or/and diastolic response to exercise (group A). LV function was normal in 6 pts. (group B). Non-homogeneous (abnormal) mIBG uptake and/or washout was observed in 11/11 pts. of group A but in only 2/6 pts. of group B ($p<0.01$). Overall, mIBG scintigraphy indicated CAN in 13/17 pts., whereas RT were abnormal in only 3/17 pts. ($p<0.01$). **Conclusion:** Subclinical diabetic cardiomyopathy is related to derangements of adrenergic cardiac innervation as assessed by mIBG scintigraphy, which is a more sensitive indicator for cardiac autonomic neuropathy than conventional cardiovascular reflex tests.

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DIABETES, NEUROLEPTIC DRUG THERAPY AND MYOCARDIAL ELECTRICAL ACTIVITY CHANGES.

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Sudden death has been reported in diabetics with Cardiac Autonomic Neuropathy (CAN) and in patients undergoing neuroleptic drug therapy, perhaps as a result of QT prolongation and ventricular arrhythmias. The effect of CAN and phenothiazine therapy on ventricular electric activity in diabetic patients was investigated by the evaluation of QT interval (corrected for the heart rate, QTc) and TmTo interval in the following groups: 1) 12 diabetic patients with CAN; 2) 13 diabetics undergoing standard phenothiazine therapy; 3) 14 non diabetic subjects with the same neuroleptic treatment; 4) 10 healthy controls. CAN was diagnosed by positive response to at least two cardiovascular tests (Deep Breathing, Cough, Standing, Valsalva Manoeuvre, Postural Hypotension). Both QTc and TmTo intervals were prolonged in group 1 ($p<0.005$ and $p<0.05$, respectively), group 2 ($p<0.001$) and group 3 ($p<0.025$ and $p<0.001$) compared to controls. QTc and TmTo were increased in group 2 ($p<0.001$ and $p<0.02$, respectively) compared to group 1 and 3, without any significant difference between the latter two groups. These data suggest a cumulative effect of diabetes and phenothiazine therapy in inducing changes of ventricular electric activity, that mainly consist in desynchronized myocardial repolarization. This could result in an increased risk for fatal ventricular arrhythmias.

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SILENT MYOCARDIAL ISCHAEMIA IN DIABETIC PATIENTS WITH AUTONOMIC NEUROPATHY.

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It is generally accepted that the incidence of both sympto matic and asymptomatic coronary artery disease is increase sed in diabetic patients. Autonomic neuropathy is associated with an increased incidence of silent myocardial infarction and sudden death. In this study we investigated the prevalence of the silent myocardial ischaemia in patients with and without autonomic neuropathy. 54 patients with type 2 (non-insulin-dependet) diabetes mellitus underwent a) four cardiovascular reflex tests (heart rate responseto Valsalva's manoeuvre, heart rate response to change in posture, heart rate response to deep breathing, and blood pressure response to change in posture) and b) exersise tests on atreadmill using the Bruce protocol with continuous ECG monitoring. Student's t test was used to assess difference between means and x^2 test with Yates correction to assess difference between proportions. 14 patients were excluded because of they didn't achieve the target of the work load. 19 patients had a mild or a severe autonomic dysfunction and 21 hadn't any autonomic neuropathy. There weren't any statistical significant difference in age, duration of diabetes and HbA1 between the two groups. 12 of 19 patients (63%) with autonomic neuropathy had painless ST depression compared with 4 of 21 patients (19%) without neuropathy ($p<0.05$). We conclude that silent myocardial ischaemia on exersise is significantly more common in diabetic patients with autonomic neuropathy than in those without.

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POSTPRANDIAL HYPOTENSION IN DIABETIC PATIENTS: A MARKER OF INITIAL AUTONOMIC DAMAGE?

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In a previous research we observed that the absolute systolic and diastolic blood pressure (BP) nocturnal minima were reached very rapidly and the increase to the morning maxima occurred slower in normo- and hypertensive diabetics with abnormal responses to cardiovascular tests than in the patients without dysautonomia. We focused subsequently on the relative BP minimum, between the morning and afternoon peaks, probably due to the postprandial hypotension, a phenomenon provoked by a large increase in splanchnic blood flow and by a fall of the systemic vascular resistances. The range of hemodynamic, hormonal, and neural responses to food ingestion are modified in autonomic failure. We studied 44 diabetics (14 insulin-dependent, 30 non-insulin-dependent; mean duration disease 6.5 ± 1.8 years) in good metabolic control (fasting glycaemia < 140 mg/dl, postprandial glycaemia < 180 mg/dl, fructosamine < 285 mg/dl), divided into two subgroups, 21 normotensives (aged 28-72 years) and 23 hypertensives (aged 32-70 years) respectively. All patients showed abnormal responses to at least two of these tests: deep breathing, lying to standing, Valsalva manoeuvre and postural hypotension. Two sex and age-matched control groups were recruited [20 normotensive (aged 26-66 years) and 20 hypertensive (aged 27-68 years) diabetics without dysautonomia]. Each patient underwent ambulatory BP monitoring for at least 24 h, using an auscultatory automatic device (Pressurometer IV, Del Mar Avionics). Data concerning biological rhythms were analysed by means of periodic functions, limiting the Fourier partial sums to the first three harmonics, considering postprandial BP decrease as a "speed" (mmHg/hour). In diabetic normotensives with autonomic neuropathy we observed that the postprandial systolic and diastolic BP minima occurred more rapidly than in controls (postprandial systolic BP decrease -2.8 versus -2.2 mmHg/h, diastolic -3.1 versus -2.4 mmHg/h). The same behaviour was observed in both hypertensive groups but the difference was more marked (systolic -3.3 versus -2.5 mmHg/h, diastolic -3.3 versus -2.3 mmHg/h). Our approach confirms that postprandial hypotension is more evident in neuropathic diabetics, first of all for the autonomic system alterations, involved in the BP control. In addition this method could give us further informations to evaluate the extent of the initial autonomic damage.

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ALTERED CIRCADIAN RHYTHM OF SYMPATHOVAGAL BALANCE IS RELATED TO ABNORMAL 24H BLOOD PRESSURE PROFILE IN DIABETES

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Circadian rhythm of sympathovagal balance (SVB) has been involved in the diurnal variation of cardiovascular accidents. In diabetic autonomic neuropathy abnormal circadian pattern of blood pressure (BP) and SVB with reduced fall of BP and prevalence of sympathetic activity during the night, has been described. To correlate the abnormalities of BP to those of SVB, we performed simultaneous 24h noninvasive monitoring of BP and ECG in 25 diabetic subjects (age 45.6±13.6, diabetes duration 17.6±9.1 yrs) with various degree of cardiovascular reflexes impairment. Autoregressive power spectrum analysis of RR interval was applied to 24h ECG recording to obtain for day and night period mean power of low- (0.03-0.15 Hz, LF) and high-frequency (0.16-0.35 Hz, HF) components, markers of sympathetic and vagal activity respectively, and their ratio (LF/HF), assumed as index of SVB. Percent day-night change in LF/HF correlated to % day-night change in systolic BP ($r=0.52$, $p<0.01$), and diastolic BP ($r=0.48$, $p<0.015$). Percent day-night change in diastolic BP was also related to night LF ($r=-0.49$, $p<0.01$), night HF ($r=0.47$, $p<0.01$) and night LF/HF ($r=-0.51$, $p<0.01$). Thus, the loss in day-night rhythm of BP is related to that of SVB. The association of decreased BP fall with sympathetic predominance during the night, might account for enhanced risk of cardiovascular accidents in diabetes.

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PROFILE OF UNMYELINATED NERVE FIBRE DYSFUNCTION IN DIABETIC POLYNEUROPATHY

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Dysfunction of the nociceptive and autonomous peripheral nerve fibres in diabetic polyneuropathy (PNP) is considered to cause trophic lesions. Because of this clinical relevance we assessed the functional deficit of the nociceptive afferents and of vasoconstrictor and sudomotor efferent nerve fibres.

Results of 29 patients suffering from a distal, symmetric PNP are compared with those of 24 age matched controls (Mann-Whitney U-Test). Nociceptive (C-)fibre function was assessed by recording of neurogenic vasodilation following histamine-iontophoresis and ischemia with Laser-Doppler-Flowmetrie and following standardized painful pinching with infrared-thermography. Compared to controls histamine induced vasodilation was reduced to 44 %, postischemic vasodilation and local warming of the pinched skin area to 42 %, on average. Sympathetic vasoconstrictor reflex function was evaluated following standardized painful pinching by means of photoplethysmography. Vasoconstriction was reduced to 31 %. Sudomotor efferents were tested by hygrometrical evaluation of the axon reflex mediated sweat response following carbacholiontophoresis. Evaporation rate was reduced to 91 %. All differences were statistically significant, except those of the sudomotor fibres.

This battery of objective tests establishs a profile of unmyelinated nerve fibres involved in PNP, which may be used for quantification of progression and regression of unmyelinated nerve fibre dysfunction with time and therapy. **(BMFT grant 0701502 3).**

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VENTILATORY RESPONSE TO PROGRESSIVE HYPERCAPNIA - ABSENCE OF INCREASE AFTER NALOXONE IN TYPE 1 DIABETIC PATIENTS

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Ventilatory response to progressive hypercapnia is reduced in diabetic patients with autonomic neuropathy (ANP) compared to healthy controls and diabetic patients without ANP. Naloxone, an antagonist of endogenous opiods improves the ventilatory response to hypercapnia in healthy probands. The aim of this study was to evaluate the influence of intravenously administered naloxone (0,1mg/kg) in healthy controls (n=8), diabetic patients with ANP (n=7) and diabetic patients without ANP (n=8). Cardiovascular tests for the assessment of autonomic neuropathy:Beat to beat variation during rest, deep breathing and a valsalva manoever, performed with the Pro-Sci-Card analyzer, orthostatic blood pressure reaction and the measurement of the QT-interval (ECG).Lung function:Spirometry, plethysmographie and specific lung compliance. The hypercapnic ventilatory responses were recorded at 30 sec intervals, following Read's method.
As a result the ventilatory response to hypercapnia (slope) increased in the controls (2,78 +- 0,55 without naloxone and 3,94 +-0,73 L/min/mmHg with naloxone), but there was no effect in the diabetic patients with or without ANP (2,12+-0,60 and 2,55+-0,47 without naloxone and 2,05+-0,65 and 2,65+-0,76 L/min/mmHg with naloxone).Impaired control of ventilation by peripheral factors (respiratory muscle dysfunction, pathologically altered mechanical impedance) were excluded by lung function analysis and electromyography of the diaphragm.From these data we can conclude, that endogenous opioids do not have any effect on ventilatory hypercapnic response in diabetic patients with or without ANP, as opposed to healthy subjects.

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PERIPHERAL AND AUTONOMIC NEUROPATHY IN DIABETIC PATIENTS WITH ERECTILE DYSFUNCTION

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Neuropathy is a frequent cause of impotence in diabetics. The aim of the study was to evaluate different neurophysiological methods in diabetics with erectile dysfunction.
In 23 patients with clinically suspect peripheral polyneuropathy we measured conduction velocity in limb nerves, bulbocavernosus reflex latencies (BCR), and cerebral somatosensory evoked potentials on stimulation of pudendal nerves (SEP). In patients with pathological values (n=20) we also tested the function of the autonomic nervous system. Control group consisted of healthy, age-matched men. Average age of patients: 49,4 years, average duration of diabetes: 10,7 years, average duration of impotence: 3,8 years. Motor conduction velocities of median and peroneal nerves were slower in diabetics (49,3m/s and 42,0m/s vs 53,2m/s and 44m/s), as well as sensory conduction velocity of median and sural nerves (41,5m/s and 39,1m/s vs 45,8m/s and 47,7 m/s). Latency of pudendal SEP and BCR were prolonged in diabetics (41,1 ms and 45,8ms vs 41,0ms and 34,6ms) respectively. 13 patients had pathologic conduction parameters in limbs, 7 in the sacral nervous system, 5 in both. Classic tests for evaluation of autonomic function (Valsava manoeuvre, handgrip test, orthostatic test, deep breathing test, face immersion test) significantly differed in patients from those in control group. Difference in spectral analysis of heart rate was also significant.
We conclude that application of several methods increases the yield of properly diagnosed neuropathy in impotent diabetics, and that the majority of patients with peripheral neuropathy also have demonstrable autonomic nervous system involvement.

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IMPOTENCE IN DIABETIC MEN

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The prevalence of impotence is 60% in our diabetics. The mean age of patients is 50.4 years, the mean duration of diabetes 11.6 years, and the mean duration of erectile dysfunction 3.9 years.
The aim of our study was to estimate the cause of impotence and to find the appropriate mode of treatment. 100 diabetics were included. After the diagnostic algorithm (history, physical examination, hormonal studies, neurophysiological tests, intracavernous application of a vasoactive substance, duplex ultrasound of cavernosal bodies, cavernosometry/cavernosography, arteriography, psychogenic evaluation) it was estimated that in 5% of patients the cause was psychogenic and in 7% it was the consequence of taking drugs. In 28% the cause was vascular (arterial insufficiency: 9%, venous leak: 19%), in 36% neurogenic, and in 24% combined: vascular/neurogenic. An intracavernous injection of prostaglandin E1 was successful in 52% of cases (46 patients). The majority of these patients were selected for autoinjections. Other patients were treated according to the cause of impotence: balloon embolization of deep dorsal penile veins (16 patients), implantation of penile prostheses (3 patients). 11 patients use vacuum devices.
We conclude that the prevailing cause of impotence in diabetics was organic, and that with different mode of treatment this disturbance could be successfully managed in the majority of patients.

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Diabetic impotence - aetiological assessment and outcomes of treatment.

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We aimed to study the causes and treatment of diabetic impotence. To assess aetiology we used autonomic neuropathy tests (acetylcholine sweatspot test; pupil test; five cardiovascular tests), vascular tests (response to intracorporeal papaverine (ICP), radioisotope phallography, penile brachial index) as well as nocturnal penile rigidity, psychiatric assessment and detailed interview. We studied 71 diabetic men complaining of impotence, median age 54 (range 26-70) years. Although 38% had a psychogenic component, and 19% predominantly psychogenic impotence, there was evidence of an organic component in 93% (vascular only, 24%; neuropathy only, 31%; vascular plus neuropathy, 38%). For therapy we compared the constriction-band type of vacuum device (Erecaid) with the "super-condom" type (Synergist) in 10 randomly-chosen patients by providing them in random order for 5 months each. Five couples achieved a satisfactory therapeutic outcome. In all five, patient and partner were unanimous in preferring Erecaid ($p<0.001$). We then used a similar protocol to compare Erecaid with ICP by offering them in random order to 15 patients. All 15 who tried the therapies experienced a degree of success (very successful 11/15(73%)). 11/15(73%) preferred Erecaid and rejected ICP; 2/15(13%) preferred ICP and rejected Erecaid; only 2/5(13%) liked both therapies one preferring Erecaid, the other ICP. The preference for Erecaid was significant ($p<0.05$). Organic impotence is common in diabetic patients who complain of impotence and vacuum constriction therapy is most commonly preferred.

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PATHOGENESIS OF SEXUAL IMPOTENCE IN DIABETIC PATIENTS

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Impotence is a major clinical problem of adult diabetic patient, with a high prevalence (between 35% and 80%). The aim of this study is to assess the causes of impotence in 14 diabetic impotent patients (15 NIDDM, 1 IDDM), mean age 54,1years (42-62), mean diabetes duration 16,5 years (5-26), mean duration of impotence 2,2 +-0,919 years.

The patients were evaluated with a nocturnal penile tumescence test (NPT), the measurement of peno-brachial index (PBI) before and after intracavernosal 10 mcg PGE1injection, the assessment of cardiovascular reflexes and measurement of the bulbocavernosus reflex latency.

NPT test results suggested that 12 (87,7%) patients were affected by an organic impotence. From these, 4 (28%) had a normal PBI (0,84+-0,07) and a prolonged bulbocavernosus reflex latency (44msec+-2,17), 2 patients (14,2%) had a reduced PBI (0,62+-0,04) and a normal bulbocavernosus reflex latency (35,5+-4,1), and 6 (42,8%) had an abnormal PBI (0,6+-0,9) and a prolonged bulbocavernosus reflex latency (44,5+-6,8). After 10 mcg of intracavernosal PGE1 patients with a normal PBI had a better response of penile rigidity than those with low PBI (86,75%+-3,86 vs 16%) (P=0,002).

We can confirm that diabetic impotence have an organic pathogenesis, that neuropathy and vasculopathy are frequently involved in the same patient and that a test of intracavernosal PGE1 injection is very useful in the assessment of the pathogenesis of the impotence.

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ANTIBODIES TO AUTONOMIC NERVOUS TISSUES IN DIABETIC NEUROPATHY.

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We investigated the presence of autoantibodies to sympathetic and parasympathetic nervous structures in: 32 patients with long-standing Type 1 diabetes (mean age 43.5 ± 11.6 years, mean duration of diabetes 29.8 ± 11.1 years) with abnormal autonomic function tests, of whom 26 had severe symptomatic autonomic and peripheral neuropathy and 6 had no autonomic symptoms; 22 patients with Type 1 diabetes, matched for age (41.6 ± 12.1 years) and duration of diabetes (23.1 ± 12.1 years) without any complications; 27 age and sex-matched healthy subjects. An indirect immunofluorescent complement-fixation technique was used, with monkey adrenal gland, rabbit cervical ganglia and vagus nerve as substrates. 3 of the 26 patients with symptomatic neuropathy (11%) showed positive staining of cell bodies in cervical ganglia and 2 (8%) showed positive staining along vagus nerve fibres. All other patients and controls were negative. Anti-adrenal medulla antibodies were detected in 3 other patients with symptomatic neuropathy (11%), in 2 patients without any complications (9%) and in 1 control (3%). Our data indicate that autoantibodies to autonomic nervous structures are present in symptomatic neuropathy. Anti-sympathetic ganglia and anti-vagus nerve antibodies seem to be more disease-specific. The low percentage of positivity may be explained by the disappearance of these antibodies after years of disease.

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A FIVE-YEAR FOLLOW-UP STUDY OF ELECTRIC TASTE THRESHOLD IN DIABETIC PATIENTS.

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In order to specify the changes in taste function throughout the course of diabetes, 30 diabetic patients without any other cause of taste impairment were screened for taste disorders initially and after 5 years. Their age was 50.4 ± 5.2 yr and duration of diabetes 12.3 ± 3.1 yr; 16 patients were treated with insulin, including 10 type 1 (insulin-dependent) diabetic patients. Electrogustometry was performed using a 3 mm diameter anode placed 15 mm from the anterior extremity of the tongue. Current stimuli began at 100 μA and went on using a dichotomic algorithm (range 5 - 500 μA; steps 5 μA). Results of 2 procedures were averaged and expressed as an electrogustometric threshold (EGT). Elevated EGT related to poor taste function. Results (m ± SE) were compared with paired t-test (logarithmic transformation of EGT) and χ^2. Throughout the study, EGT increased (53 ± 11 vs 110 ± 22 μA; $p<0.01$). Electric hypogeusia (EGT ≥ 100 μA) was found among 3 (10%) patients initially and 10 (33 %) at follow-up ($p<0.05$). Individual EGT values increased (> 10 μA) in 13 (43%) subjects, whereas it did not vary (± 10 μA) in 16 (53%) and improved in 1 (3%). Taste impairment was associated with increased incidence of of degenerative complications, especially peripheral neuropathy ($p<0.01$). These results suggest that taste is impaired throughout the course of diabetes. A degenerative mechanism could be involved.

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SMELL IMPAIRMENT AND RELATED FACTORS IN DIABETIC PATIENTS.

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In order to study smell function, 30 diabetic patients (duration: 12.3 ± 3.1 yr) and 25 healthy control subjects were screened for smell disorders. None had disease or treatment susceptible to impair smell. A score (SmS), ranging from 0 (no recognition) to 20 (perfect recognitions), was defined, based on the ability of subjects to recognize 20 different flavors from a standardized kit, using a randomized procedure. Results were analysed using chi-square, t-test and linear regression. Multivariate analysis was stepwise multiple regression. Diabetic and control subjects did not differ for age (50.4 ± 5.2 vs 50.2 ± 6.3 yr; NS), gender, tobacco and alcool consumption, whereas systolic blood pressure was higher in diabetic patients (134 ± 4 vs 123 ± 3 mm Hg; $p<0.05$). On average, SmS was higher in control subjects (14.1 ± 1.3 vs 11.4 ± 1.2; $p<0.01$). In diabetic patients, it was significantly associated with peripheral neuropathy (10.3 ± 1.1 vs 12.7 ± 0.6; $p<0.05$) and microalbuminuria (11.7 ± 0.6 vs 13.3 ± 0.6; $p<0.05$), but neither with Hb_{A1c} ($r=0.11$; NS) nor with blood glucose ($r=0.13$; NS). It correlated with age ($r=-0.29$; $p<0.05$), but did not with duration of diabetes ($r=-0.09$; NS), blood pressure, tobacco and alcool consumption. Using multivariate analysis, the strongest association was found with peripheral neuropathy ($R^2=0.09$). These results support previous findings suggesting that smell is impaired in diabetic patients. A degenerative mechanism can be involved.

PS 24
Peripheral Neuropathy

604

AUTONOMIC NERVE FUNCTION AFTER 8 YEARS OF IMPROVED BLOOD GLUCOSE CONTROL.

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We aimed to study the autonomic neural function in type I (insulin dependent) diabetic patients after 8 years with different levels of metabolic control. The 44 patients were followed prospectively for 8 years. At baseline; mean age was 26 years (range 18-42), mean duration 13 years (range 7-23) and mean HbA1 11.2 ±2.2%. During 8 years mean HbA1 was 9.5 ±1.5% , p<0.001 vs. baseline (normal <7.6%). Heart rate (RR-interval) variation during deep breathing, lying/standing (15:0 and 30:15 ratio) and Valsalva manouvre were performed after 8 years.

Heart rate variation during deep breathing was significantly decreased in patients with mean HbA1 >10% (n=12) compared to the patients with a mean HbA1 <10% (n=32) , 19.4 ±10.7 vs. 27.6 ±11.9 beats/min, p<0.04. The same was true for the 15:0 ratio; 0.78 ±0.11 vs. 0.73 ±0.05,p<0.005 and the 30:0 ratio; 1.11 ±0.12 vs. 1.20 ±0.14 , p<0.01. Multiple regression analysis showed that mean HbA1 during the last 8 years was an independent variable for the outcome of heart rate variation during deep breathing, regression coefficient(RC) -9.5, p<0.02, 15:0 ratio; RC 0.07, p<0.003 and the 30:15 ratio; RC -0.11, p<0.02.

Conclusion: Improved blood glucose control during 8 years retards the progression of autonomic neural dysfunction.

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Single fiber electromyography as a useful method to detect and evaluate diabetic neuropathy

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To examine the usefulness of single fiber EMG(SFEMG) in evaluating diabetic neuropathy(DN) SFEMG, conventional EMG and nerve conduction study were performed in 23 diabetic subjects without clinical evidence of DN(group 1) and 20 diabetic subjects with clinical features of DN(group 2). Jitter(MCD) and fiber density(FD) were measured at tibialis anticus(TA) muscle by SFEMG using the method of Stalberg. The nerve conduction studies included amplitudes of the sensory evoked potentials(NAP) and conduction velocities of sural nerve(SNCV) and amplitudes of motor evoked potentials(MAP) and motor conduction velocities of deep peroneal nerve(MNCV). Coventional EMG was examined at TA muscle. Patients in group 2 had significantly increased MCD and FD (69.3±15.3μsec, 2.79±0.84) in comparison with patients in group 1(45.5±13.1μsec, 1.88±0.37, p≤0.01, respectively). Furthermore, percentages of the abnormal values in MCD and FD were greater than those in other neurophysiological parameters in both groups(group 1:FD 22%,MCD 22%,MAP 9%,MNCV 13%,SNCV 17%,NAP 17% and EMG 9%, group 2:FD 90%,MCD 65%,MAP 60%,MNCV 55%, SNCV 55%, NAP 25% and EMG 20%). These data show that specifically MCD and FD are increased in diabetic patients indicating that axonal degeneration with reinnervation is the principal pathological finding in DN and that SFEMG is a more sensitive method to detect an early DN.

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NERVE FIBER LOSS IN DIABETIC NEUROPATHY CORRELATES WITH IMPAIRED EVOKED POTENTIAL AMPLITUDES AND NERVE CONDUCTION VELOCITY

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Diabetic neuropathy was in a recent consensus conference defined as a disease of progressive nerve fiber damage and loss. In the present study, morphometric analysis of sural nerve biopsies obtained from 53 diabetic subjects with mild to moderately severe clinical diabetic neuropathy was correlated with nerve conduction velocities and evoked potential amplitudes of the sural, sensory median and peroneal nerves. Highly significant correlations were demonstrated between sural nerve fiber density and its nerve conduction (p<0.0003) and amplitude of evoked response (p<0.008). Furthermore sural nerve fiber density correlated with peroneal nerve conduction velocity (p<0.0436) and amplitude (p<0.0220) as well as with the electrophysiological parameters of sensory median nerve conduction (p<0.0009). These data suggest that in clinical diabetic neuropathy the relentless nerve fiber loss underlies the progressive impairments in sural nerve conduction velocity and amplitude, and that a simple morphometric measure such as sural nerve fiber density is a good indicator of nerve dysfunction in nerves other than the sural nerve. These findings strongly suggest that nerve fiber damage in various peripheral nerves occurs *paru passu*, although the rate of progressive damage may vary from one nerve to the other.

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DO NEUROPHYSIOLOGICAL AND PATHOLOGICAL ABNORMALITIES DISCRIMINATE PAINFUL FROM PAINLESS HUMAN DIABETIC NEUROPATHY?

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Peripheral nerve function and structure was related to symptoms in human diabetic neuropathy. Peroneal nerve conduction (PMNCV), vibration (VPT) and thermal (TDT) perception and myelinated and unmyelinated fibre pathology was assessed in diabetic patients with painful (n=10) and painless (n=10) neuropathy and control subjects (n=8). PMNCV (P<0.01) was reduced and VPT (P<0.02) and TDT (P<0.01) were increased in diabetic patients when compared with control subjects. Only TDT was significantly greater in painless vs painful neuropathy (P<0.02). Myelinated fibre density was significantly reduced in painful (P<0.01) and painless (P<0.03) neuropathy vs controls but was no different in painful vs painless groups. Similarly unmyelinated fibre degeneration (percentage of unassociated Schwann cell profiles) was significantly greater in painful (P<0.0005) and painless (P<0.009) neuropathy when compared with controls, but was no different in painful vs painless groups. Unmyelinated fibre density did not differ amongst control and diabetic groups. However unmyelinated axon diameter was significantly reduced in painful (P<0.001) and painless (P<0.003) neuropathy vs controls but was no different between painful vs painless neuropathy. Diabetic patients exhibit reduced PMNCV and elevated VPT and TDT along with myelinated and unmyelinated fibre degeneration and axonal atrophy. However no functional or structural abnormalities distinguish painful from painless diabetic neuropathy.

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ENDONEURIAL LOCALIZATION OF MICROVASCULAR DAMAGE IN PATIENTS WITH HUMAN DIABETIC NEUROPATHY: RELEVANCE TO AETIOLOGY AND TREATMENT.

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Detailed ultrastructural, electronmicroscopic studies were conducted in each of three (endoneurial, perineurial, epineurial) capillary beds in the sural nerve of 20 diabetic patients with neuropathy and 10 age-matched control subject. Amongst control subjects, basement membrane area, endothelial cell area, luminal area and endothelial cell profile number were not significantly different between endoneurial, perineurial and epineurial capillaries. However amongst diabetic patients a number of differences were observed. Basement membrane area was significantly greater in endoneurial (directly in contact with nerve fibres) when compared with perineurial ($P<0.06$) and epineurial ($P<0.004$) capillaries. Endothelial cell size was significantly greater in endoneurial vs perineurial ($P<0.06$) and epineurial ($P<0.004$) capillaries. Luminal area was significantly less in endoneurial when compared with epineurial ($P<0.001$) but not perineurial (ns) capillaries. In contrast endothelial cell profile number was significantly greater in diabetic endoneurial ($P<0.0002$), perineurial ($P<0.003$) and epineurial ($P<0.001$) capillaries when compared with control capillaries, but did not differ between each other in diabetic patients. This study localizes basement membrane thickening, endothelial cell hypertrophy and reduction of luminal size of diabetic capillaries to the endoneurium. Accelerated damage of the microvasculature has been localized to the endoneurium of peripheral nerve and future studies on aetiology and prevention should be localized to this area.

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NERVE FIBRE AND CAPILLARY PATHOLOGY IN ASYMPTOMATIC DIABETIC PATIENTS WITHOUT NEUROPATHY.

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In order to evaluate early nerve fibre and microvascular change, diabetic patients (n=9) without clinical or neurophysiological evidence of neuropathy and normal age-matched control subjects (n=10) were studied. Diabetic patients had no symptoms and entirely normal peroneal nerve conduction and vibration and thermal perception. Myelinated fibre density, normally a very sensitive indicator of neuropathy, was no different in diabetic patients vs controls (ns). However both myelinated fibre ($P<0.02$) and axon ($P<0.01$) area were significantly reduced in diabetic patients. Teased fibre studies demonstrated an increased incidence of paranodal abnormalities ($P<0.001$) and a slight increase in segmental demyelination/remyelination ($P<0.06$) without axonal degeneration/regeneration (ns). The percentage of unassociated Schwann cell profiles (sensitive indication of unmyelinated fibre degeneration) was significantly increased in diabetic patients vs controls ($P<0.01$). Unmyelinated fibre density ($P<0.01$) was increased and axon diameter ($P<0.05$) was reduced in diabetic patients. Endoneurial capillary pathology was present in the form of increased basement membrane thickening ($P<0.02$) and endothelial cell profile number ($P<0.01$). Diabetic patients without neuropathy demonstrate early myelinated fibre demyelination with axonal atrophy, unmyelinated fibre degeneration with regeneration, and microvascular pathology. The presence of microvessel disease provides further support for the role of microangiopathy in the aetiology of neuropathy.

PS 25
Diabetic Foot

610

Epineurial new vessel formation following institution of insulin therapy.

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Neuropathy following rapid improvemovement in glycaemic control with the institution of insulin therapy (insulin neuritis - IN) is well recognised but its aetiology is unclear. We have identified 6 such subjects and compared electrophysiological parameters, sural nerve epineurial vessel anatomy, fluorescein angiography and detailed ultrastructural pathology of nerve fibres, to that of 13 subjects with chronic sensory motor neuropathy (CSMN) and 5 non-neuropathic diabetic (NND) subjects. There was a marked reduction in the electrophysiological parameters and myelinated fibre density in the CSMN group compared to the NND and IN groups. Epineurial new vessel formation was observed in all IN subjects who have undergone sural nerve photography but these changes were not present in the NND group. This contrasted with the CSMN group in which severe epineurial vessel abnormalities with widespread arterial attenuation and venous distension were observed in all patients. The nerve intensity of fluorescein and the fluorescein appearance time (FAT) which are measures of nerve blood flow were within normal range in both the IN (n=3, mean FAT 35.3 sec) and NND (n=5, mean FAT 33.4 sec) groups, but was markedly delayed in the CSMN group (n=13, mean FAT 55.4 sec). These findings show that epineurial new vessel formation similar to that found in the retina, may be a feature of IN.

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HIGH PREVALENCE OF RISK FACTORS FOR FOOT ULCERATION IN TYPE 2 DIABETIC PATIENTS: A POPULATION-BASED STUDY

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Hospital based studies may not reflect the true prevalence of lower-extremity complications in Type 2 diabetic subjects in the community. We therefore studied 811 subjects with Type 2 diabetes from 3 UK cities using standardised methods of assessment. Mean age of the subjects was 65.4 ± 11 years and mean known duration of diabetes was 7.4 ± 7.7 years. 404 subjects were male. Clinical neuropathy was diagnosed if the subject had moderate to severe neurological impairment characterised by a neuropathy disability score of > 6. Those with at least 2 absent foot pulses were diagnosed to have peripheral vascular disease. The prevalence of neuropathy was 41.9% (95% confidence limits 38.5–45.3%) and that of peripheral vascular disease was 24% (21.1–27%). 43 subjects (5.3 (3.8–6.8%) had foot ulcers (11 active). Of these, 16 subjects had purely neuropathic ulcers, 13 had mixed vascular/neuropathic ulcers, 8 had pure vascular disease and 6 were due to other factors. Subjects with ulcers had higher vibration perception thresholds (33 ± 12) compared to others (22 ± 11.2, $p < 0.05$). Foot ulceration affects 1 in 20 subjects with Type 2 diabetes; a large proportion of Type 2 diabetic subjects in the community are at risk of foot ulceration and would require preventative footcare and education.

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A MODEL FOR THE PREDICTION OF FOOT ULCERATION DATA ON A DIABETIC CLINICAL INFORMATION SYSTEM

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Annually or biennially collected neurological and vascular examination data stored on computer (validated in a previous study - in press) was used to devise optimal screening for risk of foot damage, to focus scarce foot care resources. We analysed data for 5347 patients, 1979-1991, with 354 foot problems (ulceration, gangrene, amputation). 325 had complete pulse and neuropathy data. We constructed 2 scores for each patient: Pulse Score [0-16] (1 for impaired and 2 for each impalpable lower limb pulse); Neuropathy Score [0-12] (1 for impaired, 2 for absent light touch and pinprick sensation, 1 for each absent reflex). In predicting foot problems, Pulse Score $\geq$ 1 was 73.7% specific and 71.8% sensitive and Neuropathy Score $\geq$3 was 75.2% specific and 70.9% sensitive. Sacrificing some specificity (61.8%) an improved sensitivity (87.5%) was obtained by regarding a Pulse Score $\geq$1 but a Neuropathy Score $\geq$3 as conferring risk. In this way, only 42 out of 325 (i.e. 13%) would not have been predicted. Stepwise linear regression analysis showed predictive value of Pulse Score confined to palpability of dorsalis pedis and posterior tibial pulses. There is a high correlation between the sensory components of the Neuropathy Score. The ankle reflexes were also contributors. We conclude that a limited examination of foot pulses, one sensory parameter and the ankle reflex predicts foot ulceration with 79.4% specificity and 71.2% sensitivity, allowing targeting of preventive chiropody resources in hospital clinics or in general practice.

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LASER DOPPLER FLOWMETRY EVALUATION IN DIABETIC FOOT: RELATION WITH AUTONOMIC NEUROPATHY.

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To evaluate the microcirculation of the skin -especially the foot- we performed Laser Doppler flowmetry in 90 type I diabetes mellitus patients and in 29 age and sex matched control persons. We measured basal flow, post-ischaemic hyperaemia (independent of neural mechanisms) and the hyperthermy reaction test (mediated by sympathetic axon reflex). These results were correlated with the standard tests of autonomic function (valsalva ratio, postural change in blood pressure, heart rate variation with deep breathing).
Results:

	Diabetics (n = 90)	Controls (n = 29)	p
basis (mvolt)	106 ± 43	74 ± 32	0.001
hyperthermy (mvolt)	305 ± 172	467 ± 233	0.007
Ratio therm/basis°	3.25 ± 2.30	6.77 ± 3.13	0.000
Difference therm/basis°	0.199 ± 0.165	0.394 ± 0.216	0.001

For the diabetics the basal microcirculation-flow is significantly increased, while the hyperthermy reaction is significantly lower comparing to the controls, even when the values are corrected for the basal flow (°). It was not possible to find a correlation between the Laser Doppler results and the absolute values of the standard tests of autonomic function. Conclusion: it seems that disturbed microcirculation in diabetics with loss of peripheral sympathetic function may occur in the absence of clinically detectable abnormalities of central autonomic nerve function.

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EVALUATION OF INFECTIOUS DIABETIC FOOT COMPLICATIONS WITH INDIUM-111 LABELED HUMAN NONSPECIFIC IMMUNOGLOBULIN G.

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Osteomyelitis in the diabetic foot is a major diagnostic and therapeutic problem. Recent reports suggested high accuracy of indium-111 labeled human nonspecific immunoglobulin G (In-111-IgG) scintigraphy in infectious bone and joint disease. A major advantage of In-111-IgG is the relatively easy and quick preparation of the radiopharmaceutical. In this study, the validity of In-111-IgG scintigraphy was studied in 16 diabetic patients with foot ulcers, gangrene or painful Charcot joints. In all patients plain radiographs and a bone scan scintigraphy (dynamic images after administration of 600 MBq Tc-99m-MDP, early images 5 min. p.i., delayed images 3 and 24 hours p.i.) were performed. The next day, 4, 24 and 48 hours after i.v. administration of 75 MBq In-111-IgG images of the feet were obtained from four different angles. The results were verified by histologic examination of surgical specimens (10 lesions) or long-term clinical follow-up (16 lesions). On the bone scan all 7 osteomyelitic foci were detected. However, 19 additional foci, not due to osteomyelitis were seen. On the plain radiographs 4 of 7 osteomyelitic foci were detected, on the In-111-IgG scintigraphy 6 of 7 (sensitivity 57% and 86% respectivily). Plain radiographs correctly ruled out osteomyelitis in 15 of 19 lesions, In-111-IgG scintigraphy in 16 of 19 (specificity 79% and 84%, respectivily). Penetrating ulcers and recent fractures in a Charcot joint gave false-positive results. A false-negative In-111-IgG study was observed in a patient with severe arterial angiopathy. ***Conclusion:*** **In-111-IgG scintigraphy can contribute to adequate evaluation of osteomyelitis in diabetic foot complications, as it improves specificity and sensitivity.**

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A COST EFFECTIVENESS STUDY OF TWO APPROACHES OF HEALING DIABETIC FOOT ULCERS.

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The aim of the study was to evaluate the healing and cost efficacy as well as limb salvage over a 4 1/2 years of two approaches to the treatment of diabetic foot ulcers. Patient's treatment in the conventional (C) approach (n=28) included predominantly total contact cast, debridement, antibiotics, split thickness skin grafts, irrigation, and orthotic shoes. The wound care center program treatment (WCC) (n=79 patients), included vascular surgery (if needed), debridement, and topical growth factor therapy. Outpatient, ambulatory surgery, and inpatient charges were added together. The charge for the growth factors was included in the WCC tabulation. It was found that the average grand total charge per patient was $19,431 for conventional treatment versus $14,024 at the wound care center (p=0.05). Only 24% of the conventional patients were healed whereas 79% of the wound care center patients were healed (p<0.0001). More conventional patients needed amputation (60%) versus WCC patients (19%) (p<0.001). The wound care center amputations tended to be toes versus BKA and AKA amputations in the conventional program. This study documented that the comprehensive wound care center protocol including growth factors was less expensive, had increased healing efficacy, and fewer amputations.

PS 26
Smoking

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INDICATORS OF POOR METABOLIC CONTROL AMONG NEWLY DIAGNOSED TYPE I DIABETIC PATIENTS AFTER 2 TO 3 YEARS OF DIABETES

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After participation in an intensive treatment and teaching programme (ITTP) not all type I diabetic patients have good metabolic control. In order to identify indicators of poor control in newly diagnosed type I diabetic patients we reevaluated 165 subjects (age 22±7 years; mean ± SD) 2 to 3 years after diagnosis and participation in a 5-day-inpatient ITTP. At follow-up, HbA_{1c} of the whole patient group was 7.3±2.1% (normal ≤5.5%) and the incidence of severe hypoglycaemia was 0.05 cases/patient/year. Stepwise multiple regression analysis revealed that a high HbA_{1c} is associated with the following indicators in descending order: low school education, young age, smoking and duration of diabetes. Patients with low level of education (n=57) had a mean HbA_{1c} of 8.2%, those with an intermediate level (n=54) of 7.1% and those with a high level (n=54) of 6.5% (ANOVA, $p<0.001$). Smokers (46%) had significantly higher HbA_{1c} levels than non-smokers (7.9% versus 6.8%; $p<0.002$). This study shows that among newly diagnosed type I diabetic patients with overall satisfactory quality of diabetes care, a low level of school education and smoking are strong indicators of a less favourable degree of metabolic control after 2 to 3 years of diabetes duration. It has to be shown whether patients with low school education and smokers will achieve better metabolic control by interventions in addition to the ITTP.

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SMOKING INDUCES INSULIN RESISTANCE

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The risk for cardiovascular complications is increased by smoking. It is not fully evaluated if smoking reduces the sensitivity of insulin.

7 healthy habitual smokers, age 31±2 yrs, BMI 21±0.7 kg/m^2, participated in a study of insulin sensitivity. After one night's fasting short acting insulin Actrapid 0.15 IU/kg was given subcutaneous in the region of abdomen. Every subject was investigated 3 times, randomized to smoking 1 cigarett/h, 1 amount of portion-bag-packed snuff/h or abstinence. The metabolic effect of insulin was evaluated during 6 hrs euglycaemic clamp. Glucose-turnover was studied by 3-^{3}H-glucose.

B-glucose and P-insulin were similar before and during 6 hrs clamp. B-nicotine levels were comparable during smoking as well as during snuffing, during nicotine abstinence there was undetectable levels. A reduced glucose need of 12% was found during smoking; GIR (glucose infusion rate) during smoking was 5.9±0.4 versus nicotine abstinence 6.6±0.4 mg/kg x min ($p<0.05$). During snuffing GIR was 6.6±0.4 mg/kg x min (versus nicotine abstinence, n.s.). Plasma-free fatty acids were similar in the three groups. Insulin-antagonistic hormone GH was increased during smoking (7.33±2.89 versus abstinence 2.41 ±1.02 nmol/l, $p<0.01$). We conclude that smoking induces a significant insulin resistance. Insulin-antagonistic hormone seems to be of importance.

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THE ASSESSMENT OF TOBACCO INTAKE IN YOUNG ADULTS WITH DIABETES

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Smoking is an independent risk factor for cardiovascular disease and nephropathy in diabetes and an objective smoking marker is important. Smokers vary widely in tobacco intake. Nicotine measurement in blood or urine reflects the degree of addiction and smoking load over 24 hours. We measured urine cotinine/creatinine (COT/Cr) ratios (a metabolite of nicotine) in 141 young adult smokers with diabetes (85 male, mean age 30.2 yrs., 17-45 yrs., 79% insulin dependent) and assessed the value of the smoking history (cigarettes/24 hrs.) and breath carbon monoxide measurement (C.O.) 35 patients were 'mild' smokers (COT/Cr < 3 ug/mg); 58 'moderate' smokers (COT/Cr 3-7 ug/mg) and 48 'heavy' smokers (COT/Cr > 7 ug/mg). Smoking history and breath C.O. were poor indicators of urine COT/Cr status: mild smokers, mean 14.7 (9.5, S.D.) cigarettes/24 hrs; moderate, mean 18.5 (9.5) cigarettes/24 hrs; heavy, mean 18.3 (8.1) cigarettes/24 hrs; mild smokers, mean C.O. 11.8 (6.7) p.p.m.; moderate, mean C.O. 21.6 (9.4) p.p.m.; heavy, mean C.O. 23.7 (11.9) p.p.m. 45 patients were reassessed after 12 months and COT/Cr was similar; mean 5.7 ug/mg (3.5 S.D.) v 7.5 ug/mg (6.4). Therefore 24 hour smoking history and clinic breath carbon monoxide do not detect heavy tobacco intake. Numbers of cigarettes smoked does not reflect individual differences in the inhalation of nicotine. Breath carbon monoxide only indicates cigarettes smoked in the preceeding 4 hours. Measurement of nicotine (or metabolites) in blood or urine is mandatory to study the influence of smoking on diabetic complications.

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SMOKING AND PROGRESSION OF DIABETIC NEPHROPATHY

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We have carried out a prospective, controlled, follow-up study over 1 year to investigate whether smoking is an additional risk factor for progression of nephropathy in Type 1 diabetes. 96 consecutive hypertensive Type 1 diabetic patients (62% smokers) with incipient or overt diabetic nephropathy were enrolled into the study after participation in a hypertension teaching and treatment programme to assure continuous intensified antihypertensive treatment. Two patients died during the study period. All remaining 94 patients, 36 non-smokers, 34 smokers and 24 ex-smokers were re-investigated after 12 months. Blood pressure was measured with a random-zero-sphygmomanometer in supine, sitting and standing position, metabolic control was assessed by HbA1c measurements and protein intake was calculated from 24 hour BUN urine excretion. In patients with incipient nephropathy (n=37) progression of nephropathy was defined as an increase of 24-hour-proteinuria of more than 20% of the initial value or in patients with overt nephropathy (n=57) as increase of more than 20% in serum creatinine and/or decrease of more than 20% in glomerular filtration rate. Progression of nephropathy was less common in non-smokers (n=4; 11%) than in smokers (n=18; 53%) and ex-smokers (n=8; 33%), $p<0.002$. The odds ratio for progression of nephropathy calculated for patients with and without a history of smoking and was 6.5 (95% CI 2.0-21.0). Age, gender, diabetes duration, blood pressure control, sort of antihypertensive drug treatment, protein intake and metabolic control were not different between the groups. In conclusion, in Type 1 diabetic patients with well controlled blood pressure and good metabolic control smoking represents an additional risk factor for progression of diabetic nephropathy.

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PROSPECTIVE CONTROLLED RANDOMISED TRIAL ON SMOKING CESSATION IN DIABETIC PATIENTS

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Smoking represents an additional risk factor for cardiovascular and renal complications in diabetes mellitus. We have investigated the efficacy of a smoking cessation programme based on behaviour therapy in diabetic patients. Between 1986 and 1990 2674 consecutive insulin treated diabetic patients completed a questionare about their smoking habits. 13% of Type 2 diabetic patients and 29% of Type 1 diabetic patients reported regular smoking of more than 5 cigarettes per day. 794 smoking patients (Type 1 diabetes: 79%; males: 60%) were invited to participate in a smoking cessation programmme. 89 patients (11%) (Type 1 diabetes: 84%; males: 61%) agreed to participate. These 89 patients were randomised in two groups: 44 patients took part in a behaviour therapy anti-smoking intervention consisting of 10 weekly sessions á 90 minutes supervised by a psychotherapist. 45 patients were randomised to a control group and recieved a single unstructured 15 minutes anti-smoking advise given by a physician. In this group nicotine replacement was offered to patients who reported severe nicotine addiction. Both groups were comparable with regard to age, sex, type of diabetes, diabetes duration and presence of diabetic complications. All patients were followed for 6 months. Confirmed nonsmoking was considered when a patient stated not to smoke any more and urine cotinine concentration was below 20 ng/ml. 57% of patients participated in all sessions at the behaviour therapy intervention and 70% participated in the short physician's advise. After 6 months 9 patients (9%) did not smoke, 2 (5%) from the behaviour therapy intervention group and 7 (16%) from the control group (difference statistically not significant).
In diabetic patients an extensive behaviour therapeutic intervention in smoking cessation is no more successful than an unstructured short physician's advise. In diabetic patients effective interventions in smoking cessation are lacking.

PS 27
Hypertension

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HYPERTENSION IN HEREDITARY HYPERTRIGLYCERIDEMIA AND SODIUM TRANSPORT ABNORMALITIES

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Diabetes and hypertension are common chronic diseases that frequently coexist and are significant risk factors of cardiovascular morbidity and mortality. Both diabetes and hypertension are characterized by multiple metabolic abnormalities and the alterations of ion transport are one from those. Hereditary hypertriglyceridemic (HTG) rats were used to characterize them as a new model of hypertension. Principal Na^+ transport pathways in erythrocytes and intracellular Na^+ content were also measured. Direct mean arterial pressure (MAP) measurement revealed that HTG rats are hypertensive in comparison with Wistar control rats (MAP 132±2 vs 106±2 mm Hg, $p<0.001$). In addition the high-carbohydrate diet increased MAP in both HTG males and females about 8 mm Hg. There was a significant correlation between MAP and plasma triglyceride concentration ($r = 0.523$, $n = 40$, $p<0.001$). The intracellular sodium content was significantly increased in erythrocytes of HTG rats compare to controls (3.65±0.11 vs 2.95±0.05 mmol/l cells, $p<0.01$). All principal pathways for Na transport were significantly increased in erythrocytes of HTG rats. We concluded that this new genetic model could be used for hypertensive research and the alterations of ion transport may play a role in blood pressure rise. The relation between hypertension and diabetes and analysis of disturbances in glucose and lipid metabolism in this rats are in progress in our laboratory.

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BRADYKININ-STIMULATED INTRACELLULAR CYTOSOLIC FREE CALCIUM IS HIGHER IN CULTURED FIBROBLASTS FROM NON INSULIN-DEPENDENT DIABETICS WITH HYPERTENSION AND INSULIN RESISTANCE.

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Intracellular cytosolic free-Ca^{2+} [Ca_i^{2+}] in large populations is positively correlated with blood pressure. Increased [Ca_i^{2+}] my also cause insulin resistance. It has been postulated that hyperinsulinemia (e.g. insulin resistance) leads to hypertension. Conversely altered [Ca_i^{2+}] rather than hyperinsulinemia may underlie the association between hypertension and insulin resistance. We investigated the baseline and bradykinin-stimulated (a growth factor which stimulates [Ca_i^{2+}] via protein-kinase C) [Ca_i^{2+}] in cultured fibroblasts after 5-7 passages from skin biopsies in 5 controls (C), 5 non-insulin-dependent diabetics without hypertension and with normal whole body glucose uptake (Rd) during euglycemic hyperinsulinemic (100 µU/ml) clamp (D_1) and 5 non-insulin-dependent diabetics with hypertension (>145/90 mmHg) and with impaired RD (D_2) (Rd: Cvs D_1 vs D_2: 7.9±1.1 vs 7.8±0.8 vs 5.1±0.55, $p<0.05$ and $p<0.05$, Mean ± SD). [Ca_i^{2+}] was measured with the fluorescent indicator fura-2 using a Perkin-Elmer LS5 spectrofluorimeter equipped with a thermostatically controlled cuvette holder. Baseline [Ca_i^{2+}] was similar in C, D_1 and D_2 (108±16 vs 88±22 vs 130±32 nM, Mean ± SD). Bradykinin-stimulated [Ca_i^{2+}] was lower in C and D_1 than in D_2 (327±58 vs 252±130 vs 406±89, $p<0.05$ and $p<0.05$, respectively). Conclusions: Our results demonstrate that [Ca_i^{2+}] is higher in cultured fibroblasts from non-insulin-dependent diabetics with hypertension and insulin resistance after bradykinin stimulation.

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RELATIONSHIPS AMONG RENAL FUNCTION, HYPERTENSION AND SODIUM HOMEOSTASIS IN NON-INSULIN-DEPENDENT DIABETES.

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Little information is available on the relationships among microalbuminuria, hypertension, insulin-resistance (e.g.hyperinsulinemia) and sodium homeostasis in non-insulin-dependent diabetes. We investigated glomerular filtration (GFR); extracellular fluid volume (EFV), using multiexponential analysis of ^{51}Cr-EDTA plasma decay after intravenous injection and sodium excretion rate (SER). 52 patients on antidiabetic oral agents, 13 normotensives (≤140≤85 mmHg), normoalbuminurics (<15 mg/min)(D), 17 hypertensives (≥145≥90 mmHg), normoalbuminurics (DH); 13 microalbuminuric (>20 µg/min)(DM) with normal serum creatinine (≤1.4 mg/dl) and 9 microalbuminurics (DN), with altered serum creatinine >1.6 mg/dl); 13 healthy subjects served as controls (C). GFR was higher in DM and lower in DN but not in D and DH patients than in C (DN vs DM vs DH vs D vs C: 58 ± 7, p<0.01, 123±8, p<0.05, vs 107±10, n.s., vs 108±7, n.s., vs 102±5 ml·1,73 m^{-2}). EFV was larger in DN, DM and DH but not in D patients than in C (DN vs DM vs DH vs D vs C: 14.4±0.8, p<0.05, vs 15.2±0.6, p<0.01, vs 15.2±0.6, p<0.01, vs 13.6±0.3, n.s., vs 12.6±0.911·1,73 m^{-2}) (Mean ± SE). On the contrary SER was lower in DN, DM and DH but not in D patients than in C (DN vs DM vs DH vs D vs C: 100±10, p<0.05, vs 101±11, p<0.05, vs 90±8, p<0.05, vs 123±11, n.s., vs 119±10 mmol·day^{-1}·1,73 m^{-2}). Glucose filtered load was increased in all patients, whereas baseline insulin levels were higher in DN, DM and DH but not in D than in C. SER was negatively related to glucose filtered load and to insulin levels. Conclusion: 1) Hyperglycemia, via high glucose filtered load, leading to sodium reabsorption, causes sodium retention in all patients. 2) Impaired natriuresis, higher EFV and GFR, hypertension and microalbuminuria were observed only in patients with hyperinsulinemia. Thus hyperinsulinemia (e.g. insulin-resistance) determines sodium retention in a cohort of patients, further deteriorating the antinatriuretic effect of hyperglycemia, which operates in all diabetics.

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INCREASED VENOUS WALL STIFFNESS IN TYPE I DIABETES WITH AND WITHOUT MICROALBUMINURIA.

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The venous system plays an important role in volume and blood pressure homeostasis. In essential hypertension reduced venous compliance may be an initiating factor in the development of hypertension. We studied venous distensibility (VDIST) and the effect of nitroglycerin (NTG,0.4 mg sl.) on resting venous tone in normotensive type I patients with and without microalbuminuria. Patients: 6 females/9 males; mean age: 34.3±2.6 (SEM), duration of diabetes: 18.2±2.2 yrs.; fasting blood glucose: 13.1±1.5 mmol/l; HbA1c: 8.3±0.4 %. Seven patients had microalbuminuria. Healthy controls: 5 females/9 males; mean age: 35.9±2.1 yrs. Methods: forearm volume (strain gauge plethysmography) and i.v. pressure changes were determined during externally applied pressure (25, 30, 35, 40, 50 mmHg). The slope of the delta-pressure/delta-volume curve is a measure for forearm VDIST. Results: VDIST was lower in patients vs controls (slope: 0.049 ±0.002 vs 0.059±0.003, P=0.02). VDIST was similar in patients with and without microalbuminuria. No differences were found in the effects of NTG. Conclusions: VDIST is decreased in long-term normotensive type I diabetic patients. As the effect of NTG on venous tone was similar in patients and controls the decreased VDIST is probably due to increased vascular stiffness. VDIST was similarly decreased in normo- and microalbuminuric patients, therefore, changes in venous stiffness do not play an important role in the development of hypertension in patients with microalbuminuria.

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METABOLIC RESPONSE TO INTRAVENOUS GLUCOSE IN ESSENTIAL HYPERTENSION

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Abnormalities of CHO metabolism frequently occur in hypertensive patients. Aim of this study was to evaluate the metabolic response to intravenous glucose (glucose 33%, 0.33 g/kg). in healthy and hypertensive subjects. 12 young, nonobese, patients with essential hypertension and 7 normal subjects entered the study. In hypertensive *vs* control subjects we observed: **(1)** a lower *k* of glucose utilization (1.24±0.07 *vs* 1.51±0.13, p<0.05); **(2)** higher plasma glucose (PG) values at 30 (10.7±0.4 vs 8.5±0.6, p<0.01); 40 (9.1±0.4 vs 7.2±0.8, p<0.04) and 50 min times (7.8±0.4 vs 6.9±0.8 mM, p< 0.05); **(3)** lower plasma values of insulin (IRI) at 3 (266±44 vs 398±60, p< 0.02) and 8 min. (211±30 vs 301±42 pM, p< 0.04); **(4)** lower C-peptide (CPR) plasma values at 3 min. (1.5±0.1 vs 2.3±0.1 nM, p< 0.05); **(5)** higher PG incremental areas (321±20 *vs* 286±16 mM x min.-1 x 60 min); lower IRI areas by considering the first phase of insulin release (0-10: 1435±216 *vs* 2382±402 pM x min. -1x 60 min, p<0.04) and unmodified by considering the whole curve; lower CPR areas by considering both the first phase and whole curve (0-10: 8.4±0.9 *vs* 12.8±1, p< 0.01 and 0-60: 43.5±4 *vs* 61±7, nM x min.-1 x 60 min, p<0.05); **(6)** the presence in hypertensive subjects of higher glucose and lower plasma insulin values resulted in an higher glucose/insulin ratio at 20, 30 and 40 min. times; **(7)** the CPR to insulin molar ratio was unmodified. Thus, in hypertensive subjects we studied both glucose tolerance and insulin release were impaired. Furthermore, the impairment of insulin release was more evident during the first phase of insulin release.

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THE RELATION BETWEEN CARDIOVASCULAR RISK FACTORS AND THE EVOLUTION OF GLUCOSE TOLERANCE

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The aim of this study is to analyze the relation between hypertension, obesity and the evolution of impaired glucose tolerance. The data of 209 subjects with an initial impaired glucose tolerance were used. The tolerance test was repeated at 5 years and the patients grouped according to its evolution in the following groups (1) normalization, (2) maintenance of the impaired tolerance and (3) diabetes mellitus. We compared the incidence of hypertension and obesity in each group. Hypertension was present in 25.27% of the subjects in group (1), 22.27% in group (2) and 53.13% in group (3). Great obesity (ponderal index >35 kg/m2) was found in 5.49% of the subjects in group (1), 13.63% in group (2) and 10.94% in group (3). Of the subjects with hypertension who evolved toward diabetes 11.7o% had important obesity whereas of those who had a normal or impaired glucose tolerance at 5 years only 5.88 had an important obesity. It appears that hypertension is twice as frequent in the subjects developing in the short time (5 years) diabetes as compared with those whose glucose tolerance normalizes or remains impaired. Obesity is more frequent in the subjects who develop diabetes or have constantly an impaired glucose tolerance. This 3 factors may have a common pathogenic mechanism.

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CONTROL OF BLOOD PRESSURE IN PERSONS WITH DIABETES: A POPULATION BASED STUDY IN A RURAL AREA OF AUSTRIA.

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Control of hypertension is particularly important in diabetes. However, data on the quality of hypertenison care in diabetes are lacking. Objective: audit of detection, treatment, and control of hypertension in diabetes in a geographically defined rural area with 7871 inhabitants. Methods: Using a mobile ambulance 95% of all known diabetic patients (age 66±13 years, diabetes duration 8±7 years) were examined, and blood pressure (BP) was measured twice in the sitting position by a nurse; the mean of the two measurements was used for analysis. Results: 51% of patients had hypertensive BP (≥160 and/or≥95 mmHg); prevalence of known hypertension was 54%; a further 15% with previously unknown hypertension had hypertensive BP; 10% had a BP of ≥200 and/or≥115 mmHg. Of the 202 patients with known hypertension 22% were not treated with antihypertensive drugs, 46% were on drug treatment, but their BP was uncontrolled, 32% had their BP controlled. Use of ACE-inhibitors was associated with worse BP control (182±25/91±12 mmHg, n=30, versus 167±24/89±13 mmHg, n=127, $p < 0.01$). Conclusions: In this survey only a low percentage of diabetic patients had their blood pressure controlled despite high degrees of hypertension detection and treatment. There appears an urgent need for the implementation of programmes to improve patient compliance to recommended antihypertensive treatment.

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AMBULATORY BLOOD PRESSURE IN TYPE 1, INSULIN-DEPENDENT DIABETIC SUBJECTS WITH MICROALBUMINURIA.

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To evaluate the interest of Ambulatory Blood Pressure (ABP) in incipent diabetic nephropathy, we compared ABP measurements of 10 type 1, insulin-dependent diabetic subjects with microalbuminuria (30-300 mg/24h, group A), to those of 29 normoalbuminuric ones (group B). Blood pressure was measured every 15 min from 7:00 a.m. to 10:00 p.m. (diurnal) and every 30 min from 10:00 p.m. to 7:00 a.m. (nocturnal) (Spacelabs 90202®). Groups were comparable for age (36±17 vs 42±15 years), sex (4F/6M vs 7F/22M), HbA1c (8.4±0.1 vs 8.1±0.3%), and diabetes duration (16±5 vs 12±8 years). No subject had orthostatic hypotension, or took anti-hypertensive drug. Diurnal pressures (Systolic/Mean/Diastolic Blood Pressure : SBP/MBP/DBP) were not different between the 2 groups (131±12 / 96±11 / 84±10 vs 124±10 / 90±8 / 79±7 mmHg ; ns), while nocturnal pressures were higher in group A (125±13 / 87±11 / 74±10 vs 112±12 / 78+9 / 67±8 mmHg ; $p = 0.008$ / $p = 0.01$ / $p = 0.03$). The nocturnal declines of MBP (-9±9 vs -12±5 mmHg) and DBP (-10±7 vs -12±5 mmHg) were similar, but SBP declined less in group A (-6±11 mmHg), than in group B (-12±6 mmHg ; $p<0.03$). Diurnal pulse rate was comparable between groups (84±11 vs 79±12 beats/min), and it declined similarly during night (-11±4 vs -13±6 beats/min). In conclusion, the reduced decline of SBP seems the most discriminant parameter of ABP between type 1, insulin-dependent diabetic subjects with, or without incipient nephropathy.

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DIABETIC RENAL AND RETINAL MICROVASCULAR DISEASE: ARE ALL ANTIHYPERTENSIVE AGENTS EQUAL?

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The effects of antihypertensive agents on retinal albumin permeability and albuminuria were assessed in an experimental model combining diabetes with genetic hypertension. Streptozotocin diabetes was induced in spontaneously hypertensive rats (SHR) aged 8 weeks. Rats were randomised to receive no treatment, the ACE inhibitor, perindopril [PER], triple therapy [TT: hydralazine, reserpine and hydrochlorothiazide] or the calcium antagonist, lacidipine [LAC]. After 16 weeks, retinal albumin permeation [double isotope technique], blood pressure, glycated haemoglobin and albuminuria [radioimmunoassay] were measured in the 4 groups [n=8-12/group]. All treatments reduced blood pressure to an equal degree [untreated, 176±5; PER, 129±3; TT, 126±4; LAC, 130±3mmHg], did not influence glycated haemoglobin and reduced retinal albumin permeation to a similar extent [untreated, 184±31; PER, 110±6; TT, 88±25; LAC, 61±6% control non-diabetic SHR]. In contrast, perindopril and triple therapy were more effective than lacidipine in retarding albuminuria in diabetic SHR [untreated, 28.8x/÷1.2; PER, 5.3x/÷1.2*†; TT, 6.9x/÷1.2*†; LAC, 17.8x/÷1.2mg/24hrs; *$p<0.01$ vs untreated, †$p<0.01$ vs LAC]. It is concluded that the protective effect of these agents may be both drug and organ specific.

PS 28
Nutrition

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NUTRITIONAL INTAKE IN IDDM PATIENTS. FIRST RESULTS FROM THE EURODIAB IDDM COMPLICATIONS STUDY.
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One goal of the EURODIAB IDDM Complications Study is to define regional variations in the environmental and personal risk factors for diabetic complications. Different patterns of nutritional intake could help to explain regional variations in the prevalence of diabetic complications. Therefore nutritional intake was analyzed centrally using 3 day dietary records in the IDDM population samples of 31 participating centres. The following results were obtained from Athens, Bukarest, Cork, Düsseldorf, Gent, Helsinki, Leiden, Lisbon, Luxemburg, Milan, Paris/Valenciennes, Pisa, Vienna and Zagreb who completed a sample size of 100 IDDM patients/centre in 1991: (intake $\bar{x}$/14 centres) 2346±602 kcal (18% protein, 39% fat, 42% carbohydrate, 1% alcohol), 18±7g fibre, 16% of total energy as saturated fat, 70% of total protein as animal protein. Marked differences were seen between centres: (intake $\bar{x}$/centre) mean protein intake ranged from 94±31 to 118±30g, fat from 86±28 to 121±39g, carbohydrate from 198±65 to 309±102g, fibre from 13±3 to 22±8g, saturated fat from 13 to 21% of total energy and animal protein from 62 to 75% of total protein. In many aspects the nutritional intake of IDDM patients in the European centres does not meet the recommendations, however, it could be shown that regional variations exist which can now be related to regional variations in the prevalence of diabetic complications e.g. nephropathy or macroangiopathy.

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COMPUTER SIMULATION OF GLUCOSE LEVELS FOLLOWING EXERCISE AND CHANGES IN DIET.
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We previously reported a computer simulator which allows diabetic patients to practice adjusting insulin doses, and predicts resulting blood glucose (BG) changes. Our aim was to extend the program to incorporate BG changes following exercise alone, or with compensatory adjustments in diet and insulin dosage. The program's parameters were adjusted to match 5 specialists' BG predictions following exercise ± dietary or insulin dose adjustments in 7 hypothetical patient cases. The program's responses were then tested against 18 other specialists in Australia using 7 different cases. In the developed program, mild or moderate exercise can be simulated starting 2 hours after meals. Multiple insulin regimens are possible and diet can be altered in 15g carbohydrate portions at 7 different times of day. Following adjustment, the program's predicted change in 54 BG levels (7 cases) correlated well with the corresponding mean predictions of the 5 specialists ($r=0.98$; slope=0.86; $p=0.0001$). The analogous correlation for the 18 other specialists was $r=0.97$; slope=1.17; $p=0.0001$. All but one of the program's BG predictions were within one standard deviation of the corresponding mean of the 18 specialists. We conclude that it is possible to successfully simulate specialists' predictions of BG levels following exercise and dietary changes.

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POCKET COMPUTER SYSTEM FOR DIETARY PLANNING AND GUIDANCE FOR DIABETICS AND OBESE.
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For a patient-oriented 14,9 x 9,4 x 2,6 cm pocket computer system (Diacomp) with a working memory of 314 kB, CPU-intern real time clock and 4 lines display a program for dietary planning and guidance for diabetics and obese was developed in Turbo Pascal 6.0. For different caloric categories 30 breakfasts, 60 lunches and 30 dinners may be chosen. By modular structure of day-sections the meals of different sections may be freely combined resulting in 54000 day combinations with identical carbohydrate and energy distribution during the day. To motivate the patient for optimal nutrition the computer memorizes which dishes were selected and balances the intake of following nutrients: cholesterol, total fat, portion of saturated, mono- and polyunsaturated fatty acids, protein, fibre, iodine, iron, vitamin E. These targets are optimized by a guidance system. It counterregulates deficiences and overloads weighing the targets and ranking all dishes of the next meal section according to their priority. The efficiency of this guidance system was tested by simulation studies. If dishes of first choice were eaten (e.g. jelly + joghurt, pork + beans, corned beef meal) the priority is changed after 6 days to dishes with higher iodine content. In conclusion an optimal nutrition of diabetics may be achieved at broad variability and easy use.

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ASSESSMENT OF PHYSICAL ACTIVITY IN TYPE II DIABETIC PATIENTS: A COMPARISON OF METHODS.
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A sedentary life style is thought to represent a risk factor for type II Diabetes and cardiovascular disease. Reliable techniques for measuring everday physical activity are necessary prerequisites for patient counselling and research. We compared a questionnaire (q), a 7 day activity protocol (ap) and wearing a motion recorder (mr; Kenz Calorie Counter, Suzuken Co, Japan) in 39 type II diabetic patients (20 females & 19 males, age 58 (9) years (mean (SD)), diabetes duration 14 (10) years, HbA_{1c} 9.3 (2.3) %, BMI 27.9 (4.6) kg/m^2). Initial testing of the motionrecorders demonstrated a 4 % variance between instruments. Patients filled out the activity questionnaire, then after a one day run-in period they wore the motion recorder and filled out the activity protocol for 7 consecutive days in their everday environment. Daily physical activity as assessed by questionnaire was 5 (3-6) hrs/day (median (interquartile range)). Activity as noted in the activity protocol summed to 16.2 (11.9-20.4) kcal/kg body weight/day. Activity as measured by the motionrecorder was 4.4 (2.2-9.1) arbitrary units/day. Spearman rank correlation coefficient between q vs. ap was 0.34 ($p<0.05$), between q vs. mr 0.45 ($p<0.005$), and between ap vs. mr 0.59 ($p<0.001$). We recommend motion recorders as reliable and easy to use instruments for measuring physical activity. Questionnaires are a cheap and simple alternative. In contrast, activity protocols are cumbersome and often difficult to interpret.

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DEVELOPMENT OF A BARCODE SYSTEM FOR NUTRIENT ANALYSIS IN THE U.K.

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Dietitians need to assess the diets of newly-diagnosed patients and to monitor the affect of dietary education. The aim of the current study is to develop a reliable time-saving accurate tool for dietary assessment. A new approach to dietary assessment is the Food Meter System originally developed in Italy. This system comprises a credit card sized barcode reader (with memory for 28 days food records) and a booklet of barcodes for the most commonly consumed foods and for the weights eaten. Food intake data are recorded by scanning the appropriate barcodes and downloaded directly to a computer analysis programme. In order to develop a British barcode booklet 280 of the most commonly eaten British foods were identified from a study of British adulrs in which 244 foods accounted for 84% of foods consumed by 112 subjects over 4 days. A further 34 foods were identified as frequently eaten following discussions with local dietitians. Six foods were unnamed to allow extra entries to be made. The nutrient analyses were derived from The Composition of Foods. Food portion weights ("average", "small", "medium", "large") were also incorporated into the Booklet. It is concluded that the studies currently being undertaken to assess accuracy, time-saving aspects and reliability of the U.K. Food Meter System will provide data on its feasibility for use in the U.K.

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Dietary Evaluation in Type II Diabetic Subjects Randomly Allocated to Insulin, Sulphonylurea or Diet Therapy

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132 patients (120 Caucasian, 12 Asian) from the UK Prospective Diabetes Study (UKPDS) with Type II diabetes of 3 to 6 years duration, stratified for gender, obesity and allocated therapy, were selected at random from five of the 23 participating centres. Each subject was asked to complete a three day food diary which was then checked at their subsequent clinic visit by a UKPDS dietitian. Completed diaries were analysed using a computer based version of McCance & Widdowson's tables (NIBBLES). 83% (111)of the diaries were returned of which 97% (108) were satisfactorily completed.Mean values for these 108 patients were age: 55.1 y (SD 7.7), HbA1c: 7.1 % (SD 1.5), total cholesterol: 5.7 mmol/l (SD 1.1) and BMI: 27.9 kg m^{-2} (SD 4.3). Dietary analysis gave a mean admitted energy intake of 1650 kcal (SD 424) with protein 21 % (SD 4), carbohydrate 43 % (SD 7), fat 37 % (SD 7), fibre 22 g (SD 9) and p/s ratio 0.62 (SD 0.49). No therapy related or regional differences were observed in admitted macronutrient intakes. Asian subjects derived fewer calories from protein (13.7 *v* 21.3 %, $p<0.0001$) and more from fat (46.4*v* 37.2 %, $p<0.01$). Patients receiving structured dietary advice in UKPDS clinics have similar dietary intakes despite different therapeutic allocations and regional locations. The average admitted dietary intake is closer to the current BDA recommendations than the usual UK diet.

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NUTRITIONAL STATUS AND LIPID,APOLIPOPROTEIN AND Lp(a) LIPOPROTEIN PROFILES IN DIABETICS ON CHRONIC HAEMODIALYSIS (HD).

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Nutritional assessment has not been clearly defined in diabetic (D) patients on HD. Nutritional status and lipid profile of 14 D patients (10 males, 4 females) on HD were compared to a group of 17 non-diabetic (ND) patients (11 males, 6 females) well matched for age and duration of HD. Protein intake was higher in D than in ND patients on HD (1.16±1.0 vs 0.87±0.07 g/kg/d; $p<0.01$)while caloric intake was not different. Vitamin B_{12} was lower (824±88 vs 1636±126 pg/ml; $p<0.05$) and plasma carnitine (PC) higher (71.2±6.7 vs 62.1±7 mmol/L; $p<0.05$) in D patients. PC was correlated with BMI ($p<0.01$) and arm circumference($p<0.05$). Folic acid, transferrin, C_3, C_4 were similar in both groups. The valine/glycine ratio (0.95±0.05 vs 0.65±0.07; $p<0.01$) and plasma levels of isoleucine, proline were higher in D patients. Triglyceride levels were higher ($p<0.05$) and apolipoprotein A_1 lower ($p<0.05$) in D patients. Lp(a) concentration was similar in both groups. In conclusion diabetics on HD are in better nutritional status but present more lipoproteins abnormalities compared to the non diabetics. Plasma carnitine and transferrin are good predictors of nutritional status in diabetics on HD.

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DIETARY FIBRE PREVENTS THE UNTOWARD EFFECTS OF A HIGH CARBOHYDRATE DIET ON GLUCOSE METABOLISM AND ON PERIPHERAL INSULIN SENSITIVITY.

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A high carbohydrate-low fibre diet increases plasma glucose, insulin, triglycerides and insulin resistance in type 2 diabetic patients. However, these deleterious effects might be reversed if dietary fibre is also increased. To test this hypothesis 8 type 2 diabetic patients (7M/1F) (age 56.1±10.5 yrs, BMI 25.2± 3.1kg/m2, M±SD) treated with diet alone or diet + glibenclamide, were randomly assigned for 15 days to either a high carbohydrate-high fibre (carbohydrate 60%, fat 20%, fibre 54g/d) or a low carbohydrate-low fibre diet (carbohydrate 40%, fat 40%, fibre 16g/d) and then were crossed-over to the other diet for 15 more days. Diets were similar for all other components. The dosage of hypoglycemic drugs was mantained constant throughout the study, performed in a metabolic ward. The high carbohydrate-high fibre diet significantly decreased postprandial plasma glucose (209±91 vs 242±74 mg/dl,$p<0.05$) while no difference between the diets was observed in postprandial insulin (25±8 vs 41±32 mU/L) and insulin mediated glucose disposal (4.72±1.14 vs 4.22±0.45 mg/kg/min). However the high carbohydrate-high fibre diet significantly increased plasma triglycerides (178±83 vs 120± 56 mg/dl $p<0.03$) and significantly decreased HDL-cholesterol (31.2±5.7 vs 34.6±4.6 mg/dl, $p<0.04$) while plasma total cholesterol remained unchanged (181±38 vs 180±49 mg/dl). In conclusion dietary fibre can prevent the untoward effects of a high carbohydrate diet on glucose metabolism but not on plasma triglycerides and HDL.

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EFFECTS OF DIFFERENT MEAL FREQUENCIES ON GLUCOSE AND INSULIN RESPONSE IN NON-INSULIN-DEPENDENT DIABETICS.

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To study the effects of different meal frequencies on glucose, insulin and free-fatty-acids (FFA) responses, 12 non-insulin dependent diabetics took two isocaloric diets, with the same composition on two separate days. The patients were randomized to study A (two identical, large meals in eight hours) or study B (six identical, smaller meals over eight hours) first. During the first four hours the blood glucose response areas (A: 475.5 ± 70.2 vs B: 327.8 ± 67.9 mmol/l x 240 minutes; 2p< 0.02) and the serum insulin response areas (A: 36062 ± 5943 vs B: 23290 ± 4442 pmol/l x 240 minutes; 2p< 0.02) were significantly lower in study B than in study A. No difference was found between blood glucose response areas during the entire eight hour observation period, whereas the insulin response areas were still significantly lower in study B as compared with study A (53321 ± 9702 vs 85595 ± 19833 pmol/l x 480 minutes; 2p<0.03). Mean FFA for the entire eight hour observation period was lower in study B than in study A (2p< 0.02). The present results demonstrate that a higher mealfrequency decreases glucose and subdues blood glucose excursions in the morning, and reduces insulin levels throughout the day in NIDDM.

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Effects of different hypocaloric diets on insulin sensitivity and oxidative glucose disposal in obesity.

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The aim of the study was to investigate the effects of two hypocaloric (800 cal) diets on body weight reduction, insulin sensitivity and proteolysis in 11 normal-glucose tolerant obese women. The diets had the following composition: (A;6 subjects) 60% complex carbohydrate (C-CHO) and 20% protein, (B;5 subjects) 45% protein (P) and 35% CHO. Before and after 21 days of diet, an euglycaemic hyperinsulinaemic (25 mU/kg/h) clamp and indirect calorimetry lasting 150 min were performed. Both diets induced similar decrease in body weight and FFM and an increase in fasting Fat Oxidation (FO). Blood glucose and insulin levels remained unchanged in both diets, while FFA levels increased by 18% only in A ($p<0.05$). During clamp period, both M-value (3.2±0.2 vs 2.5±0.2 mg/kg.FFM/min, $p<0.05$) and Glucose Oxidation (GO: 2.2±0.1 vs 1.7±0.2 mg/kg.FFM/min, $p<0.05$) decreased by 20% in A , while M-value remained unchanged and GO increased by 10% in B. FO was less inhibited in A ($p<0.05$ vs B). 3-Methil-istidine excretion was decreased by 10% in A and 45% in B ($p<0.05$). Conclusions: 1) hypocaloric C-CHO diet induces a state of insulin resistance decreasing the GO disposal, while P diet seems to show the opposite results. 2) Proteolysis is decreased more during P diet.

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VITAMIN E SUPPLEMENTATION IMPROVES THE VITAMIN E STATUS IN NON-INSULIN-DEPENDENT DIABETICS AND REDUCES IN VIVO LIPID OXIDATION

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Free radical (FR) oxidation of LDL may increase its atherogenicity. Vitamins C (Vit C) and E (Vit E), are FR scavenging antioxidants. As the major lipid soluble antioxidant, Vit E status is better assessed by Vit E: lipid concentration ratio (Vit E : cholesterol (Ch) + Triglyceride (TG)) than absolute concentrations. We investigated the effect of Vit E and Vit C supplementation on LDL oxidation. Vit C (1g) and Vit E (300mg) were taken for three weeks (with an intervening 3 week washout) by nine Type II diabetics and ten healthy controls. In-vivo oxidation was assessed by Thiobarbituric Acid Reactive Substances (TBARS), susceptibility to oxidation by the time lag (LAG) before development of di-ene conjugated species during copper stimulated LDL oxidation in-vitro. Vit E : (Ch + TG) was significantly lower in diabetics than non-diabetics at baseline [Mean (SD) 2.1 (0.4) vs 2.5 (0.4); p=0.03]. Vit C supplementation was without effect. With Vit E supplementation Vit E : (Ch + TG) rose significantly in diabetics [Mean rise (SD): 0.8 (0.5); p=0.01] and non-diabetics [0.9 (0.6); p=0.03] TBARS fell significantly in the group as a whole [2.1 (0.3) $\mu mol.l^{-1}$ vs 2.3 (0.5); p=0.05]. Similar non-significant changes occured within the sub-groups. [Diabetics: Mean fall (SD) 0.2 (0.5) $\mu mol.l^{-1}$; p=0.3. Non-diabetics: 0.2 (0.4) $\mu mol.l^{-1}$; p=0.11] LAG was not significantly altered. No correlation between Vit E: (Ch+TG) and TBARS or LAG at baseline nor between changes in these factors existed. Vit E status is impaired in diabetes exposing lipids to greater risk of oxidation. Vit E status can be improved by supplementation. Vit E not Vit C supplementation reduces in-vivo oxidation.

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FISH INTAKE AND DEATH OF CORONARY HEART DISEASE IN NORMOGLYCEMIC AND GLUCOSE INTOLERANT SUBJECTS

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The association between habitual fish intake and 17-year mortality of coronary heart disease was compared between normoglycemic and glucose intolerant elderly subjects. In 1971, 272 men and women aged 64-87 yr were examined in a general practice in the town of Rotterdam, The Netherlands. Glucose intolerance (impaired glucose tolerance or diabetes mellitus diagnosed from a 50 g glucose tolerance test) was observed in 83 subjects, and 189 subjects were found to be normoglycemic and free of clinically diagnosed diabetes mellitus. Information on usual dietary intake was obtained using the cross-check dietary history method. The vital status of the subjects was verified in 1988. Habitual fish use was found in 58.7% of the normoglycemic subjects, and among 62.4% of the glucose intolerant subjects. Among normoglycemic subjects, the age and sex adjusted 17-year mortality of coronary heart disease amounted to 10.9/1000 person-years for fish users, and to 25.1/1000 person-years for non-fish users. Adjusted for differences in age and sex by survival analysis, the Risk Ratio (RR) was 0.44 (p=0.017). For the glucose intolerant population the coronary heart disease death rates rates amounted to 20.6/1000 and to 31.2/1000 respectively. Adjusted for age and sex a RR of 0.78 (p=0.57) was observed. After adjustments for other confounders such as body mass index, smoking, alcohol use, and intake of energy, poly-unsaturated fat and carbohydrates, the RR for normoglycemic subjects was 0.34 (95%-confidence interval (CI): 0.16-0.72). For glucose intolerant subjects an adjusted RR of 0.80 (95%-CI:0.31-2.05) was observed. Although the difference in RR's between the two sub-populations was not statistically significant (p=0.19), these results suggest that fish intake is inversely associated with coronary heart disease death in a normoglycemic population, but not in a population of glucose intolerant subjects.

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EFFECT OF VITAMIN C ON THE ALTERED FREE RADICAL DEFENCE MECHANISM IN LIVER AND KIDNEY OF DIABETIC RATS.

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In the present study we have examined the effect of vitamin C (ascorbic acid) on the altered oxygen-radical related processes seen in short-term (4 week) diabetes induced in rats by streptozocin. The activities of superoxide dismutase (SOD), catalase (CAT), glutathione perodidase (GSH-PX) and glutathione reductase (GSSG-RD) were assayed in liver and kidney of control, diabetic and vitamin C treated diabetic rats, together with measurements of levels of key enzymes and metabolites of glucose utilization involved in $NADP^+$/NADH redox mechanisms. Diabetes was associated with decreased activities of SOD(-20%), CAT(-55%), GSH-PX(-36%), malic enzyme (-61%) and the levels of glutathione (-30%) and glucose 6-phosphate (-26%) in liver. Vitamin C treatment, in the dose of 200 mg/kg/day in drinking water, for 4 weeks, resulted in the full recovery of SOD activity (to 95% of control value) and in the significant increase in the activities of CAT and malic enzyme (by 60% and 37%, respectively). In kidney, -32% decrease in SOD and -48% decrease in CAT activities were observed in diabetes. These changes were not effected by vitamin C treatment. Our results support the previous findings of increased oxidative stress in uncontrolled diabetes shown by marked alterations in tissue antioxidant enzyme activities, and they show that vitamin C treatment could be beneficial to the delay or the prevention of chronic complications occurring in diabetes.

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NUTRITIONAL REGULATION OF PYRUVATE DEHYDROGENASE EXPRESSION IN RAT ADIPOSE TISSUE AT WEANING.

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We have shown that pyruvate dehydrogenase (PDH) activity in rat adipocytes is low during the suckling period and increases after weaning onto a high-carbohydrate diet. In the present study, we have quantified, in rat white adipose tissue during the suckling-weaning transition, the PDH complex concentration in isolated mitochondria, using a rabbit polyclonal antiserum raised against the 4 subunits of pig heart PDH complex. We have compared 15 day-old suckling rats and 30 day-old rats weaned at day 19. Weaning onto a high-carbohydrate diet induced a 18-fold increase in the E1α subunit concentration (the target of regulatory phosphorylation) of the PDH complex, whereas the concentration of other subunits was less significantly affected. A significant increase of E1α subunit was already seen after 48h of weaning. This increase was precluded by weaning onto a high-fat diet. Weaning on a high-carbohydrate diet induces a 11-fold increase in the mRNA concentration of the E1α subunit as analysed by Northern-blot hybridized with a 1.6 kb cDNA of human E1α subunit (B.H. Robinson, Toronto). It is concluded that in rat adipose tissue, the expression of the E1α subunit of PDH complex is under nutritional control and that this involves a pretranslational mechanism.

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IINCREASED POST-PRANDIAL CHOLESTEROL SYNTHESIS IN DIABETIC PATIENTS WITH CORONARY ARTERY DISEASE.

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Vascular disease is a common complication of diabetes. This study examines the relationship between endogenous cholesterol synthesis, lipoprotein (a) [Lp(a)] levels and atherosclerotic coronary artery disease (CAD). Five Type 2 diabetic patients with angiographically normal coronary arteries and ten with atheromatous vessels were examined fasting and 4 hours after a 1300 k.cal. high fat meal . They were matched 1:2 for age, sex and BMI. Cholesterol synthesis was measured by [^{14}C]-acetate incorporation into mononuclear leucocyte cholesterol and Lp(a) by an Elisa method. There was no significant difference in serum cholesterol (Mean ± SE 5.4±0.54 mmol/l in patients without CAD and 6.5±1.1mmol/l in CAD patients $p>0.05$)High density lipoprotein, triglycerides and haemoglobin A_1 were also similar in both groups. Cholesterol synthesis increased post-prandially in both groups but diabetic patients with CAD had a significantly greater increase compared to those with no CAD (48% vs 28%) ($p<0.05$ using paired t Test). Median fasting Lp(a) values of 6.0mg/dl, range 0.4-91.5 mg/d for diabetics with no CAD 1(40% > 30mg/dl) and 38.35 mg/dl, range 0.2-67.6 mg/dl in CAD (60%>30mg/dl) did not change post-prandially. The study demonstrates a decreased ability of diabetic patients with CAD to down-regulate cellular cholesterol synthesis after a meal. This abnormality may contribute to the development of CAD in diabetic patients.

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CHOLESTEROL METABOLISM IN TYPE 1 DIABETIC WOMEN; THE INFLUENCE OF THE MENSTRUAL CYCLE

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Oestrogen may be the protective against atherosclerosis in the pre-menopausal, non-diabetic woman. This protection is lost in diabetes. Our aim was to examine the effect of the menstrual cycle on cholesterol metabolism in Type 1 diabetic patients. Nine female, non-diabetic and 9 age-matched Type 1 diabetic, pre-menopausal women, with regular cycles were compared. Fasting blood samples were taken weekly for 4 consecutive weeks. Cellular cholesterol synthesis measured in peripheral blood mononuclear leucocytes (PBMC) by [^{14}C]acetate incorporation into cholesterol, and cellular cholesterol content by an enzymatic fluorometric assay. Low density lipoprotein (LDL) and high density lipoprotein (HDL) were isolated by ultracentrifugation, cholesteryl ester transfer protein (CETP) measured by transfer of [^{14}C]cholesteryl oleate from LDL to HDL and lipoprotein (a) [Lp(a)] measured by an Elisa method. Serum lipoproteins, oestrogen, progesterone, cell cholesterol and Lp(a) were normal for both groups and there was no cyclical variation . Cholesterol synthesis was increased in diabetic patients at week 3 (220±18 vs 144±14 cpm/min/mg protein, $p<0.05$). Oestrogen ($r=-.36$, $p<0.05$,$n=36$) and progesterone ($r=-.33$, $p<0.05$, $n=36$) both correlated negatively with cholesterol synthesis in control subjects but not diabetic patients. CETP activity was significantly higher in diabetic patients (mean 184±11 vs 143±7nmol/ml/h, $p<0.05$, $n=36$) reflecting an altered cyclical LDL esterified/free cholesterol ratio.These findings may help to explain the loss of protection against atheroma in the pre-menopausal diabetic patient.

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Postprandial lipoprotein metabolism in type 2 diabetes mellitus

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To investigate relationships between hypertriglyceridaemia and lipoprotein metabolism, we performed postprandial studies on patients with type 2 diabetes mellitus with or without hypertriglyceridaemia (HTG and NTG) and controls. Diabetic subjects were matched for age, gender, body mass index and glycaemic control. Lipoproteins were separated by ultracentrifugation and subfractionated by gradient gel electrophoresis. Intestinal-derived lipoproteins were identified by adding retinyl palmitate to a standard test meal. The extent of postprandial lipaemia measured by the area under the triglyceride curve over 12 hours was inversely correlated with lipoprotein lipase activities ($r=-0.57$) and was higher in the HTG group (56.88 ± 10.61(SD) mmol/l.12 hours vs 30.78 ± 6.87, $p<0.05$). This was mainly due to an increase in chylomicron remnants as shown by the elevation of retinyl palmitate in the non-chylomicron fraction (52.17 ± 20.24 mg/l.12 hours vs 31.36 ± 12.07). Small dense LDL-III concentrations correlated with fasting triglyceride and the extent of postprandial lipaemia, $r=0.74$ and 0.73 respectively. LDL-III concentrations were higher in HTG group ($202\ \pm76$ mg/dl vs 114 ± 62, $p<0.05$). The activities of the enzyme lecithin:cholesterol acyltransferase and cholesteryl ester transfer protein both increased significantly after meal. These various changes associated with postprandial lipaemia may partly explain why hypertriglyceridaemia is a risk predictor for coronary heart disease in type 2 diabetes mellitus.

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LIPOPROTEIN(a),ACUTE-PHASE PROTEIN REACTANTS AND ENDOTHELIAL FUNCTION IN MICROANGIOPATHIC TYPE 1 DIABETIC PATIENTS.
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Lipoprotein(a) is an independent risk-factor for cardiovascular complications in diabetes mellitus.The aim of the study was to evaluate Lp(a),together with acute-phase protein pattern(fibrinogen,α1 acid glycoprotein,ceruloplasmin, haptoglobin,α1 antitrypsin),fibronectin,Von Willebrand factor antigen(VIII-RAG)and the other lipid features in 107 long-term Type 1 diabetic patients.Higher plasma Lp(a)(IEMA assay)was demonstrated in diabetic patients than in healthy controls(35.3+9.2 mg/dl,vs,9.73+4.3 mg/dl,p<.001).The highest values of Lp(a)were found in patients with microangiopathic and neurological complications,compared to uncomplicated patients(52.1+15.3 mg/dl,vs,16.9+11.4 mg/dl, p<.001).The increase of Lp(a) plasma concentration was independent of serum apolipoprotein A1 and B and HDL-cholesterol changes and of the occurrence of blood lipid alterations;whereas changes of Lp(a) were associated with increased serum concentrations of acute-phase proteins and with plasma fibronectin and VIII-RAG levels.In effect,a positive correlation between Lp(a),fibrinogen(r=.41,p<.001) and fibronectin(r=.55,p<.001)was observed in microangiopathic patients.Moreover Lp(a) correlated with urinary albumin excretion in diabetic patients with incipient(r=.47,p<.01) and overt nephropathy(r=.51,p<.01).Lp(a) and hyperfibrinogenemia were significantly associated with the occurrence of arterial hypertension in our study. Lp(a)was suggested to be a risk-factor,in addition to fibrinogen,fibronectin and VIII-RAG,for the development of microvascular complications in Type 1(insulin-dependent)diabetic patients,independently of blood lipid changes.

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APOLIPOPROTEIN A-IV LEVELS AND ISOFORMS IN TYPE 2 DIABETES.
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Apolipoprotein (apo) A-IV is a 46 kd apolipoprotein found associated with HDL and triglyceride rich lipoproteins. Different apoA-IV isoforms have been described. Since apoA-IV is supposed to play a role in the reverse cholesterol pathway, its study is of interest in type 2 diabetes. Apo A-IV levels have been measured by competitive enzyme immunoassay, and their isoforms have been determined by isoelectric focusing followed by immunoblotting in 46 type 2 diabetic patients (24 males[M] and 22 females[F]), as in 80 controls (40 M,40 F). In both sexes, apoA-IV levels were found to be significantly elevated in type 2 diabetic patients compared with controls (M: 194 ± 83 vs 131 ± 31 mg/dl, $p<0.01$; F: 214 ± 105 vs 138 ± 21 mg/dl, $p<0.01$). ApoA-IV level was not significantly different between males and females in diabetics as in controls. ApoA-IV levels were not correlated with fasting glycemia, mean glycemia or HbA1c. Among the diabetic population, apoA-IV levels were significantly higher in patients with triglyceride(TG) levels ≥200 mg/dl than in those with TG levels <200 mg/dl (241 ± 97 vs 181 ± 85 mg/dl;$p=0.03$) and in patients with HDL-cholesterol levels ≥60 mg/dl than in those with HDL-chol. levels <60 mg/dl (238 ± 36 vs 196 ± 80 mg/dl, $p=0.04$). On the other hand, diabetic patients with TG levels less than 200 mg/dl and HDL-chol. levels less than 60 mg/dl did not have significantly different levels of apoA-IV than controls. ApoA-IV isoform repartition was not significantly different between type 2 diabetics and controls: isoform 1-1 in 85% of diabetics and 88% of controls, isoform 1-2 in 15% of diabetics and 12% of controls. In conclusion: 1) Apo-A-IV levels are significantly increased in type 2 diabetic patients. 2) ApoA-IV isoform repartition is not different in diabetics than in controls. 3) High apoA-IV levels are found in diabetic patients with either high HDL-chol. or high TG levels. Further studies are needed to know whether high apoA-IV levels associated with high HDL levels have the same meanings and metabolic consequences than high apoA-IV levels associated with high TG levels.

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GEMFIBROZIL INDUCED CHANGES OF LDL PARTICLES IN TYPE 2 DIABETIC PATIENTS

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LDL particles vary in their size, composition and in their atherogeneity. To study the effects of gemfibrozil therapy on the properties of LDL 16 type 2 (non-insulin-dependent) diabetic patients were randomized into two groups, matched for age, BMI, HbA1c and serum triglycerides, receiving either placebo or gemfibrozil 600 mg twice daily for three months. Gradient gel electrophoresis was used to determine particle size of the major LDL peak. LDL density distribution and composition were determined by density gradient ultracentrifugation. During gemfibrozil treatment serum triglycerides decreased from 2.93 ± 1.11 to 1.81 ± 0.50 mmol/l, (mean±SD, $p<0.05$) and HDL and LDL cholesterol concentrations increased from 1.16 ± 0.21 to 1.28 ± 0.23 ($p<0.05$) and from 3.44 ± 0.93 to 4.03 ± 0.83 mmol/l ($p<0.05$), respectively. Diameter of major LDL peak increased from 244 ± 7 to 251 ± 5 Å ($p<0.05$) and the increase was inversely correlated with the change of serum triglycerides ($p<0.01$). The mean peak density of LDL decreased from 1.0371 to 1.0345 g/ml and the mass concentration of light LDL (d=1.0232-1.0352 g/ml) increased from 141 ± 49 to 183 ± 44 mg/dl, ($p<0.05$), in the gemfibrozil group. The cholesteryl-ester-to-triglyceride-ratio weight ratio in LDL increased by 27% ($p<0.05$) consistently with an increase in LDL particle size and lowered LDL density. In conclusion, the gemfibrozil induced increase of LDL cholesterol and mass occurred in the light LDL which is considered to be the least atherogenic LDL particle.

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INFLUENCE OF SIMVASTATIN ON INSULIN SENSITIVITY IN TYPE 2 (NON-INSULIN-DEPENDENT) DIABETES MELLITUS (NIDDM).

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NIDDM is often characterized by both insulin resistance and dyslipidemia. To test wether lipid lowering therapy with a HMG-CoA-reductase-inhibitor (Simvastatin) improves insulin sensitivity 16 mildly hypercholesterolemic NIDDM subjects (aged 66 ± 4 (±SD) years, BMI 28 ± 4 kg/m^2) were, in a double-blind design, randomized to either placebo or Simvastatin (10-20 mg/day) for 18 weeks. A euglycemic glucose clamp (insulin infusion rate 1.0 mU/kg/min) was performed twice in all subjects. Following Simvastatin therapy total-cholesterol and LDL-cholesterol were significantly reduced from 6.7 ± 0.6 to 5.2 ± 0.6 mmol/l and 4.5 ± 0.7 to 2.9 ± 0.7 mmol/l respectively (both $p < 0.01$). Serum NEFA and triglycerides were unaltered. No lipid changes were observed in the placebo group. The glycemic level and Hb_{A1c} were similar in the two groups. Isotopically determined insulin-stimulated glucose disposal was similar before and during therapy in the placebo (4.1 ± 1.9 and 3.8 ± 0.7 mg/kg/min) and the Simvastatin group (3.0 ± 1.6 and 3.1 ± 1.8 mg/kg/min). Equivalently no differences were found in glucose and lipid oxidation. Furthermore, the suppressive action of insulin on hepatic glucose production was not influenced by Simvastatin (-0.7 ± 0.8 vs -0.7 ± 0.5 mg/kg/min). In conclusion, despite marked improvement of the dyslipidemia in NIDDM subjects Simvastatin failed to reduce insulin resistance.

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PRONOUNCED BLOOD GLUCOSE LOWERING EFFECT OF THE ANTILIPOLYTIC DRUG ACIPIMOX.

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Acute administration of the antilipolytic nicotinic acid analogue Acipimox to Type II diabetic patients is associated with increased peripheral and hepatic insulin sensitivity. However, long term Acipimox treatment (250 mg x 3) to Type II diabetic patients does not improve blood glucose control, possibly due to a rebound phenomenon of lipolysis. The aim of this study was to assess influence of *intensive frequent* Acipimox administration (125 mg x 12) on diurnal profiles of plasma glucose, insulin, non-esterified free fatty acid (NEFA) and triglyceride (TG) during a 3 days study period. Eight Type II diabetic patients (age 58.9±7.9 years, BMI 31.4±4.5 kg/m^2) were included in a randomized, double-blinded, placebo controlled, cross-over study. Blood samples were collected every second hour during 3 days of Acipimox and placebo treatment. The treatments were separated by a two weeks washout period. Acipimox treatment was associated with reduced diurnal profiles of NEFA (0.26±0.03 vs 0.63±0.06 mmol/l, $p<0.001$), triglyceride (1.74±0.21 vs 2.10±0.18 mmol/l, $p<0.03$), glucose (12.7±1.0 vs 15.8±1.2 mmol/l, $p=0.002$) and insulin (21.9±2.9 vs 28.8±3.7 mU/L, $p<0.05$). During Acipimox treatment NEFA increased from day 1 to day 3 (0.18±0.03 vs 0.34±0.04 mmol/l, $p=0.001$). However, from day 1 to day 3 during Acipimox treatment plasma glucose (13.4±1.2 vs 12.3±0.9 mmol/l, $p<0.03$) and insulin (23.4±3.2 vs 20.6±2.4 mU/l, $p<0.04$) were reduced. In conclusion we have showed a pronounced blood glucose lowering effect as well as a lowered plasma insulin profile in response to treatment with the antilipolytic drug Acipimox.

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EFFECT OF ACIPIMOX AND BEZAFIBRATE ON LIPOLYSIS *IN VITRO* IN RAT EPIDIDYMAL ADIPOCYTES.

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Hypertriglyceridaemia is common in non-insulin-dependent diabetes and is a significant cardiovascular disease risk factor. Nicotinic acid, its analogue acipimox and fibrates such as bezafibrate are commonly used to treat diabetic hypertriglyceridaemia, but the precise mechanism of action remains elusive. We have therefore examined their effects on lipolysis in epididymal adipocytes isolated from Wistar rats (170-190g) by collagenase digestion. Lipolytic rate was monitored as glycerol release following a 20min incubation. Under conditions of maximal stimulation (100nM isoproterenol, 96 ± 6 (SEM) nmol glycerol/ml medium/20min (n=10)) the rate of lipolysis was decreased by 12 ± 4% (n=4), 21 ± 6% (n=7) and 35 ± 8% (n=4) by 1mM nicotinic acid, acipimox and bezafibrate, respectively. However, at 10mM, nicotinic acid decreased lipolysis to 34 ± 5% (n=4), while acipimox and bezafibrate suppressed lipolysis to basal levels. A time course study showed that the isoproterenol-stimulated glycerol release was totally inhibited within 5min by both acipimox and bezafibrate at 10mM. The release of lactate dehydrogenase into the incubation medium was used as an indicator of cellular integrity and remained at basal levels during the incubation. These findings indicate that acipimox and bezafibrate are acute inhibitors of lipolysis in adipose tissue, although the molecular mechanisms remain to be established.

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IMPROVEMENT OF LIPOPROTEIN LIPID COMPOSITION IN HYPERLIPIDEMIC TYPE II DIABETICS BY ACIPIMOX (OLBETAM) TREATMENT: RESULTS OF A MULTICENTER TRIAL

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To study the effect of acipimox (ACI) on hyperlipoproteinemia, diabetes compensation and insulin action in Type II diabetes under conditions of a routine clinical practice, 121 patients were recruited in 10 participating centers, randomly divided into two groups, and treated for 3 months either with ACI or placebo (PLA) (open study design). ACI administration (250 mg t.i.d.) led to a decrease (by 31 %) of fasting serum total triglycerides level (3.30±0.20 vs 2.27±0.11 mmol.l^{-1}; $p<0.01$) already after 1 month of treatment, prevailing up to the end of the study (2.26±0.12). Serum total cholesterol levels declined (by 12 %) (7.15± 0.15 vs 6.29±0.13 mmol.l^{-1}; $p<0.01$), and those of HDL-Ch increased (by 18 %) (1.11±0.04 vs 1.31±0.04, $p<0.01$). Changes in serum lipids were accompanied by improvements of glucose metabolism: a decrease of fasting glycemia (8.30±0.3 vs 7.33±0.2 mmol.l^{-1}; $p<0.05$) and an increase ($p<0.05$) in insulin sensitivity (euglycemic clamp) after 3 months of treatment. Moderate adverse events of transient character (skin reactions, gastric disturbances) were reported in 3 cases (out of 81) treated with ACI. Thus, a 3 months treatment of hyperlipidemic Type II diabetics with ACI was associated with significant improvement of lipoprotein lipid composition accompanied also by a beneficial action on carbohydrate metabolism. Moreover, ACI was well tolerated by the patients who remained free of serious side effects.

PS 30
Rheology

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HAEMOSTATIC ABNORMALITIES PERSIST DESPITE GLYCAEMIC IMPROVEMENT IN TYPE 2 DIABETES.

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Patients with poorly controlled type 2 diabetes are at an increased risk for cardiovascular events, which is associated with disturbances of metabolic, lipid and haemostatic systems. In order to investigate the influence of glycaemic optimisation, we studied in 62 diabetic patients with secondary sulfonylurea failure prior and 6 months after induction of sc. insulin therapy and 45 control subjects the following parameters: HbA1c [HPLC], fructosamine (FA) [colorimetry], lipid parameters, apolipoproteins [standard methods], plasma fibrinogen (FIB) [Clauss], coagulation factor VII:C (FVII:C) [coagulometry], proteins C (PC) and S (PS): Ag, vonWillebrand factor:Ag (vWF), D-dimer (DD) [ELISA]. Prior to insulin therapy metabolic parameters were elevated compared with controls (HbA1c 10.1±0.2 (mean±SEM) vs 4.5±0.1 %, p<.0001; FA 403±11 vs 270± 9 mmol/L, p<.0001), as well as FIB (346±16 vs 287±12 mg/dl, p<.0001), FVII:C (1.11±0.05 vs 0.93±0.04 U/L, p<.01), vWF (1.85±0.12 vs 1.32±0.07 U/L, p<.0001), PC (1.26±0.04 vs 0.94± 0.03 U/L, p<.0001), PS (1.16±0.04 vs 0.91±0.03 U/L, p<.0001), and DD (162±24 vs 109±11 µg/L, p<.05). After 6 months of insulin therapy metabolic parameters were significantly decreased (HbA1c 7.5±0.2 %, p<0.1; FA 294±17 mmol/L, p<.01), but not haemostatic parameters (FIB 320±14 mg/dl, ns; FVII:C 1.13±0.06 U/L, ns; vWF 1.74±0.15 U/L, ns; PC 1.39±0.06 U/L, ns; PS 1.23±0.06 U/L, ns; DD 160±30 µg/L, ns). Our data present an increased atherogenic and prothrombotic risk in poorly controlled type 2 diabetic patients. Induction of insulin therapy reduces metabolic and lipid parameters, but coagulation abnormalities remain. Therefore, metabolic optimisation does not necessarily result in a reduction of haemostatic disturbances and cardiovascular risk.

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HAEMOSTATIC FACTORS IN TYPE 1 DIABETES WITH MICROALBUMINURIA:EFFECTS OF GLUCOSE CONTROL

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The aim of this study was to investigate if the presence of microalbuminuria (MCA) is associated with coagulation and fibrinolytic abnormalities. We studied Type 1 diabetics patients,16 with MCA (urinary albumin excretion 20-200 ug/min) before and after six months of optimised metabolic control (intensive conventional treatment) and 13 without MCA. Both groups were similar in age, sex distribution, diabetes duration, body mass index, HbA1 and retinopathy. Statistical evaluation was performed with non-parametric test, correlation with multiple regression. Patients with MCA were found to have a higher post stasis tissue-type plasminogen activator (s t-PA, 8.49±4.72 vs 5.30±3.62 ug/L,p=0.02) and Fibrinopeptid A (FBA, 5.43±3.71 vs 2.80± 3.71,p=0.04). No significant differences were found in fibrinogen,antithrombin III,protein C, protein S,basal t-PA,plasminogen activator inhibitor 1,thromboxane B2 and platelet factor 4. In group with MCA the basal value of thromboxano was correlated with MCA (r=0.70,p=0.00). After six months, 12 patients showed improvement of blood glucose control (HbA1: basal: 11.57 ± 0.71, six months: 9.45 1.18%,p=0.00). Despite metabolic control improvement, s t-PA and FBA values remained higher. In conclusion: our results suggest disturbances in fibrinolytic capacity in Type 1 patients with MCA compared with diabetics with normal urinary albumin excretion. Improvement of metabolic control was not able to correct them.

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Urinary albumin excretion, cardiovascular disease and endothelial dysfunction in non-insulin-dependent diabetes mellitus.

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An elevated urinary albumin excretion (UAE) indicates an increased risk of cardiovascular disease (CVD) in non-insulin-dependent diabetes. To examine the role of endothelial dysfunction, we followed a cohort of 94 patients (median, 3 yr) and investigated the relationships among UAE, new CVD and von Willebrand factor plasma level (vWf; an indicator of endothelial dysfunction). At baseline, 66 patients had normal UAE (<15 µg/min), which remained normal in 33 (Group 1) and increased in 33 (to (median) 55 µg/min; Group 2). In 28 patients, baseline UAE was abnormal (56 µg/min; Group 3). vWf (% normal) did not change in Group 1 (129% at baseline and 127% at follow-up), but increased in Group 2 (from 121 to 212%, P<0.0001) and Group 3 (from 157 to 203%; P=0.0005). Baseline level of and change in vWf were related to the development of microalbuminuria (R^2=0.60; P<0.0001), but classical CVD risk factors were not (R^2=0.14, P=0.87). An elevated baseline UAE was associated with an increased risk of new CVD only in patients with vWf above the median (relative risk [95% confidence interval], 3.7 [1.3, 11.9]). In addition, the CVD risk associated with an elevated UAE was modified by the HDL-cholesterol level, the relative risk being 2.9 (1.03, 8.5) when HDL-cholesterol was below the median. Conclusion: Dysfunction of vascular endothelium may be a pathophysiological link between albuminuria and atherosclerotic cardiovascular disease in non-insulin-dependent diabetes.

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ELEVATED PLASMA ENDOTHELIN IN NON-INSULIN-DEPENDENT DIABETIC SUBJECTS WITH MICROALBUMINURIA

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It has been suggested that increased immunoreactive endothelin (ET) in human plasma, presumably derived from damaged endothelial cells may be related to the complications of diabetes. We measured the ET in 16 normotensive, microalbuminuric type 2, non-insulin dependent diabetic subjects (8 males, 8 females, age 55.9 ∓ 6.4 years) and in 14 normotensive, normoalbuminuric type 2 diabetic subjects (7 males, 7 females, age 56.9 ∓ 10.2 years) and compared with the plasma ET in 20 control subjects (10 males, 10 females, age 57.6 ∓ 4.3 years). ET was measured in plasma by radioimmunoassay, using Endothelin-1.2 RIA kit (Amersham, London) after prior extraction by sep-pack cartridges. Plasma ET level was significantly higher in non-insulin dependent diabetic subjects with microalbuminuria than in those with normoalbuminuria (17.28 ∓ 7.86 vs. 12.50 ∓ 1.63 fmol/ml, $P < 0.05$), or in controls (10.73 ∓ 0.97 fmol/ml, $P < 0.01$). Normoalbuminuric diabetic subjects also had higher ET levels than controls ($P < 0.05$). Plasma ET was not related to age, duration of diabetes and HbA1 ($P > 0.05$). In conclusion, our results indicate that endothelin is highly elevated in type-2, non-insulin dependent diabetic subjects, especially in those with microalbuminuria. The elevated plasma ET may be a marker of vascular complications of diabetes.

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PLATELET AGGREGATION IN NON-INSULIN DEPENDENT DIABETES CORRELATES WITH PLASMA LIPIDS NOT GLYCAEMIC CONTROL.

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The abnormal platelet aggregation found in diabetes is not restored to normal by improving glycaemic control and may be due to other factors. We investigated relationships between *in vitro* platelet aggregation and blood lipids and glycaemic control in non-insulin-dependent diabetic patients - 16 men and 4 women, mean (± SEM) age 59 ± 8 years, duration of diabetes 7 ± 4 years, Body Mass Index 27.0 ± 4.2 $Kg.m^{-2}$, HbA_{1c} 9.1 ± 0.4%, free of significant complications. Seven were treated by diet alone, 8 sulphonylurea alone and 5 sulphonylurea plus biguanide. Platelet aggregation was measured using a modified Born photometric technique, after the addition to platelet rich plasma of collagen, 1.0 ug/ml high dose (ColH), 0.15 ug/ml low dose (ColL) and ADP, 10.0 umol/l high dose (ADPH), 0.5 umol/l low dose (ADPL). Using simple linear regression significant correlations were found between LDL-cholesterol and ColH ($r = 0.50$, $p<0.05$), ColL ($r = 0.73$, $p<0.001$) and ADPL ($r = 0.56$, $p<0.01$); the correlation between LDL-cholesterol and ADPH was non-significant ($r = 0.23$). Similar correlations were found between total cholesterol and platelet activity. There were no significant correlations between platelet activity and fasting blood glucose, $HbA1_c$ or plasma triglycerides. These positive associations between platelet aggregation and cholesterol suggest that reducing lipid levels is as important as glycaemic control in attempts to reduce cardiovascular disease.

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PLATELET LEVELS OF ANTIOXIDANT GLUTATHIONE-UTILIZING ENZYMES IN TYPE 1 (INSULIN-DEPENDENT) DIABETES.

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Tissue levels of antioxidant enzymes which utilize glutathione as substrate are altered in both experimental and clinical diabetes. Whether platelet content of such enzymes are modified in Type 1 (insulin-dependent) diabetes mellitus is however unknown. Aim of this study was to assay spectrophotometrically the platelet content of glutathione transferase (GST), glutathione reductase (GSSG-RD) and glutathione peroxidase (GSSG-Px) in a group of 13 Type 1 (insulin-dependent) diabetic patients in good metabolic control (plasma glucose < 9.74 mM/L and HbA1c < 8%), as compared with 8 age and sex-matched healthy controls. Mean(±SD) GST was similar in both diabetcs and controls (224.17±103.5 vs. 207.04±89.48 nM/min/10^9 platelets), GSSG-RD was higher in diabetics (130.76±46 vs 76.90±39.28 nM/min/10^9 platelets; p=0.01) and GSSG-Px was significantly lower in diabetics (41.34±21.6 vs. 137.68±44.54 nM/min/10^9 platelets; p=0.0001). Each enzymatic activity was unrelated with age,duration of diabetes or body weight, being correlated with HbA1c (r=0.58; p=0.03 for GSSG-RD, r=0.56; p=0.04 for GSSG-Px and r=0.65; p=0.01 for GST). In conclusion platelet contents of antioxidant glutathione-utilizing enzymes are deeply altered in Type 1 (insulin-dependent) diabetes, all of them being significantly induced by long-term metabolic control.

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INSULIN INCREASES CYCLIC-GMP IN PLATELETS INDEPENDENTLY OF NITRIC OXIDE.

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Insulin exerts its anti-aggregating effect on human platelets increasing 3',5'-cyclic guanosine monophosphate (cGMP) concentrations. Since an enhancement of intraplatelet cGMP levels is induced also by nitric oxide (NO) and platelets contain a constitutive L-arginine-dependent NO synthase, we aimed at investigating whether insulin increases intraplatelet cGMP by influencing NO synthesis. Platelet-rich plasma (PRP) samples, obtained from 7 male healthy volunteers (mean age: 30.4±1.8 years, BMI: 24.2±0.59), were incubated for 3 min with increasing insulin concentrations (0, 40, 80, 120, 160 and 320 µU/ml) both in the presence and in the absence of the NO synthase inhibitor N^G-monomethyl L-arginine (LNMMA) at a final concentration of 30 µM. PRP responses were stopped with ice cold 30% trichloracetic acid; samples were submitted to 10 ether extractions and cGMP was determined by RIA. cGMP levels (pmol/10^9 platelets, m±SEM) at the different insulin concentrations were: with insulin alone 18.3±5.3, 25.8±6.4, 25.9±7.2, 36.7±7.6, 37.7±8.8 and 39.6±8.0, respectively (ANOVA: p=0.000); with insulin + LNMMA: 11.3±2.1, 18.6±4.2, 18.0±3.9, 22.9±4.0, 31.4±6.2 and 33.8±9.9, respectively (ANOVA:p=0.009). In conclusion, we demonstrated that the insulin-induced increase of cGMP in human platelets is not mediated by an insulin effect on nitric oxide synthesis.

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ACTIVATION OF MONOCYTES AND RHEOLOGICAL ERYTHROCYTE ABNORMALITIES IN DIABETIC PATIENTS.

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Activated monocytes (AM) may be involved in arterial disease in diabetic patients. The aim was to test the link between activation of monocytes and rheological erythrocyte abnormalities. 60 patients, 18 type 1 (insulin-dependent) and 42 type 2 (non-insulin dependent) diabetics were compared with 27 healthy controls. Monocytes were separated and surface antigens were tested by cytofluorometry. The very high proportion of My4 positive cells (90.3 ± 0.7% (sem) in diabetics, 91.8 ± 0.5% in controls), shows this separation is very effective. The proportion of AM bearing CD 25 (interleukin 2-receptor) was highly increased in diabetics (42.6 ± 1.4% vs 0.3 ± 0.1%, $p < 0.001$), type 1 and type 2 patients, but higher in the 18 hypertensive patients (49.2 ± 2.5%) and in the 7 with macroangiopathy (46.3 ± 3.4%) than in the 14 normotensive patients free of any vascular complication (39.6 ± 1.8%, $p < 0.01$ and $p = 0.07$). The rigidity index (RI) of erythrocytes (hemorheometer) was higher in the diabetics (13.7 ± 0.4 vs 12.1 ± 0.4, $p < 0.025$), as well as the erythrocyte aggregation (Myrenne aggregameter) (6.83 ± 0.32 vs 4.36 ± 0.27, $p < 0.001$). The mean cell transit time (cTT) through a micropore filter was not different in diabetics and controls. The proportion of AM correlated with RI ($r = 0.45$, $p < 0.001$) and with cTT ($r = 0.37$, $p < 0.01$). These results demonstrate 1) the high proportion of AM in diabetics, particularly in those with hypertension or macroangiopathy, correlating with rheological erythrocyte abnormalities, and 2) the possible involvement of monokines in these abnormalities.

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ABNORMAL GRANULOCYTE DEFORMABILITY IN TYPE 2 (NON-INSULIN-DEPENDENT) DIABETES MELLITUS.

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Previous rheologic studies in diabetes have focused on blood and red cells. Granulocytes, however, are larger and less deformable than erythrocytes, and when activated become more rigid and prone to capillary entrapment leading to tissue damage. We have thus studied the deformability of granulocytes from three groups of Hispanic subjects: Type 2 (non-insulin-dependent) diabetes (n=38); impaired glucose tolerance (IGT, n=9); normal controls of comparable age and sex (n=14). Granulocyte deformability was tested using the Cell Transit Analyzer (8 μm pores) under three separate conditions: 1) resting state; 2) following activation with 1nM fMLP; 3) after incubation with 20μM Cytochalasin B (CB) to dissociate F-actin from the cytoskeleton. Compared to controls, granulocytes from diabetics exhibited greater rigidity at rest (+22%, $p<0.001$) and after CB treatment (+16%, $p<0.001$) but not following fMLP; IGT values were between those for controls and diabetics. No differences in granulocyte count or volume existed among the three groups. Analyses of pooled data indicated that resting granulocyte rigidity increased with HbA_{1c} ($r=0.34$, $p<0.02$), cholesterol ($r=0.58$, $p<0.001$), and triglycerides ($r=0.36$, $p<0.05$) but not with fasting glucose. These results demonstrate lower granulocyte deformability in diabetes mellitus which may contribute to the development of microcirculatory disturbances and vasculopathy.

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DYNAMICS OF ATP RELEASE FROM DIABETIC RED BLOOD CELLS (RBC) AND ITS CORRELATION WITH HbA1c.

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Aim of this study was to investigate if modifications of diabetic RBC membrane may influence ATP release following osmotic shock. Venipuncture-collected RBC were submitted to osmotic shock by NaCl solutions (0.9-0.63-0.49-0.36-0.18-0.045%) and ATP release values , determined by bioluminescent method, were expressed as percentage of maximum ATP release. 20 Type 1-(Insulin-Dependent) (IDDM) (mean age 34.2±8.6 yr) and 18 Type 2-(Non-Insulin-Dependent)(NIDDM)(mean age 61.7±11.4 yr) diabetic subjects and two control groups (C1-C2, 5 subjects each, age comparable) were investigated. The % ATP release at 0.49% NaCl (%R) was significantly lower in both diabetic groups (IDDM = 31.18±17.54 ; C1=58.12±14.08 %R; $p<0.05$; NIDDM =34.22±21.20 ; C2 = 50.64±11.07 %R; $p<0.05$). These data demonstrate an increased resistance of ATP release to the osmotic shock in diabetic RBC. In the same subjects HbA1c has been found inversely correlated with ATP %R ($r=.3316$; $p<0.05$), suggesting a possible relationship between Hb glycation, ATP release and RBC membrane protein skeleton glycation. In a preliminary study we investigated membrane protein skeleton in 6 diabetics (2 IDDM + 4 NIDDM) with increasing HbA1c values (from 7.7 up to 15.3%) and 3 controls, by negative-stained electron micrograph (1.15x10^5 magnification). A mantained spectrin hexagonal lattice of RBC membrane skeleton in diabetics was observed in spite of its progressive glycation and no morphological alterations were there evidentiated.

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THE ROLE OF PLASMA, ERYTHROCYTE, AND PLATELET MYO-INOSITOL LEVELS IN THE DEVELOPMENT OF DIABETIC MICROANGIOPATHY.

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In this study the plasma, erythrocyte, and platelet myo-inositol levels in 24 type I, 24 type II diabetics, and in 15 healthy controls were determined. The diabetics were devided into two groups with microangiopathy (n:26) and without microangiopathy (n:22). The plasma, erythrocyte, and platelet myoinositol levels in the whole diabetic group and control subjects were as follows. Diabetic group: 46.5±38.9 μmol.ml^{-1}, 23.3±19.8 nmol.ml^{-1}, 2,6±2.8 nmol.ml^{-1} (10^5 cells)$^{-1}$. Control group: 17.4±57 μmol.ml^{-1} 12.2±5.2 nmol.ml^{-1}, 1.5±0.9 nmol.ml^{-1} (10^5 cells)$^{-1}$. The values of the diabetic group were significantly higher than the values of the control group ($p<0.01$). In patients with HbA_{1c} levels more than 9% plasma, erythrocyte, and platelet myo-inositol values were significantly higher than the values of the group with HbA_{1c} levels less than 9% ($p<0.05$, $p<0.05$ and $p<0.01$). In diabetics without complications plasma and erythrocyte myo-inositol levels were higher than the values of the control group ($p<0.01$), whereas there was no significant difference between the platelet myo-inositol values. In diabetics without complication all these three values were higher than those of the control group ($p<0.01$, $p<0.01$, $p<0.01$). The most profound increase in the plasma, erythrocyte, and platelet myo-inositol levels were seen in complicated diabetics with a diabetes duration of 6-10 years ($p<0.01$). After 10 years duration the values declined. In the group without complication there was no difference according to the duration of diabetes.

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ALTERED MEMBRANE FLUIDITY AT DIFFERENT DEPTHS OF LIPID BILAYER IN PLATELET MEMBRANES FROM DIABETIC JUVENILE SUBJECTS

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Previous studies showed that the lowered platelet membrane lipid fluidity in diabetic subjects, attributed to the enhanced glycation of membrane proteins, was associated with platelet hypersensitivity to thrombin. As the modulation in membrane lipid fluidity may determine the degree of accessibility of membrane receptors and their response to fluidity changes, we examined the gradient in membrane lipid fluidity and the possible association of lipid bilayer dynamics with the amount of platelet membrane glycoproteins GPIIb and GPIIIa. We compared 12 diabetic juvenile subjects with 12 age- and sex-matched controls. To monitor the possible changes in fluidity at different depths of lipid bilayer we employed two fluorescent labels anchoring in different regions of lipid bilayer: 1,6-diphenyl-1,3,5-hexatriene (DPH) located in lipid hydrocarbon core, and 1-anilino-8-naphthalenesulphonate (ANS), located at membrane surface. The mean steady-state fluorescence polarization (p) values of both DPH and ANS in membranes from diabetic subjects were significantly greater than from control subjects, thus indicating for the reduced membrane lipid fluidity in diabetic platelets in both membrane regions; the alterations of p_{DPH} significantly exceeded those of p_{ANS}. Furthermore, the amounts of these glycoproteins were significantly reduced in diabetic subjects and correlated inversely with p_{DPH} and p_{ANS}. We concluded that the total changes in membrane fluidity and the greater rigidification of diabetic platelet membranes in their deeper regions might be responsible for the altered dynamics of membrane proteins, and hence might underlie the hypersensitivity of diabetic platelets.

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PERIPHERAL AUTONOMIC NEUROPATHY (PAN) DOES NOT INFLUENCE FOOT TRANSCUTANEOUS OXYGEN TENSION (TcPO2)

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Recurrent foot ulceration is a frequent finding in diabetics with peripheral neuropathy. The influence of PAN on TcPO2 and on difference of Arterial and Venous Oxygen content (A-VΔO2) of the foot was studied in patients without peripheral vascular disease (ankle/brakial index 0.9-1.1). The presence of PAN was identified by the absence of Galvanic Skin Response (GSR). The following age matched patients were selected and evaluated in both legs: controls (C=7), NIDDM (D=19) and NIDDM with recurrent foot ulceration (F=11), (duration of disease D=11.8±7, F=19.2±7yrs; p<0.01).

The results are expressed in Tab. 1 and Tab. 2.

	TcO2	A-VΔO2
C (n=14)	62.8±16*/°	6.2±5°°
D (n=38)	46.5±16	5.1±3.5
F (n=22)	49.5±15	2.26±2

Tab. 1: C vs D *<0.01; C vs F °<0.05, °°<0.01, §<0.01

GALVANIC-SKIN RESPONSE		
	TcO2	A-VΔO2
present (n=36)	62.4±20	5±4*
absent (n=14)	51.6±16	1.7±3

Tab. 2: present vs absent *<0.05

a) the TcPO2 is decreased in D and F and apparently is not influenced by the presence or absence of PAN.

b) the A-VΔO2 is reduced in F and in diabetic patients with absent GSR.

PAN does not affect the TcPO2 and appears to be associated with low values of A-VΔO2. Therefore the reduced TcPO2 values found in D cannot be explained by the presence of PAN, but probably are related to a reduced oxygen diffusion through the tissues.

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PRECLINICAL ALTERATIONS OF MICROCIRCULATION IN TYPE 1 (INSULIN-DEPENDENT) DIABETES MELLITUS

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To verify early functional microangiopathic alterations, transcutaneous (tc) PO_2 and cutaneous blood flow (Laser Doppler) were monitored in the submalleolar area of male patients (n=10 ea., aged <35y., no neuropathy), diabetes duration (1) 2±1y. and (2) 14±3y. without or (3) 19±6y. with pre-proliferative retinopathy ± microalbuminuria, or (4) 19±5y. and treated for various angiopathic complications. - **Protocol:** run-in, 8 min basal monitoring, 8 min O_2-breathing, 3 min ischaemia, 3 min reactive hyperaemia. **Results:** $tcPO_2$ in groups (1) - (4) without/with O_2-breathing was 38±10(SD)/50±15, 39±17/50±21, 34±8/44±13, 30±6/39±10 mm Hg vs 35±8/45±19 mm Hg in controls (intraindividual SD's <1.0 mm Hg at 0.25 s intervals). $tcPO_2$ recovery from ischaemia was delayed, flow (without/with O_2-breathing) was reduced, and hyperaemia was smoothed in (2), (3) and (4). Power spectral analysis of flow in controls showed averages of 1.2 and 0.6 significant oscillations per patient, related to arteriolar (5 to 15 s periods) and to arterial (periods >50 s) function, respectively. Oscillations decreased during O_2-breathing but were not altered in hyperaemia. In diabetics (3) and (4), rhythms were generally reduced under basal conditions and during hyperaemia but were paradoxically elevated during O_2-breathing in (2) and (3). - **Conclusion:** Early circulatory hyperfunction is confirmed; oscillation analysis contributes to diagnosis of functional microangiopathy.

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REGIONAL CEREBRAL BLOOD FLOW IN PATIENTS WITH INSULIN-DEPENDENT DIABETES MELLITUS.

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High occurrence and worse outcome of strokes in patients with diabetes mellitus are well known. The aim of this investigation was to study the regional cerebral blood flow rate in patients with insulin-dependent diabetes mellitus. Study included 40 patients aged 18-39 yrs. Blood flow was examined in occipital,parietal and temporal regions,its rate being determined from the rate of i.v.administered radioactive 133Xe clearance. The level of glycemia did not exceed 10 mmol/l during a day. Indices of 10 healthy persons of analogous age served as control. Deceleration of blood flow rate was found in all study regions of the cerebrum. Thus,in occipital region the blood flow rate was 56.4±1.35 vs 59.9±1.10 ml/100 g.min^{-1}(p<0.05) of control level in left hemisphere and 52.6±1.73 vs 58.9±1.53 ml/100g.min^{-1}(p<0.05) in the right; in frontal regions: 52.8±2.3 vs 65.1±1.28 ml/100 g.min^{-1}(p<0.01) from the left and 52.2±2.19 vs 64.8±1.05 ml/100g.min^{-1}(p<0.01) from the right; in temporal regions: 54.2±1.92 vs 60.7±1.10 ml/100 g.min^{-1}(p<0.01) from the left and 54.4±1.42 vs 59.6±1.18 ml/100 g.min^{-1}(p<0.01) from the right. No differences in the blood flow rate were found in patients with a varying disease duration. In addition, patients were divided into 2 groups in relation to the presence of diabetic retinopathy and nephropathy. There were no significant differences in study parameters between these groups. Revealed was also the interregional asymmetry of cerebral blood flow in patients under study. In conclusion, patients with diabetes mellitus showed a decrease in the rate of regional cerebral blood flow that may be a link of pathogenesis of central neuropathy.

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REGIONAL COURSE ON DIABETES FOR PATIENTS AND THEIR RELATIVES: ONE YEAR'S RESULTS.

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After having educated 1230 patients and relatives in 12 years with 90 systematic courses on diabetes, we started two new courses sponsored by Public Assistance: for insulin-treated patients (INS, 21h) and for non-insulin treated patients (OHA, 14h). In 1991, 103 patients, 45 relatives and 6 student nurses took part in 16 courses. Knowledge was evaluated with two similar questionnaires of 75 items each, before and after the course. Answers (true/false/unknown) were scored 0-2 and analyzed with parametric and nonparametric statistics. Global score (shown in tenths) rose from 5.8±0.04 to 6.7 ± 0.04, p<0.001. Percentages of right/unknown/wrong answers changed respectively from 37.8 ± 1.7% to 58.2±1.9%, p<0.001; from 39.9± 1.5% to 18.3±1.1%, p< 0.001; from 22.3±1.4% to 23.5±1.4%, ns. Individually, 26/69 INS, 33/79 OHA and 1 student nurse improved significantly. Younger people, INS and those with higher schooling got better scores. There was no difference between patients and relatives. The topics with best results were; foot care (score from 5.5±0.3 to 8.2±0.3: percent R/?/W answers from 43.3/41.0/24.6 to 77.3/10.0/12.7) and insulin treatment (from 5.9±0.2 to 7.1±0.2: percent R/?/W answers from 40.5/37.6/21.9 to 64.7/13.1/22.2). Questionnaires allowed to measure patients' learning and to concentrate teaching efforts on harder topics.

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DIABETES CARE AND GLYCEMIC CONTROL OF DIABETIC PATIENTS IN FINLAND.

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The present study examined glycemic control in diabetic patients in three hospital districts in Finland. In districts A and B regional planning of diabetes care had been going on for several years. District A was characterized by mainly specialist care and district B by mainly primary care. District C has so far no regional planning of diabetes care. From the three areas patients, aged 15-64 years, were randomly sampled from the drug reimbursement registry (n=896). HbA_{1c} (reference range 4.0-6.0%) was determined for the patients in districts A-C. The average HbA_{1c} was 8.9±0.1% with only slight differences between the areas. 13.4 % of patients were in good control (HbA_{1c} <7.0%) and 45.0% in poor control (HbA_{1c} >9.0%), (district A 43.5 %, B 39.7 %, C 53,3 %; A vs C p<0.05, B vs C p<0.001).The respective proportions for patients whose onset of diabetes was before the age of 30, were: A 42.9%, B 34.2%, C 52.0% (A vs C n.s., B vs C p<0.01). The risk of poor control was assessed by a logistic regression among patients using insulin injections (n=579). The following variables were entered into the model: age, sex, age at onset of diabetes, years since the onset of diabetes, the average insulin dose (U/day), specialist vs. primary care, and number of daily insulin injections. In this model only the dose of insulin was a significant (p=0.001) predictor of poor glycemic control: the higher the dose the lower the risk of poor control. Conclusions: these analyses suggest that good planning and organization of diabetes care and the quantity of the insulin dose are related to better glycemic control.

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DELAY OF PROGRESSION OF DIABETIC LATE COMPLICATIONS IN TYPE 1 DIABETES BY 6 YEARS IMPROVEMENT OF METABOLIC CONTROL IN GENERAL PRATICE.

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This study was designed to evaluate the impact of functional insulin therapy (FIT; Group 1; N = 55), a form of intensified insulin treatment, versus conventional insulin treatment (CIT; Group 2; N = 55) in type 1 diabetic patients. Patients were matched for age (35 ± 13 vs 36 ± 15 yrs; n.s.) and duration of diabetes (14 ± 9.7 vs 13.8 ± 9.7 yrs; n.s.) and observed during a 6 year follow - up period. All patients were on CIT when entering the study. 55 patients were introduced to FIT six years prior to evaluation but received their further outpatient care by general practitioners. Metabolic state and distribution of diabetic late complications was similar betweeen both groups before entering the study protocol (Group 1/Group 2: Mean blood glucose (MBG, mmol/l) 11.6 ± 3.8 / 11.8 ± 4.2; HbA1c (%) 8.3 ± 1.9 / 8.0 ± 1.7; retinopathy (% of total) 32 / 43 %; nephropathy 5 / 8 %; peripheral angiopathy 0 / 14 %, p < 0.001). After 6 yrs patients on FIT presented with improved metabolic state (MBG 8.5 ± 5.0 vs 12.5 ± 5.7 mmol/l, p < 0,001; HbA1c 6.9 ± 1.4 vs 8.4 ± 2.0, p < 0.001) and reduced progression of late complications (retinopathy 38 vs 64 %, p < 0.005; nephropathy 27 / 44 %, p< 0.01; peripheral angiopathy 2 / 37 %, p < 0.001) as compared to patients on CIT . It is concluded that long term improvement of metabolic control (1) is achievable in general practice and (2) delays progression of late diabetic complications.

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IMPLEMENTATION OF THE ST. VINCENT DECLARATION IN AN URBAN INTER-HOSPITAL QUALITY NETWORK.

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The DIABCARE Monitoring Group of the St. Vincent Declaration has developed a Basic Information Sheet (BIS) for Europe in 1991. It is the aim of this study to implement an urban quality network using this BIS for feedback communication in order to improve health care for people with diabetes.

For this purpose 18 hospitals founded the 'Speciality Commission of Diabetes in Munich'.

The international BIS, however, allows the assessment of own process quality (monitoring, metabolism, complications) only when the patient is under continuous care in one service. Therefore, this BIS was adapted to local requirements of monitoring discontinuous care (ambulatory, hospital). As the result of a feasibility phase seven percent of datafields were added. The duration of data collection for one sheet was 15-30 minutes. The anonymized data were collected and analyzed with an adapted computer program. Each service gets back its own results as histograms and percentile-plots and cross sectional data for external comparision. There is no comparision between the institutions from outside. The implementation process is finished; data collection and aggregation have been started.

The internal evaluation and implementation of changes remains under the resposibility of the institutions themselves.

Members of the SCDM: Intern. Dept.: 3. Med. Abt. KH Neuperlach, 3. Med. Abt. KH Schwabing, 3. Med. Abt. KH Bogenhausen, Med. Klinik Innenstadt, 2., Med. Abt. Klinikum Großhadern, 2. Med. Abt. Klinikum rechts der Isar, 1. und 2. Med. Abt. KH dritter Orden, Schreiber Klinik,; Pädiatr. Dept.: KH Harlaching, KH Schwabing, v. Haunersches Kinderspital,; Gyn. Dept.: KH Schwabing, KH Neuperlach, 1. Univ. Frauenklinik; Ophthalm. Dept.: KH Harlaching; Nephrolog. Dept.: KH Harlaching, KH Schwabing

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International Diabetes Programme - New Model of Health Care Delivery for Diabetic Patients in Newly Independent States of Former Soviet Union. A.AMETOV. CIAMS, Moscow, Russia.

Our present situation is characterized by a certain lag between the scientific advances in the treatment of diabetes and their practical implementation. In this respect situation with developing and implementation of national standards of diabetes care delivery is a very difficult task especially in a multinational country, with variable levels and standards of different living traditions and national nutritional habits, climatic conditions and the level of education. We seriously think that diabetes could be used as a good model indicating the necessity to reach a consensus in political, economical, medical and social matters.

Last year we established international non-governmental (partially) intersectoral effort with activities-education, diagnostic, patients consultation and implementation of special training programmes for health professionals. We have created a network of Educational-Diagnostic-Consulting Centers in 17 diverse cities of the former USSR. All these centers will be connected using telecommunication network for operative management and for data collection and subsequent analysis. We think that the magazine "Diabetes and Life Style", which we have issued in cooperation with scientists from more than 26 countries as well as a package of educational materials, including lectures, videotapes, etc., will be very useful instrument of information distribution and what is more important for international exchange of information.

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STAGED DIABETES MANAGEMENT (SDM): A TECHNOLOGY-BASED APPROACH

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A systematic approach to clinical decision-making for primary care physicians was developed. Data from 3000 patients were reviewed to determine key ***decision parameters*** used to achieve optimum metabolic control. Three phases were identified: ***baseline***--establishing diagnostic criteria; ***therapeutics***-- identifying conditions for selecting a treatment modality; ***stabilization***-- achievement of the therapeutic goal. Specific stages of therapy were defined: 2 stages of meal planning; 3 stages of diet and oral agent therapy; 5 stages of increasing insulin therapeutic intensity. The phases and stages produce a roadmap of therapies reflecting the ***community's standards of care.***Twelve prototype medical sites ranging from community-based to private practice tested the efficacy of SDM. Comparison of pre to post SDM intervention revealed that: irratic and inconsistent standards were replaced by national standards for the screening and diagnosis; undocumented and discrepent practice was replaced by a systematic SDM approach; and ad hoc reliance on expert consultation was replaced by co-management between primary care physicians and specialists. These steps will result in more subjects achieving optimized glycemic control as primary care physicians adopt consistent patterns of diabetes management.

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VARIATIONS IN DIABETES PREVALENCE AND TREATMENT, AND COSTS OF PRODUCTION LOSSES.

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Antidiabetic drug consumption (ADC) in Swedish counties and communities was estimated by drug sales statistics, and diabetic prevalence (DP) figures in some of these communities were studied, or collected from existing sources. Costs of production losses due to sickness benefit days and/or to premature retirement (< 65 years) were assessed after analyses of medical and social insurance records. There were large geographic differences in overall ADC but a close correlation ($r = 0.87$; $p < 0.01$) between DP and overall ADC, suggesting large geographic variations in DP within Sweden. However, there was no correlation between DP and ADC divided in oral agents and insulin, suggesting variations in therapeutic attitudes. Non-insulin-treated diabetics did not have more sickness days than the average population, but they were prematurely retired twice as often. Insulin-treated diabetics had more than twice as many sickness benefit days and were prematurely retired more than twice as often as average. The costs of these production losses were about USD 6,000 higher than average per individual and year. This was 20 times the difference in antidiabetic drug costs per individual and year for insulin vs. oral agents.

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ECONOMICAL DIABETIC CARE

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Health economics is of growing importance as the insatiable demand for optimal care must be curtailed by limited available resources. Accordingly a cost effective system to supply the necessary long-term care for the increasing diabetic population should be established. This we have done analysing that of a hospital based urban diabetic out-patient service. In one year 1,198 patients attended with 2,232 consultations. The costing methadology assessed the following resources used. Salaries for the consultant diabetologist, junior medical staff, specialist diabetic nurses, dieticians, chiropodist, general nurses, clerical, technical and secretarial staff amounted to £49,978 (ECU 69,969.2). Additional costs were : laboratory investigations (patients having urinalysis and blood testing for urea, electrolytes, lipids, glucose and haemoglobin A1c at each consultation) £10,979 (ECU 15,370.6), stationery and postage £534 (ECU 747.6) heating, cleaning and rates for accommodation £1,750 (ECU 2464). The total cost amounted to £63,251 (ECU 88,551.4) giving an average cost for out-patient diabetic care of £52.80 (ECU 73.9). The service provides three specialist clinics per week, out-patient education facilities and an additional 5½ days open access and out of hours telephone answering system for diabetic specialist nurse consultations. This system allows patients access to expert advice at all times and is extremely economical.

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SHORT- OR LONG-TERM INITIAL HOSPITALIZATION OF CHILDREN WITH IDDM: COST-EFFECTIVENESS ANALYSIS

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We carried out a randomized prospective study where we shortened the length of initial hospitalization from 23±4 to 9±3 days in half of 61 consecutive children with newly-diagnosed IDDM. Metabolic control, psychosocial adjustment, and costs of care were compared during 24-month follow-up. After having confirmed similar medical and psychosocial effectiveness of the hospitalizations, we now compare the costs of the treatment modes. - Total costs during the first month were on average 2.3 times higher in the long- than short-term treatment (FIM 74859±15074 and 32062±8778, p<0.001, one-way ANOVA) mostly due to the costs of hospital days (FIM 54593±17026 and 22633±9041, p<0.001). Travelling costs were 1.8 and parents' opportunity costs of work time lost 1.7 times higher in the long-term treatment (FIM 1881±955 and 1067±698, p=0.003; 9393±4221 and 5373±1976, p<0.001). Mean costs of ambulatory visits were FIM 30±164 in the long-term but FIM 2492±1509 in the short-term treatment. No significant differences were found between the groups during the following 23-months (total FIM 16712±7278 and 15664±11466). - We show for the first time that shortening of initial hospitalization of children with newly-diagnosed IDDM on average by 61% leads to a 48% decrease in the total costs during the first 2 years of the disease without influencing metabolic or psychosocial outcome of care.

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STRESS AND HORMONE SECRETION IN TYPE 1 DIABETES : RELATION TO UNSTABILITY ?

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The role of acute psychological stress in the metabolic unstability in diabetes is still controversial.To investigate the effect of a psychological stress on the counterregulatory hormone secretion, we studied the effect of a public speaking videorecorded in 20 type 1 diabetic patients and in 8 matched controls.The diabetics patients were subdivised in stable and unstable (>3 hypo or hyperglycemia /week in spite of regular glucose monitoring and adaptation of insulin therapy). During the test and on a day control blood pressure, catecholamines, cortisol, ACTH and GH were recorded .In the diabetic and the control group, the hormones rose significatively (cortisol:143±21%, ACTH:156± 20%, epinephrine:250±52%).The difference between controls and diabetics was not statistically significant. The increase of hormone levels was significantly (anova, p<0.01) higher in unstable vs stable diabetics : increase of 195±15% for cortisol and of 205±20% for ACTH .Eight of the 10 unstable diabetics had 3 time more ACTH and 2.6 more cortisol during stress; this degree of reaction occurs only in 2/10 stable diabetics. Fifty % of the brittle patients report correlation between their acute metabolic unstability and personnal psychological stresses. In conclusion hormonal response to psychological stress seems more important and frequent in brittle diabetes and could be one of its numerous pathogenic factors

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Anxiety disorders in type 1 and 2 diabetes. Influence of degenerative complications.

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Our purpose is to evaluate in 34 type 1 (insulin-dependent) and 27 type 2 (non-insulin-dependent) diabetic patients the different features of anxiety disorders by comparison with 25 patients suffering from hypertension. Analysis was performed following clinical diagnosis (according to DSM III-R criteria), Hamilton Anxiety Inventory, HSCL 58 and Beck Anxiety Inventory. The three groups were homogeneous, except for age (but not duration of disease) and body mass index. HbA1c was higher in type 2 patients (8.5 vs 10.1%, p < 0.001). In all groups the two major psychiatric clinical diagnoses were anxiety disorders (respectively 53, 59 and 60%, NS) and depressive disorders (respectively 21, 22 and 20%, NS). These disorders were more common in women in the three groups (p < 0.05). Self evaluation by HSCL 58 pointed out high scores in the three groups and a trend to higher anxiety scores in type 2 diabetics (mean 33.1) when compared to type 1 (mean 19.0) as well as in women . With Hamilton and BAI (which we recently validated in French) scales, somatic signs of anxiety (palpitation, nausea,...) were significantly more frequent and severe in type 2 than in type 1 diabetics when compared to psychological signs of anxiety (fear, apprehension,...). The influence of degenerative complications was also studied. Self rated or medically assessed complications were correlated, except for nephropathy. Type 1 diabetics with objective nephropathy had a significantly higher anxiety score. In type 2 diabetics, microangiopathy, diabetic foot and poor control (HbA1c > 10%) were also associated with high anxiety scores.

We conclude that type 1 and type 2 diabetics present with a high prevalence of anxiety (around 50%), associated with depressive disorders in 20%. The severity of anxiety disorders is significantly linked to the prevalence of diabetic microangiopathy.

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WAR-INDUCED PROLONGED STRESS AND METABOLIC CONTROL IN TYPE 2 DIABETIC PATIENTS

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The effects of war as a psychological stress on glycemia control have not been assessed yet. A randomly selected sample of displaced Type 2 diabetic persons (N = 50) was compared to a sex-age-weight-duration of diabetes-type of treatment-matched group of diabetic patients that had not left their home (N = 50). The self-reported stress (SRS), depression level, fasting blood glucose, postprandial blood glucose,, lipids, cortisol, HbA_{1c} and treatment were compared. Using the Mann-Whitney U test the two groups were found to be significantly different in scores for SRS symptoms, percieved stress intensity and depression level (p < 0.001). The proportion of examinees belonging to the category of clinically significant depression was larger in the group of displaced persons (44% vs. 10%, p < 0.001). This group also had a higher proportion of extreme SRS scores (69% vs. 29%, p < 0.001). The variables measuring metabolic control were log-transformed where appropriate and tested for difference. No significant difference between the groups was found for these variables. These results are not in accordance with anecdotal evidence on the effect of protracted stress on glycemia control.

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GLUCOSE TURNOVER DURING PARTURITION IN NORMAL PREGNANCY. J.L. Chiasson*, P. Maheux, B. Bonin, and A. Dizazo, and P. Guimond . IRCM and Maisonneuve-Rosemont Hospital, Montreal, Canada.

In six normal pregnant women undergoing spontaneous labor, glucose utilisation and the pancreatic hormones were measured during the latent (A_1) and active (A_2) phase of cervical dilatation(A), during fetal expulsion (B) and during placental expulsion (C). **Plasma glucose** increased throughout labor from 4.0 ± 0.2 mmol/L (A_1) to 4.7 ± 0.2 (A2), 5.4 ± 0.3 (B) and 5.5 ± 0.4 (C) compared to 4.9 ± 0.4 in control women. **Glucose utilisation** was markedly increased at 32.2 ± 7.2 μmol/kg•min during stage A_1, A_2 and B, decreased to 21.3 ± 5.2 during stage C, still much higher than post-partum women (8.1 ± 1.8). **Glucose metabolic clearance** was also increased to 8.0 ± 1.9 ml/kg•min during stage A_1 and decreased gradually to 6.9 ± 1.2 (stage A_2), 5.8 ± 1.1 (stage B) and 4.7 ± 0.6 (stage C), still higher than non-pregnant women (1.8 ± 0.8). **Plasma insulin** remained stable at 9.4 ± 1.4 μU/ml during stages A1, A2 and B, but increased to 20.3 ± 3.7 during stage C. **Plasma glucagon** was slightly increased throughout labor at 124 ± 13.2 pg/ml compared to non-pregnant women (89.9 ± 8.6). In **conclusion** these data indicate that during labor in normal pregnancy, glucose utilisation is markedly increased despite no change in insulin. It is suggested that muscle contraction (uterine and skeletal muscle) per se is a major regulator of glucose utilisation during labor.

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ROLE OF INSULIN ON LIPOPROTEIN LIPASE ACTIVITY IN MAMMARY GLAND DURING GESTATION IN THE RAT
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Lipoprotein lipase (LPL) activity in adipose tissue is stimulated by insulin and the decrease in its activity that normally appears during late gestation has been associated to the insulin resistance occurring in this situation. As opposite to adipose tissue, LPL activity increases in mammary gland (MG) during late gestation but it is not clear whether insulin modulate (or is responsible for) this change. To study this point we used rats at day 12, 15, 19 and 21 days of gestation that were compared to virgin controls. LPL activity in MG increased already at day 12 of gestation and remained so until day 21. After 24 h starvation LPL activity decreased in all the groups, and these changes were paralleled by similar ones in plasma RIA-insulin levels. To produce a prolonged hyperinsulinemic condition without causing hypoglycemia virgin and pregnants rats were infused with either 30 or 50% glucose (35 ml/day) from days 17 until 20, at which time they were killed. LPL activity in MG enhanced in all the groups infused with glucose and the greatest effect was found in pregnant rats receiving the 50% dose that had the highest plasma insulin levels. When using all the rats studied, the regression between LPL activity in mammary gland versus plasma insulin showed a lineal and highly significant correlation (r=0.668). Present results show that insulin enhances LPL activity in MG, and this effect is clearly seen during gestation in spite of the well known insulin resistance occurring in other tissues.

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LEUCINE FLUX IS INCREASED WHILST GLUCOSE TURNOVER IS NORMAL, IN PREGNANCY COMPLICATED BY GESTATIONAL DIABETES MELLITUS.
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In gestational diabetes mellitus (GDM), increased maternal glucose and amino acid supply have been postulated to be responsible for the fetal hyperinsulinaemia and macrosomia. We have therefore determined glucose and leucine flux in normal pregnancy and GDM. Eight GDM subjects were compared with 7 normal pregnancies, both in the second trimester. Eight non-pregnant controls were also studied. Leucine kinetics were studied using 1-[1-^{13}C]-leucine with measurement of keto-isocaproate isotopic enrichment and glucose kinetics were assessed using 6,6,^{2}H-glucose. Proteolysis (leucine appearance in the circulation) was increased in GDM, whether expressed in relation to total body weight (mean±SEM, non-preg 141.6±7.0, pregnant 90.4±6.7, GDM 109.5±5.1 umol/kg/hr, all $p<0.05$) or to lean body mass (169.3±6.2, 134.8±12.0, 154.9±11.5 umol/kgLBM/hr respectively, $p<0.05$ 1or3v2). Leucine incorporation into protein was also decreased (119.4±6.2, 74.2±4.8, 93.0±4.9 umol/kg/hr respectively, all $p<0.05$). Glucose production was similar in the three groups (135±4, 130±5, 129±11 mg/min). These results confirm the reduced leucine flux of normal pregnancy; in GDM leucine flux is higher than non-diabetic pregnancy at a time when glucose turnover is normal suggesting that leucine may be more important than glucose as a fetal insulin secretagogue, or fuel source, necessary for the development of macrosomia.

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WHOLE BODY PROTEIN METABOLISM IN LATE PREGNANCY AND THE EFFECTS OF INSULIN.
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We measured protein breakdown, synthesis and oxidation during fasting, in late pregnancy both basally and during a hyperinsulinaemic-euglycaemic clamp. 6 non-pregnant controls and 6 healthy women in late pregnancy underwent a six hour primed, continuous infusion of L-^{13}C leucine and $^{2}H_5$ phenylalanine (**Phe**) (0.5 *mg/kg/hr*) with a 40 *mU/kg/hr* insulin clamp for the final 3 hours. Indirect calorimetry,$^{13}CO_2$ collection and blood sampling for plasma KIC, phenylalanine, and tyrosine enrichment (GC/MS) were performed. There were no differences in basal protein breakdown or synthesis by either method and no differences were seen during the clamp. Glucose disposal [M/I(*mg/kg/hr/mU/l*) was significantly lower for the same blood glucose level : in the pregnant group ; 0.08 (0.04) *vs* 0.11 (0.03),$p<0.05$] Protein oxidation was similar in the control group both basally and during the clamp by either method but was lower in the pregnant group when measured by the phenylalanine method [Basal-**Phe**:Pregnant 0.17 (0.12),Control; 0.77 (0.40),*g/kg/d* Clamp-**Phe**:Pregnant; 0.11 (0.1) Control; 0.63 (0.1), *g/kg/d* $p<0.01$] . We conclude *(i)* that pregnant women show insulin insensitivity for carbohydrate but not protein metabolism in the third trimester , and *(ii)* the phenylalanine model produces different results for measuring protein oxidation in pregnancy.

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INSULIN-LIKE GROWTH FACTORS AND NEONATAL WEIGHT

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To elucidate the possible contribution of insulin-like growth factors (IGF) to neonatal weight, IGF I and IGF II were measured by specific radioimmunoassays in 4 macrosomic, 5 normal, 4 dystrophic, and 5 premature offsprings of non-diabetic mothers. Blood was drawn immediately *post partum* from the umbilical artery. Results (mean±SD):

Category (n)	IGF I (ng.ml^{-1})	IGF II (ng.ml^{-1})
Macrosomia (4)	87±25#	352±52
Normal (5)	40±15*	294±59
Dystrophy (4)	32±11*	327±16
Prematurity (5)	16±14*	264±40*¶

Significant differences (Mann Whitney U-Test): # $p<0.025$ vs. normal; * $p<0.025$ vs. macrosomia; ¶ $p<0.025$ vs. dystrophy.

IGF I levels are significantly correlated with birth weight ($r=0.8$, $p<0.001$). Accelerated fetal growth is associated with increased IGF I levels whereas retarded growth and prematurity are associated with decreased IGF I levels. In contrast, IGF II levels are not correlated with birth weight of infants delivered at term. Thus, in contrast to the commonly held view that IGF II is a fetal growth factor, IGF I appears to be more important for fetal growth in the human.

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Screening for gestational diabetes in different ethnic groups

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In order to compare the incidence of gestational diabetes and pregnancy outcome in different ethnic groups and to evaluate different screening strategies, we screened all women attending the antenatal clinic over a 12 month period at 28 weeks gestation with a 2hr post-prandial blood glucose; 207 Caucasian women, 91 Asian women and 61 Afro-Caribbean women had values > 6.0 mmol/l. These women subsequently underwent either oral glucose tolerance testing (75G OGTT) or performed home glucose monitoring (HGM) pre-breakfast, pre-lunch and 2h after the evening meal for 7 days. The results of OGTT were classified by WHO criteria as normal, impaired glucose tolerance (IGT) or diabetic. The incidence of gestational diabetes was 1.8% (Caucasian), 3.8% (Afro-Caribbean) and 4.4% (Asian) whilst IGT was found in 6.4%, 7.7% and 15.5% respectively. The incidence of large-for gestational age babies was 5.8%, 6.5% and 9.9% resp. Afro-Caribbean mothers had greater body mass indices and heavier babies than other ethnic groups. No perinatal deaths or episodes of severe neonatal hypoglycaemia occured during the study. The mean blood glucose obtained by HGM proved as effective as OGTT in identifying at risk pregnancies and HGM was popular with patients from all three ethnic groups.

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INSULIN SECRETION IN GESTATIONAL DIABETES MELLITUS.

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The aim of the study was to investigate endocrine function of pancreas in pregnancy complicated by gestational diabetes (GDM). The studies were performed on a group of 12 women with GDM and 6 in normal pregnancy (control group). During the study the hemoglobin A1c and fructosamine concentrations were within normal range. Fasting and after intravenous glucagon (1 mg) plasma glucose, insulin and C-peptide as well as 24 hrs urinary excretion of C-peptide were determined in the 28th, 36th week of pregnancy, and in 7 days following delivery. In the 36th week of pregnancy glucagon induced the increase of plasma C-peptide levels from 1.47±0.16 to 4.20±1.07 ng/ml in normal pregnancy and from 1.62±0.57 to 2.50±0.93 ng/ml in GDM. After delivery plasma C-peptide concentrations were 1.40±0.23; 2.86±0.74; 1.37±0.21; 2.45± 0.51 ng/ml, respectively. 24 hrs urinary C-peptide excretion in the 36th week of pregnancy in normal and in GDM were 159±21.9; 87±15.6 ug ($p < 0.01$) and following delivery 48±10.8; 60±15.8 (n.s), respectively. 24 hrs insulin secretion was calculated as 469 and 256 nmol in the 36th week of normal and GDM pregnancy and 141, 177 nmol after delivery, respectively. The experiment revealed the correlation between a maximal increase of plasma C-peptide concentration after glucagon and C-peptide urinary excretion and the dose of exogenous insulin in GDM. The results indicate a decrease in a capacity of pancreas to secrete insulin in GDM.

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MICROALBUMINURIA IN GESTATIONAL DIABETES

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It has been suggested that microalbuminuria (MA) identifies a subset of pregnant diabetic women more prone to develop gestosis. We aimed to assess the usefulness of MA to identify gestational diabetic women who will develop pregnancy-induced hypertension (PIH) later in pregnancy. We studied 150 pregnant women with gestational diabetes (GD) according to NDDG criteria. In the second and third trimestres, nocturnal urine samples (6 h) were collected. Samples with a positive urine culture were discarded. A nefelometric assay with a detection limit of 6 mg/l was used for measuring MA. Two hundred and twenty-four samples were assayed, with MA being detectable in 31 samples (13.8%) of 28 GD women (18.7 %). Albumin excretion rate was 14.35 + 14 mcg/min. GD women with or without MA did not differ in age, body mass index, weight gain or gestational age at GD diagnosis. Five women developed PIH (3.3 %) and only one of them (20%) had measurable MA four weeks before. In conclusion, 1) MA is a frequent finding in GD pregnancies and 2) In this series, MA haves a low yield to identify women who will later develop PIH.

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GESTATIONAL DIABETES MELLITUS: A METABOLIC, IMMUNOLOGIC AND GENETIC STUDY.

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Few, contrasting data were available on HLA-linked genotypical characteristics, frequency of islet-cell-antibodies (ICA) and of other immunologic markers in Gestational-Diabetes-patients (GDM). So we studied 65 patients: **Group 1**= 40 patients (3rd trimester of pregnancy, 12 insulin-treated, 28 on a diet), age 33±0.78 yrs, fasting-glycaemia 88±2.9 mg/dl, post-prandial-glycaemia 119±4.5 mg/dl, HbA1c 5.0±0.1%; **Group 2**= 25 patients 6-12 months after delivery, age 33±1.0 yrs. OGTT revealed: Normal-Glucose-Tolerance in 21, Impaired-Glucose-Tolerance in 3 patients. 1 patient developed Type-1-diabetes. 103 women with NGT were controls. In all patients we evaluated: 1) ICA, CF-ICA, other antibodies, (Standard-Indirect-Immunofluorescent- Technique); 2) IAA (RIA); 3) circulating CD4+, CD8+, CD3+, DR+, CD57 lymphocytes, CD4/CD8 ratio (MoAbs, cytofluorimetry); 4) HLA-typing (A-B-C-D-DR-DP-DQ) (microlymphocytotoxicity test).We found: -Group 1= ICA-IgG, CF-ICA and IAA in 2 patients (5%); -Group 2= IAA in 3 patients (12%), ICA in none; -no correlation between ICA and IAA, between IAA and insulin treatment; -in all patients= thyroid-autoantibodies in 6, adrenal-antibodies in 1, parietal-cell-antibodies in 2; -significant increase in CD3+ and CD57 as against controls; -CD4/CD8 ratio within normal ranges; -frequency of histocompatibility antigen: DR3=19%, DR4=2.3%, DRw8=2.3%, B8=6.9%, B15=2.3%, not different from controls. Thus, few GDM patients showed the immunologic markers of Type-1-diabetes, and their genetic situation was not different from controls.

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GLUCOSE STIMULATED ISLET AMYLOID POLYPEPTIDE AND GESTATIONAL DIABETES MELLITUS.

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Islet amyloid polypeptide (IAPP) is a 37 amino acid polypeptide with a possible early or late pathogenetic role in Type 2 diabetes. Gestational diabetes mellitus (GDM) is considered to be a variant of type 2 diabetes, propably an early stage of permanent diabetes. We studied wether glucose stimulated IAPP is higher in GDM patients than in healthy pregnant women. We studied 5 women with normal glucose tolerance (CON) and 5 women with GDM. A 75 g oral glucose tolerance test (OGTT) was performed measuring glucose, IAPP, insulin and c-peptide at 0, 30, 60, 120 and 180 minutes. IAPP was measured by radioimmunoassay after extraction. In CON glucose increased from 4.78 ± 0.15 to 5.48 ± 0.66 mmol/l (mean ± SEM) at 120 min., IAPP 12.86 ± 2.15 to 23.46 ± 2.26 pmol/l, insulin 9.80 ± 1.58 to 79.2 ± 13.45 mE/l and c-peptide 0.81 ± 0.14 to 3.24 ± 0.62 nmol/l. In CON age is 30.4 ± 2.4 years. In GDM glucose rose from 6.44 ± 0.72 to 9.80 ± 0.24 mmol/l at 120 min., IAPP 21.22 ± 3.84 to 56.44 ± 17.01 pmol/l, insulin 35.00 ± 11.58 to 143.40 ± 31.43 mE/l and c-peptide 1.15 ± 0.12 to 5.78 ± 1.25 nmol/l. In GDM age is 31.6 ± 2.6 years. It is concluded that glucose stimulated IAPP concentrations during OGTT are higher in GDM patients than nondiabetic pregnant women. This high IAPP level may add to pathogenesis of type 2 diabetes by providing proteinfibrosis for amyloidformation around beta cells.

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POST-PARTUM GLUCOSE INTOLERANCE IN GESTATIONAL DIABETES.

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Factors which influence the frequency of post-partum glucose intolerance in gestational diabetes are subject to discussion. We studied 130 women diagnosed of gestational diabetes with a 100g oral glucose tolerance test (OGTT)(NDDG, 1979). At two-three months post-partum (without lactation) an OGTT (75g) was done and classified as (WHO, 1985): diabetes mellitus (DM), impaired glucose tolerance (IGT) and normal (N). We compared the post-partum glucose tolerance with the 100g OGTT (fasting, 1h., 2h., 3h. glucose (FG, G1, G2, G3), glycaemic area (GA)), clinical risk factors and diabetes treatment. We found 19.2% with IGT and 4.6% with DM. The presence of IGT+DM was significantly higher ($p<0.01$) in relation to FG when subdivided into three groups: <5.8 mmol/L (15.5%); 5.8-7.2 mmol/L (38.2%); >7.2 mmol/L (66.6%). There were differences between IGT and N in FG, G2, GA ($p<0.01$) and G3 ($p<0.05$). Between DM and N in FG, G3 ($p<0.001$) and GA ($p<0.01$) and between DM and IGT in G3 ($p<0.05$). Patients with previous fetal mortality, gestational diabetes ($p<0.05$), and insulin therapy ($p<0.01$) had a higher frequency of glucose intolerance. In summary, factors which most influence incidence of post-partum glucose intolerance are antecedents of gestational diabetes, fetal mortality and insulin treatment together with FG, G3 and GA values during pregnancy.

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LOWER DIABETES INCIDENCE AFTER IMPAIRED GLUCOSE TOLERANCE IN PREGNANT THAN IN NONPREGNANT WOMEN.

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Impaired glucose tolerance (IGT) is a risk factor for type 2 (non-insulin-dependent) diabetes, but as the glucose load and criteria for IGT in pregnant and nonpregnant women often differ, the relative risk of subsequent diabetes is unclear. This risk was evaluated in 15-40 year old Pima Indian women in Arizona, U.S.A., who develop type 2 (non-insulin-dependent) diabetes at young ages and in whom a 75 g glucose tolerance test is used in both pregnant and nonpregnant women. IGT (World Health Organization criteria) was first recognized in 229 nonpregnant women with at least one previous pregnancy and in 63 pregnant women. After follow-up (mean=6 years) diabetes developed in 49% of nonpregnant women but in only 17% of women with IGT identified during pregnancy (χ^2=19.3, df=1, $p<0.001$). Controlled for age, degree of glucose intolerance, and number of pregnancies by proportional hazards analysis, the risk of diabetes was less than half (rate ratio=0.45) when IGT was first detected during pregnancy ($p=0.015$). Number of pregnancies and degree of IGT were also significant risk factors for diabetes. Women who develop IGT in the absence of pregnancy are at greater risk of subsequent diabetes than those who have IGT under the stress of pregnancy.

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DOPPLER UMBILICAL ARTERY VELOCIMETRY IN PREGNANCY COMPLICATED BY DIABETES MELLITUS

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To determine the relationship between the peak systolic to diastolic umbilical artery ratio (S/D) and (a) White's classification, (b) glycemic control in diabetic pregnancies. S/D was measured in 631 Doppler studies performed during third trimester in 115 diabetics (A_1=39, A_2=46, B=12, C=5, D=9, R=3, F=1). Mean S/D were in groups A:2.6 ±0.4, B-C (pregestational diabetics without vasculopathy) 2.7±0.3, D-R-F (pregestational diabetics with vasculopathy) 3.2±0.5. These values were significant ($p<10^{-4}$) with linear increase related to the severity of disease. The percentages of abnormal S/D (≥3) in the above groups were 7.1%, 17.7%, 53.9% respectively (linear trend x^2=19, $p<10^{-5}$). Mean HbAic were 4.8±0.8, 6.3±1.7, 6.0±1.3 respectively. Subdivision of diabetics into well-controlled (n=87, HbAic=4.6±0.6) and poor-controlled (n=23, HbAic =6.9±1.1) revealed significant difference in S/D values (2.6±0.4 v.s. 2.9±0.5, p<0.002). A significant positive correlation between S/D and HbAic was found (r=0.30, p<0.001).Using multiple regression analysis a significant positive correlation ($p<10^{-4}$) between S/D and White's classification adjusted for maternal age and parity was found. (a) Normal S/D can be expected in gestational well-controlled diabetics. (b) Increased risk of abnormal fetoplacental resistance is associated with the severity of diabetes mellitus, especially in combination with poor control.

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DIFFERENCES IN MATERNAL AND NEONATAL INSULIN-GLUCOSE AXIS IN ETHNIC GROUPS AT DIFFERENT RISKS OF TYPE 2 DIABETES

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Maori (M) and Pacific Islanders (PI) have 4 times more diabetes than Eurpeans (E). This study compared the glucose-insulin axis in non-diabetic expectant mothers and their neonates from these ethnic groups. Consecutive mothers had a 3 hour 100g glucose tolerance test (32 weeks gestation) and fasting venesection (36 weeks gestation) and neonates had umbilical cord samples and anthropometric measurements taken. Mothers with any medical condition, who smoked heavily or had a 'difficult' delivery were excluded from the study. 31E, 31M and 32PI completed the study. M were younger (23 v 27 years, p<0.01) and PI fatter (BMI(mean±SEM):E,M,PI: 25.2±0.9, 27.7±1.2, 30.3±1.0 kg/m^2 p<0.001). PI mothers had the highest 1 hour glucose (6.8±0.2, 6.9±0.2, 7.5±0.2 mmol/l; p<0.05), insulin concentrations (median (interquartile range): 19.5(13.0-22.5); 20.0(15.0-29.0); 25.5(18.0-37.5) mU/l, p<0.01) and insulin:c-peptide ratios (45.7(39.8-56.9), 57.8(43.2-82.8), 67.5(48.9-101.5), p<0.01).Other measures were similar. Among neonates, birthweights (3.5-3.6kg), cord fructosamine (203-209 umol/l), cord insulin (12-14mU./l) and cord glucose (5.2-5.5 mmol/l)concentrations were similar. However, E had smaller triceps skinfold (36±1.8, 42±1.4, 42±1.3 mm, p<0.05) and PI higher cord insulin:c-peptide ratio (41.9(35.1-56.3), 48.4 (40.6-52.6), 56.4(45.8-65.0) p<0.05). These results suggest that insulin handling in PI neonates is abnormal at birth. Whether this is due to exposure to minor hyperglycaemia while in utero or a genetic predisposition is unclear.

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Medical care of pregnant women in Białystok.

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To assess indices for diabetic control and pregnancy outcome all diabetic pregnancies from Jan'86 to March'92 were reviewed. We treated 116 patients aged 29±7,5 : with IDDM, 5 with NIDDM and 34 gestational diabetes. According to White classification the group consisted of 1 patient with A, 49 with B, 34 with G, 18 with C and 12 with D. All patients had participated in the educational programme. Intensified care was begun in 8±5 weeks of pregnancy and 22 patients planning pregnancies intensified care was begun earlier. Patients were treated with intensive insulin therapy and continued selfmonitoring. Patients performed 4 point home reflectometr profile aiming at pre and postprandial glycemia 90mg% and 140mg% respectively. The mean value of fructosamine significanty decreased during pregnancy. Algorithm of insulin therapy in all groups varied significantly during pregnancy. There were no spontanious abortions. Mean gestation of delivery was 38± 1 weeks. Ceasarean section rate was 60%.Perinatal mortality decreased from 16% in 1986 to 2,87% in the following years. Congenital malformation rate was 8,7% in 1986 and 7,2% in 1991. Mean birth weight was 3526±788 g, 86% newborns had 10 points on theAbgar scale.

The majority of diabetics (82%) would accept the whole management programme again, and 96% could advise other pregnant diabetic women. 82% of patients are continueing intensive insulin therapy after delivery.

The precision of care and current management stratigies for diabetic pregnancy were assessed by patients questionnaires which were sent to 100 patients, The response rate was 90 %.

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COMPUTER ASSISTED ANALYSIS OF INSULIN REQUIREMENT IN DIABETIC PREGNANCY.

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Insulin requirement was monitored by computer assistance in pregnant type 1 diabetic patients (N = 40) employing functional insulin therapy, i.e. the basis-bolus principle with multiple insulin injections and blood glucose self control (N > 6/d). Thereby near normoglycemia was maintained throughout pregnancy as judged by HbA1c < 6 %. Self-monitored BG-values, daily carbohydrate intake as well as daily insulin requirement were monitored. Constancy of daily insulin dose at constant glycemia was seen throughout the first five months of pregnancy (1st month: 40.7 ± 13.6 U/d, 5th month: 45.8 ± 14.6 U/d. N.S.) increasing thereafter to 72 ± 29.1 U/d (p < 0.01). Daily insulin need did not decline until 8 days before delivery (day -8: 69 ± 33 U/d, day -3: 56.8 ± 20.6 U/d). However, a subgroup of 8 patients with suspected placental dysfunction showed a "premature" linear decrease in daily insulin requirement from day -28 (80 ± 27 U/d) to day -3 (58.3 ± 13.9 U/d; p < 0.01). Mean daily intake of carbohydrates (144 ± 36 g) and mean daily blood glucose (101 ± 22.8 mg/dl) did not change significantly during the last four weeks of pregnancy. Since a premature decrease in insulin need 4 weeks before delivery could indicate placental dysfunction with the danger of fetal asphyxia, computer assisted analysis of metabolic parameters seems to be a valuable tool in the management of pregnant type 1 diabetic women.

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MICROALBUMINURIA IN TYPE 1 DIABETIC WOMEN BEFORE CONCEPTION, DURING THE COURSE OF PREGNANCY AND AFTER DELIVERY.

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The aim of present study was to evaluate the influence of pregnancy on renal function in a group of type 1 diabetic women without vascular complications. Twenty-four type 1 insulin-dependent diabetic (mean age 27.13±5.38 years;duration of diabetes 10.36±7.93 years)(DM1) and 7 normal women (30.33±4.33 years)(NGT) were examined during pregnancy. Before conception, at 12th, 20th and 34th week of gestation and 2 months after delivery, glycosilated haemoglobin levels (HbA1c), day and night urinary albumin excretion (RIA method)(dUAE and uUAE), and creatinina clearance (CrCl) was determined. The CrCl is increased respect to pre-pregnancy values from 12th week until 34th week and decreased 2 months after delivery in both groups. The dUAE and nUAEa is higher at pre-pregnancy control and at 12th week and 2 months after delivery respect to 20th and 34th weeks in DM1 group and there is no difference between the two groups examined. Glycosilated haemoglobin is higher at pre-pregnancy control and 12th week and 2 months after delivery in DM1 group (p<0.05,36th vs 2mpp). In conclusion, the pregnancy does not determine a worsening of microalbuminuria in diabetic pregnant women, if the glycaemic control is optimized. Before conception and after delivery, when the glycaemic control is not optimized, microalbuminuria > 20 mcg/min may appear.

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METABOLIC AND CLINICAL CORRELATIONS WITH CORD-BLOOD C PEPTIDE IN TERM AND PRETERM NEWBORN INFANTS OF DIABETIC MOTHERS.

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We have previously reported higher cord-blood C peptide levels (CPR) associated with higher fetal morbidity in IDMs. In this study we investigated CPR and glucagon (IRG) plasma levels in a large population of preterm and term infants of diabetic (IDMs) and non-diabetic (C) pregnant women to see their correlations with fetal morbidity. CPR values (pmol/l) and CPR/IRG molar ratios in 57 term IDMs vs 72 C were respectively 329±43 vs 233±17 and 7.7±1.2 vs 3.7±1; CPR values and CPR/IRG molar ratios in 71 preterm IDMs vs 77 C were respectively 616±69 vs 260±100 and 22.2±3.7 vs 3.7±1.0. The differences were statistically significant at all times. Among IDMs, the CPR values were higher in 34/36-week than in 37/38-week premature infants: 868±135 vs 523±76. Preterm IDMs showed a higher prevalence of foetal morbidity and, among them, higher CPR values (>616 vs <616) were associated with higher prevalence of hypocalcaemia (36% vs 8.9%), hypoglycemia (56% vs 29%) and RDS (40% vs 9%). Ordering all CPR values of IDMs in three groups: <233 (mean of controls), 233-932 and >932 (mean +2SD), we found a progressive and significant increase of jaundice, hypocalcaemia, hypoglycemia, RDS, malformations. This increase was significantly higher in preterm vs term IDMs.

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PANCREATIC ISLET FUNCTION IN INFANTS OF DIABETIC MOTHERS AND INFANTS WITH RHESUS HEMOLYTIC DISEASE.

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The feature of neonatal hypoglycemia assimilates the infants of diabetic mothers (IDMs) and those with Rhesus Hemolytic Disease of the newborn (HDN). To investigate the A- and B-cell functions of these infants during foetal life, we collected the amniotic fluid (AF) from 92 diabetic (34-36 wk), 73 Rh (25-39 wk) and 48 control (C) pregnant women in basal conditions (AF-BAS) and two hours after an intravenous arginine test (AF-ATT). We assumed that AF insulin (IRI) and glucagon (IRG) were of foetal origin and that the ATT could influence them. In AF-BAS, IRI (μU/ml), IRG (pg/ml) and their molar ratios (I/G) were respectively: 6.2±0.6, 66±9.6, 4.8±1.7 (C); 11±2, 24±6, 21±7 (IDMs); 7.7±0.7, 66±5, 3.4±0.4 (HDN). In AF-ATT, IRI, IRG and I/G were: 7±1, 66±5, 3.4±0.4 (C); 28±5, 30±4, 68±26 (IDMs); 18±6, 19±7, 30±9 (HDN). In AF-BAS, the differences between IDMs and the other two groups were statistically significant. In AF-ATT the IRI, IRG and I/G values were unchanged in C, whereas in both IDMs and HDN they were significantly changed. These results indicate that a derangement of A- and B-cell function is demonstrable already during foetal life in both IDMs (more evidently) and HDN (after ATT amplification) in whom B-cell hypertrophy is present despite the absence of hyperglycemia.

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LOW SERUM GLUCOSE CONCENTRATIONS INDUCES GLUCAGON COUNTERREGULATION IN HEALTHY NEWBORNS.

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Before feeding regulatory mechanisms maintain blood glucose concentrations of the newborn to meet the demands of the central nervous system. The role of glucagon in this process has been investigated. Healthy, term babies without clinical signs of hypoglycemia were studied. A capillary blood sample was obtained at 3-15 hours (median 6 hours) of age, a second sample 24 hours later and serum concentrations of glucose, insulin and glucagon were measured. Glucose concentrations at the first sampling averaged 2.1 ± 0.5 mM (mean ± SD; n=51) and were positively correlated with postnatal age. At the second sampling glucose concentrations had increased to 3.0 ± 0.5 mM. Glucagon concentrations were 541 ± 219 pg/ml at the first sampling and inversely correlated with glucose concentrations. At the second sampling glucagon concentrations had decreased to 406 ± 163 pg/ml. Insulin concentrations were 12.1 ± 2.4 and 10.6 ± 2.1 μU/ml, respectively, and did not correlate with glucose concentrations. In a multiple regression analysis glucose concentrations were inversely correlated with glucagon concentrations and positively correlated with birth weights but not correlated with insulin concentrations or other neonatal and maternal characteristics. The results suggest that glucagon is part of normal counterregulation against hypoglycemia and that neonatal energy stores, as indicated by birth weight, influence the ability to maintain normoglycemia.

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CHANGES IN INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS IN DIABETIC PREGNANCY.

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Intrauterine growth retardation has been related to changes in circulating insulin-like growth factor binding proteins (IGFBP's). To investigate whether the changes in fetal development found in diabetes could be related to IGFBP's we have examined the plasma profiles of IGFBP-1 and 3 in rats. Normal (N) (blood glucose 6.3 mmol/l) and streptozotocin diabetic (D) (blood glucose 18.7 mmol/l) rats and their fetuses were bled on day 19 of gestation. Samples were incubated with ^{125}I-IGF-I and subjected to high performance size-exclusion chromatography. The profiles obtained were related to molecular weight standards and expressed as % total label applied. Diabetes resulted in a significant decrease in IGFBP-3 peak (N = 13.3 ± 2.2 (SD)%, D = 8.2 ± 1.9%, $p < 0.03$) in the mothers. In the fetuses IGFBP-3 accounted for approximately 1% and was not different between D and N, but was significantly different from the mother in both groups (N $p < 0.02$, D $p < 0.01$). Diabetes significantly increased the IGFBP-1 peak in the mother (N = 48.5 ± 7.7%, D = 63.1 ± 4.4%, $p < 0.05$). This peak accounted for 44.6 ± 10.5% in N and 57.2 ± 4.2% in D (p=NS) in the fetuses and were not different from maternal values. These changes in the profiles of IGFBP's could reflect alterations in the bioavailability of IGF's in diabetic pregnancy.

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EFFECTS OF EXPOSURE TO MATERNAL TYPE I DIABETES IN UTERO ON GLUCOSE DISPOSAL AND INSULIN SECRETION IN THE CHILDREN.

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Exposure to maternal hyperglycemia during fetal development has been reported to impair insulin secretion and glucose disposal in the offspring and may predispose them to NIDDM. To investigate this hypothesis, 11 normoglycemic children of IDDM mothers were compared with 25 normoglycemic children of IDDM fathers. While both have an IDDM parent, only offspring of IDDM mothers have been exposed to hyperglycemia in utero. The groups were equal with regard to age (mean 23 years, range: 12-39), percent ideal body weight (110%), fasting glucose (4.2 mM) and glucose disposal rate (Kg -2.1) . Fasting insulin was slightly lower in the offspring of IDDM mothers (76 as compared to 95 pM, p=0.20), but insulin response to glucose were similar.

Bergman's minimal model of glucose disposal and insulin secretion was fitted to glucose and insulin values from a 3-hour intravenous glucose tolerance test done in each individuals. Means for indices of insulin sensitivity (S_I), insulin independent glucose disposal (S_G) and first and second phase insulin secretion were:

Parent with IDDM	S_I	S_G	Phi_1	Phi_2
Mother	8.0	2.1	0.3	2.5
Father	7.4	2.6	0.5	2.6
t-tests, p value:	0.71	0.23	0.25	0.52

Conclusion: Exposure during gestation to the maternal hyperglycemia of type I diabetic mothers does not appear to have a lasting effect on the ability of the exposed offspring to dispose of intravenous glucose or on its beta cell secretion capacity (Phi_2).

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Muscle insulin sensitivity is drastically impaired during insulin-induced hypoglycemia

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The metabolic events occurring in skeletal muscle during insulin-induced hypoglycemia have not been directly explored. In the present study, the metabolic response of muscle tissue to mild hypoglycemia has been examined in 11 normal subjects by using the forearm perfusion technique. Insulin was infused i.v. at a rate of 0.5 mU/Kg/min for 4 hours. In 5 subjects blood glucose was maintained constant at its basal level (E) while in 6 subjects it was clamped at 50 mg/dl (H). Forearm glucose uptake (FGU) increased 8-9 fold from baseline (0.75±0.2 mg/l/min) during euglycemia, whereas it remained very close to the basal value (0.92±0.3 mg/l/min) throughout the hypoglycemic study. The difference in FGU between E and H was statistically significant at all time points ($p<0.05$-0.001) and remained significant even when normalized by the glucose concentration (clearence) ($p<0.05$-0.001). Arterial lactate concentrations remained substantially unchanged in both studies. Forearm lactate balance was similar in the basal state in E and H and increased during insulin infusion in both groups. However, by the 3rd hour of the study, the release of lactate in H was significantly higher as compared with E (9.0±1.7 and 3.6±1.1 µmol/l/min, $p<0.05$). The ratio of lactate release to glucose uptake was 7±2% in E and 64±18% in H ($p<0.02$). Forearm blood flow remained unchanged during E whereas it increased by 40 % during H. These results indicate that during insulin-induced hypoglycemia a state of insulin resistance develops in muscle tissue and that the intracellular glucose metabolism is directed preferentially towards non-oxidative pathway with increased lactate formation.

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DECREASE IN REGIONAL CEREBRAL BLOOD FLOW IN TYPE 1 (INSULIN-DEPENDENT) DIABETES: RELATION TO HYPOGLYCEMIC EVENTS ?

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Regional cerebral blood flow (rCBF) was studied in Type 1 (insulin-dependent) diabetic patients and related to prevalence and severity of their former hypoglycemic episodes; rCBF was determined semiquantitatively by brain-SPECT after IV-injection of 99mTc-hexamethyl-propyleneamine-oxime (HMPAO). Diabetic patients on intensified insulin treatment were subdivided in three groups (A,B,C). Group A comprised patients (age 34±10yrs; HbA1c 7.8±1.1% - N 4.0-7.5% - mean±SD; n=6) with diabetes duration of <2yrs and without history of hypoglycemic coma but with impending hypoglycemia (≥ 2/week to daily). B differed from A by duration of disease (17±10yrs; age 39±9yrs; HbA1c 8.7±1.3%; n=12). C-patients had experienced at least one hypoglycemic coma within the last five years (diabetes duration 24±7yrs; age 43±10yrs; HbA1c 8.1±1.5%; n=10). rCBF of normal volunteers (age 31±5yrs; n=12) provided reference. All patients in C showed a significant decrease in rCBF, defined as count values lower than mean±2SD in controls, in at least one cerebral zone. In A no abnormalities were encountered. In B regional hypoperfusion was demonstrated in 9/12 patients; particularly among these 9, 4 patients had rather short duration of disease (i.e. 3-8yrs). Conclusion: decreased rCBF can be evidenced by HMPAO-SPECT in Type 1 (insulin-dependent) diabetes even early in its course. Its prevalence in patients on intensified insulin treatment with impending hypoglycemia urges further evaluation.

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ACUTE COMPLICATIONS IN INSULIN DEPENDENT (TYPE 1) DIABETES: THE EURODIAB COMPLICATIONS STUDY.

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The frequency of keto-acidosis and severe hypoglycaemia has been assessed in 3296 insulin-dependent (Type 1) diabetic patients attending 31 diabetes centres in 16 European countries. Patients were in the age range 15-59 years (mean ± sd; 32.3 ± 10.2) and had an average duration of diabetes of 14.2 ± 9.5 years. At least one episode of keto-acidosis requiring hospital admission in the previous year had occurred in 8.5% of subjects. The between-centre frequency of keto-acidosis varied considerably from 0% to 50%. One or more episodes in the previous year of severe hypoglycaemia, requiring the assistance of another person, were reported by 32% of patients, with a between-centre variation of 12% to 50%. Risk of severe hypoglycaemia was higher in patients with good control; adjusted odds ratio 2.1 (95% CI 1.5-3.0) for patients with mean HbA1 (A1c) ≤ 8% (6%), compared to patients with HbA1c (A1c) ≥ 12% (9%). Compared to patients with normal autonomic function tests (lying-to-standing heart rate and systolic blood pressure changes), patients in whom both tests were abnormal had a higher risk of severe hypoglycaemia (adjusted odds ratio 1.7, 1.2-2.4). These potentially avoidable acute complications of Type 1 diabetes remain a serious problem in some parts of Europe and efforts should be directed towards their prevention.

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SEVERE HYPOGLYCAEMIA IN TYPE-1 DIABETES IS RELATED TO HbA_1c, DIABETES DURATION AND INSULIN THERAPY STRATEGY

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In the DCCT feasibility study (Diabetes Care 10:1,1987) the occurence of severe hypoglycaemia (SHG) was 3 times higher in the experimental group than in the standard group suggesting that improvement of diabetes control increases the risk for SHG Thus, it was of interest to reanalyze, whether the degree of diabetes control (HbA_1c), strategy of insulin treatment, and duration of diabetes influence the risk for development of SHG. In total 178 type-1 diabetic patients (mean age: 34+13 yrs; mean HbA_1c: 8.0%+1.8; mean duration of diabetes: 13+12 yrs) were evaluated concerning the occurence of SHG during an observation period of 18 months. We compared 159 patients-years under basal-bolus-insulin-treatment (BBIT) with 170 patients-years under conventional-insulin-treatment (CIT). In the total group frequency of SHG (per patient-year) was significantly ($p<0.05$) related to the degree of diabetes control (HbA_1c <6.5%: 0.44; HbA_1c 6.5-8%: 0.32; HbA_1c 8-10%: 0.24; HbA_1c>10%:0.17. In the higher HbA_1c-range (6.5-10%) frequency of SHG was significantly lower in the BBIT group than in patients with CIT ($p<0.05$), whereas at low HbA_1c values(<6.5%) frequency of SHG (per patient-year) was very similar (p:NS) in the BBIT-(0.46) and in the CIT-group (0.42). The frequency of patients presenting with SHG was significantly higher in patients with longlasting diabetes (>10 yrs: mean duration of diabetes: 22.3+9.7 yrs; n=90) than in patients with short duration (<10 yrs: mean duration: 3.7+2.7 yrs; n=88). During the observation period 25 out of 90 (27.7%) patients in the former group, but only 9 out of 88 (10.2%) in the latter group experienced a SHG ($p<0.0003$). Our data indicate that (1) the frequency of severe hypoglycaemia in type-1 diabetic patients is rather high despite the use of intensified education programs. (2) Patients with low HbA_1c values and longlasting diabetes have the highest risk. (3) Basis-bolus-insulin therapy strategy can lower the SHG risk in patients with HbA_1c values above 6.5%, whereas (4) in patients with near-normoglycaemia the SHG-risk is not different from patients with conventional insulin treatment.

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EXERCISE AND ALCOHOL - INDUCING NOCTURNAL HYPOGLYCEMIA ?

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Exercise of long duration may be expected to induce hypoglycemia, but possibly post-exercise consumption of alcohol contributes to hypoglycemic episodes by inhibition of hepatic gluconeogenesis. Therefore we monitored blood glucose levels of eight Type 1 diabetic patients during a night at rest and a night after 3 hours bicycle ergometry (19.00 - 22.00 hours) at a work load of 30% maximum. Fluid and carbohydrate intake were standardized, regular insulin was reduced according to exercise, whereas basal NPH insulin and the usual evening snack remained unaltered. For comparision the same measurements were performed on the same individuals under equal conditions with the additional ingestion of alcohol (0,7g/kg body weight, 22.00 - 23.30 hours). Blood glucose results are presented as means ± SEM [mmol/l], statistics by multiple analysis of variance MANOVA. During the exercise nights (with/without alcohol) glucose levels declined from 9,2±1,3/9,0±1,2 at 19.00 hours to 4,7±0,6/4,1±0,2 at 22.00 hours. Later on they increased to 8,0±1,1/7,3±0,9 at 2.00 hours and 9,3±0,9/7,8±1,1 in the following morning. During the nights at rest blood glucose levels were 9,9±1,5/9,8±1,3 (19.00 hours), 8,7±1,4/10,4-±1,2 (22.00 hours), 5,8±0,8/6,1±1,2 (2.00 hours) and 8,3±1,1/ 8,4±1,7 (6.00 hours). Blood glucose levels remained higher after exercise compared to the resting nights ($p<0,05$). Alcohol up to 0,7g/kg body weight does not affect the blood glucose levels.

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COGNITIVE FUNCTION IN TYPE 1 DIABETIC PATIENTS IS UNCHANGED AFTER NOCTURNAL HYPOGLYCAEMIA

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Eight Type 1 (insulin-dependent) diabetic patients without any diabetic complications were studied thrice. The aim was to evaluate the influence of nocturnal hypoglycaemia on neuropsychological and reaction time tests the following morning. Hypoglycaemia was induced by iv-insulin infusion, blood glucose nadir was 1.5 (1.1-2.5) mmol/l. Duration of hypoglycaemia (blood glucose < 3 mmol/l) was 101 (45-180) min. Whole night sleep statistics for all patients showed no statistical differences between normoglycaemic and hypoglycaemic nights. Each patient was then used as his own control and periods with blood glucose concentration < 3 mmol/l were compared to exactly the same periods from nights where the blood glucose level was > 5 mmol/l. The amount of deep sleep was reduced and replaced by superficial sleep and arousals of short duration during hypoglycaemia. Further, the reduction in deep sleep was replaced later at night. Neuropsychological test scores and visual reaction time measurements in the morning showed no differences between the normoglycaemic and hypoglycaemic nights. In conclusion: Nocturnal hypoglycaemia led to sleep disturbances but did not have measurable impact on cognitive function the following morning in Type 1 (insulin-dependent) diabetic patients.

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INTRANASAL GLUCAGON TREATMENT OF HYPOGLYCAEMIA IN INSULIN-DEPENDENT DIABETIC CHILDREN

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The aim of the study was to compare the hyperglycaemic effect of intranasal-administered glucagon with subcutaneously-injected glucagon in Type 1 (insulin-dependent) diabetic children with insulin-induced hypoglycaemia. In eleven children, aged 7-12 years, hypoglycaemia (blood glucose concentration 2 mmol/l), was induced twice with an interval of one week. An insulin clamp was used and the children were randomized to be given either 1 mg of intranasal (i.n.) or 0.5 mg of subcutaneous (s.c.) glucagon on the first occasion. The induction of hypoglycaemia was almost identical on the two occasions. Equal blood glucose and plasma insulin concentrations were achieved before the glucagon treatment was given. A significant rise of the blood glucose concentration was obtained in both groups 15 min after glucagon was given, 1.3 $\pm$ 0.3 mmol/l (i.n.) v.s. 1.4 $\pm$ $\pm$ 0.3 mmol/l (s.c.). There was no significant difference between the rise of blood glucose concentration in the two groups until 45 min after glucagon treatment. Nausea was observed in 10/11 children given s.c. glucagon compared to 1/11 given i.n. glucagon. Minor nasal irritation was observed after i.n. glucagon treatment.
It is concluded that intranasal glucagon is an efficient treatment of insulin-induced hypoglycaemia in diabetic children.

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SUSTAINED EFFECT OF INTRANASAL GLUCAGON ON GLUCOSE RECOVERY AFTER HYPOGLYCAEMIA.

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To investigate the effect of intranasal glucagon on glucose recovery after insulin induced hypoglycaemia twelve healthy subjects were studied. The subjects received apart from an insulin bolus: Somatostatin and propranolol to eliminate counterregulation and 3-^{3}H-glucose to estimate glucose turnover. When hypoglycaemic the subjects received either A: Intramuscular glucagon from pancreatic extraction, 1 mg or B: Intranasal genetically engineered glucagon, 2 mg.

Five min after treatment, A showed a 0.3 mmol/l increase in p-glucose, whereas p-glucose decreased 0.1 mmol/l after B, $p=0.001$. P-glucagon and glucose appearance rate (Ra) measured at the same time were lower after B than after A, $p=0.04$ and $p=0.003$. Incremental AUC for p-glucose and Ra for the whole study period after treatment (90 min) did not differ for the preparations. AUC for p-glucagon was significantly larger after B than after A, $p=0.009$. Both preparations had a sustained effect on p-glucose with end values of p-glucose of 7.7 mmol/l for B and 9.7 mmol/l for A, (ns).

In conclusion: (1) Glucose recovery started within 5 min after A and within 10 min after B. (2) Apart from the slightly later start of glucose recovery after B, the preparations were equally potent in augmenting p-glucose as well as Ra (3) Both preparations proved a sustained effect on p-glucose.

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THEOPHYLLINE ENHANCES GLUCOSE RECOVERY AFTER HYPOGLYCAEMIA.

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The principal mediators of glucose counterregulation (glucagon and adrenaline) utilize intracellular cyclic AMP to mediate glucose release. Since theophylline increases cyclic AMP (by inhibiting its decomposition) we investigated the effect of theophylline on glucose recovery after hypoglycaemia. 11 healthy subjects (A) and 8 type 1 insulin-dependent patients (B) participated in two blinded experiments in randomized order receiving on both days an insulin bolus of 0.15 IU Actrapid Human and 3-^{3}H-glucose to estimate glucose turnover. One day theophylline (i.v. bolus 220 mg followed by i.v. infusion 1 mg/kg/h) was administered from 1 h before induction of hypoglycaemia till the end of the study period, on the other day NaCl.

P-glucose before hypoglycaemia was equal on the two study days. P-glucose area under the curve (AUC) was larger with theophylline than with NaCl, $p=0.04$ (B), $p=0.003$ (A). Glucose appearance rate was greater 15-60 min after insulin with theophylline for B, $p=0.02$. AUC for cyclic AMP was larger with theophylline for B, $p=0.01$. For A cyclic AMP was augmented with theophylline 30 min after insulin, $p=0.04$.

In conclusion: Glucose recovery after hypoglycaemia is significantly increased when theophylline is administered in asthma dosage before hypoglycaemia is induced. This may be due to a significant enhancement of cyclic AMP response.

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SYMPTOMATIC AUTONOMIC RESPONSES TO ACUTE HYPOGLYCAEMIA ARE PRESERVED IN DIABETIC PATIENTS WITH AUTONOMIC NEUROPATHY

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Hypoglycaemia unawareness has often been attributed to autonomic neuropathy in diabetic patients. To determine whether autonomic neuropathy modifies the symptomatic response to hypoglycaemia, an infusion of soluble insulin (2.5 $mUkg^{-1} min^{-1}$) was used to induce hypoglycaemia in two age and sex-matched groups each of 8 insulin-dependent diabetic patients : Group A had symptomatic autonomic neuropathy while Group B had normal cardiovascular autonomic function. Co-incidental increments in sweating and heart rate were used to identify the onset of the autonomic reaction (R). Serial measurements of plasma glucose and counterregulatory hormones were made and questionnaires administered to characterise the symptomatic response. Mean plasma glucose was similar at R in both groups (Group A: 1.7 $\pm$ 0.2 v Group B: 1.6 $\pm$ 2 mmol/l, p=ns). The mean intensity of autonomic symptoms (Group A: 12 ± 1 v Group B: 16.2 ± 2, p=ns) and neuroglycopenic symptoms (Group A: 11.7 ± 2.2 v Group B: 12.0 ± 1.6, p=ns) was also similar at R. Haemodynamic and sweating responses did not differ. The magnitude of the adrenaline response was significantly lower in the neuropathic patients (Group A) from R to R+60 minutes (MANOVA $F=19.4$, $p<0.001$). Despite subnormal secretion of adrenaline secretion patients with diabetic autonomic neuropathy had preservation of the symptomatic and physiological responses to hypoglycaemia, suggesting that hypoglycaemia unawareness and autonomic neuropathy are not causally related.

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HYPOGLYCEMIA REDUCES LIVER INSULIN SENSITIVITY.

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The effect of insulin concentration on whole body glucose uptake (GU), glycolysis, overall hepatic glucose production (HGP) hepatic glycogenolysis and gluconeogenesis was examined, under identical conditions of hypoglycemia, in three groups of rats. 6h fasted conscious rats were infused with Phloridzin (PHLOR: 3.0 mg/kg·min), or low insulin (LOW: 4.0 mU/kg·min) or high insulin (HIGH: 20 mU/kg·min) + octreotide (100 ng/kg·min), [3-^{3}H]-glucose and [U-^{14}C]-lactate. Hypoglycemia (3.3 mmol/L) was reached in 20 min and clamped for 40 min with variable glucose infusion. The ratio between ^{14}C-UDPG (reflecting ^{14}C-G6P) and 2x^{14}C-PEP (both purified by HPLC from liver samples) measured the contribution of gluconeogenesis. Both GU and glycolysis significantly increased with the augmenting insulin dose (GU PHLOR: 34.6±3.0 µmol/kg·min; LOW: 58.2±3.7 µmol/kg·min, p<0.01; HIGH: 116.1±8.8 µmol/kg·min, p<0.01). HGP was unaffected by low insulin (PHLOR: 58.7±1.3 µmol/kg·min; LOW: 56.3±3.7 µmol/kg·min) but was markedly reduced by high insulin (HIGH: 28.9±5.4 µmol/kg·min, p<0.01). Liver glycogenolysis was gradually but significantly reduced by insulin (PHLOR: 46.7±1.4 µmol/kg·min; LOW: 36.2±3.1 µmol/kg·min, p<0.01; HIGH: 14.3±4.8 µmol/kg·min, p<0.01). On the contrary, gluconeogenesis significantly increased with low insulin (PHLOR: 10.8±2.2 µmol/kg·min; LOW: 26.8±4.5 µmol/kg·min, p<0.01) but was suppressed again by high insulin (HIGH: 16.3±2.0 µmol/kg·min). These data indicate that, in presence of identical hypoglycemia: 1) insulin concentration has a major effect on relative gluconeogenesis and glycogenolysis; however 2) the sensitivity of overall HGP to insulin is reduced.

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Gluconeogenesis is the Main Component of Hepatic Glucose Production during the Early Response to Hypoglycemia in Diabetic Rats.

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The time-course of gluconeogenesis (GNG) and glycogenolysis (GLG) was examined during insulin (8 mU/kg·min)-induced hypoglycemia (HYPO) in control (C) and diabetic rats (D). Two groups of conscious 6 h fasted rats were studied: 1) C (PG=7.2±0.1 mM); 2) D (90% pancreatectomy;PG=15.6±1.1 mM). All rats received an infusion of [3-^{3}H]- or [2-^{3}H]-glucose and [U-^{14}C]-lactate; hepatic glycogen concentration, ^{14}C-PEP and ^{14}C-UDPG specific activities (SA) were meastured at baseline and at 40, 60 and 90 minutes of HYPO. The ratio between ^{14}C-UDPG SA and 2x^{14}C-PEP SA (the former reflecting G6P SA) measured the per cent of glucose output derived from PEP-GNG. Basal hepatic glucose production (HGP) was 62% higher in D compared to C (107.8±7.4 vs 67.2±2.9 µmol/kg·min; p<0.01). In response to HYPO, HGP in C was significantly increased at 40 (79.7±4.2 µmol/kg·min) and 60 min (80.7±5.4 µmol/kg·min). In D, HGP decreased at 40 (63.4±8.3 µmol/kg·min) and 60 min (68.7±8.4 µmol/kg·min) and returned to basal by 90 min (99.7±7.1µmol/kg·min). In C GLG was dramatically enhanced at 40 and 60 min and represented 100% and 68%, respectively, of HGP. In D, GLG failed to increase at 40 min and GNG represented 84% and 65% of HGP at 40 and 60 min. Glucose cycling was 2-3 fold increased in D compared to C at all time points.

These data indicate that the glycogenolytic response to HYPO is severely blunted and delayed in this diabetic model and gluconeogenesis represents the only early response to severe hypoglycemia.

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AUTONOMIC MEDIATION OF PANCREATIC POLYPEPTIDE AND GLUCAGON RESPONSES TO 2-DEOXY-D-GLUCOSE AND HYPOGLYCEMIA IN THE MOUSE.

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Neural control of pancreatic polypeptide (PP) release has not been previously investigated in the mouse. PP was measured with a new radioimmunoassay which selectively detects PP in rodent plasma. In addition, it is not known if increased glucagon secretion during hypoglycemia in mice is neurally mediated or due to a direct effect of hypoglycemia on the islet. Neuroglucopenia induced by 2-deoxy-D-glucose (2-DG, 500 mg/kg) increased plasma PP in fasted mice to 44 ± 4 vs 25 ± 2 pmol/L with saline (p<0.01). This increase was abolished by atropine or hexamethonium (both p<0.01). Similarly, the PP response to insulin-induced hypoglycemia (43 ± 3 vs 22 ± 1 pmol/L with saline, p<0.01) was abolished by atropine or hexamethonium (p<0.01). In addition, increased plasma glucagon levels during hypoglycemia (1750 ± 275 vs 507 ± 48 ng/L with saline, p<0.01) were reduced by atropine (779 ± 85 ng/L, p<0.01), or combined adrenergic blockade with phentolamine + propranolol (1274 ± 112 ng/L, p<0.05), and nearly abolished by atropine + combined blockade (571 ± 40 ng/L) or hexamethonium (both p<0.01). We conclude that in the mouse: 1) Plasma PP is increased by a cholinergic mechanism during neuroglucopenia produced by 2-DG or hypoglycemia 2) The glucagon response to hypoglycemia is partly mediated by muscarinic and partly by adrenergic mechanisms and is largely the result of autonomic activation rather than a direct effect of low glucose levels.

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INSULIN ACTION AND COUNTERREGULATION TO HYPOGLYCAEMIA IN PATIENTS WITH CHRONIC ORGANIC HYPERINSULINAEMIA. EFFECT OF INSULINOMA REMOVAL.

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Insulin sensitivity and counterregulation to hypoglycaemia was evaluated in 6 insulinoma patients (INS: M/F=2/4; age=39±8 yrs), before and after adenoma removal. Hypoglycaemia was allowed to occur after 100min euglycaemic hyperinsulinaemic clamp (1mU/kg/min). Glucose recovery was examined over 60min following interruption of insulin infusion. Adenoma removal induced body weight reduction (BMI=25.7±1.9 vs 23.0±1.6 kg/m^2; p<0.05), normalization of basal plasma glucose (PG=2.9±0.2 vs 4.8±0.1 mmol/l) and insulin (162±24 vs 48±12 pmol/l) levels, and hepatic glucose production (HGP) (7.6±0.8 vs 12.2±1.1 µmol/kg/min). During clamp, PG (4.7±0.1 vs 4.9±0.1 mmol/l) and insulin (534±36 vs 540±48 pmol/l) were comparable. Before removal, the insulin-mediated glucose disposal was lower (30.9±3.2 vs 48.3±3.9 µmol/kg/min; p<0.01) than in a control group (M/F=3/4; age=38±6 yrs). After removal, glucose disposal remained lower (30.4±3.1 µmol/kg/min; p<0.01). HGP was suppressed in both occasions. PG decline and nadir (2.0±0.1 vs 2.2±0.2 mmol/l) were similar. Glucose recovery rate was markedly altered in the first study (0.7±0.2 vs 2.5±0.4 µmol/min; p<0.01) with significantly lower final PG (2.4±0.1 vs 4.0±0.2 mmol/l; p<0.001) and it was associated with lower HGP (3.3±1.6 vs 10.2±2.7 µmol/kg/min; p<0.05) and larger exogenous glucose infusion (11.8±3.2 vs 3.8±3.5 µmol/kg/min; p<0.05). Insulin remained higher during first study (174±24 vs 84±24 pmol/l; p<0.05). Glucagon (+49±15 vs +95±27 pg/ml; p<0.05) and growth hormone (+16±6 vs +30±3 ng/ml; p<0.05) increment were reduced, suggesting a suppressive role of hyperinsulinaemia in both HGP and counterregulatory hormones. In conclusion, in INS, restoration of normal plasma insulin: 1) improves counterregulation to acute hypoglycemia, but 2) does not restore normal insulin-sensitivity.

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REDUCED COUNTERREGULATORY RESPONSE OF GLUCAGON, ADRENALIN AND CORTISOL, BUT NOT GROWTH HORMONE, DURING HYPOGLYCAEMIA IN INSULINOMA PATIENTS

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Disturbances of hypoglycaemia counterregulation have been described in Type 1-diabetic patients especially with near-normoglycaemic metabolic control. If adaptation to recurrent hypoglycaemic episodes is the cause, the same abnormalities could occur in insulinoma patients. Nine insulinoma patients (8 benign, 1 malignant) with fasting hypoglycaemia, 5 of these patients after successful surgical removal of the tumor, and 11 control subjects without fasting hypoglycaemia were compared. After a euglycaemic (4.4 to 5.0 mmol/l) hyperinsulinaemic (80 mU/kg/h) clamp experiment, plasma glucose was allowed to fall to 2.2-2.5 mmol/l within 30 min and was maintainted at this level for further 60 min. Glucagon, cortisol, growth hormone (RIA) and catecholamines (HPLC, fluorescence detection) were measured. In control subjects, glucagon, adrenalin, cortisol and growth hormone increased significantly. This response was reduced in insulinoma patients (glucagon: by 89%, $p = 0.001$; adrenalin: by 76%, $p = 0.0006$; cortisol: by 69%, $p = 0.0003$); but growth hormone: no significant difference). After surgery, counterregulatory hormone responses were at least partially normalized (all: $p = 0.04$). In conclusion, repeated exposure to hypoglycaemia leads to a reduction in counterregulatory hormone response at the adrenal and pancreatic level, whereas growth hormone secretion does not appear to be subject to this adaptation in insulinoma patients. Similar mechanisms may be operative in insulin-treated diabetic patients who experience recurrent hypoglycaemic episodes.

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HYPOTHALAMIC ACTIVATION IS NOT DIFFERENT DURING HUMAN OR PORCINE INSULIN-INDUCED HYPOGLYCAEMIA

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Although pituitary hormones play only a minor role in acute hormonal counterregulation during insulin-induced hypoglycaemia, their concomitant secretion with the profound sympatho-adrenal response provides an indicator of hypothalamic activation. The release of different amounts of beta-endorphin, a biological active peptide with a common precurser with ACTH, during human (HI) and porcine (PI) insulin-induced hypoglycaemia, would be a pointer towards a different insulin species effect on hypothalamic-pituitary response. We performed a controlled, double-blind study with randomized application of either HI or PI to compare the insulin species effect on hypothalamic activation during developing and established hypoglycaemia. The glucose clamp technic was applied to stepwise lower the blood glucose concentration (3.3, 2.2, 1.7 mmol/l) in 10 IDDM patients in similar time intervalls. At each of the abovementioned plateaus, arterialized blood was drawn form a dorsal hand vein for hormone analysis. Beta-endorphin was determined by a N-terminal specific radioimmunoassay (^{125}I-beta-endorphin). A different action of HI or PI on glucose metabolism and sympatho-adrenal response could not be detected. Accordingly, we found a significant increase in beta-endorphin secretion during hypoglycaemia (at 3.3 mmol/l: 29.8 ± 3.2 vs. 28.4 ± 2.2, at 2.2 mmol/l: 30.3 ± 3.2 vs. 36.4 ± 4.0, at 1.7 mmol/l: 42.5 ± 6.6 vs. 51.2 ± 14.1, [pmol/l], ANOVA for hypoglycaemia effect: $p<0.02$), but without any effect of insulin species being used. Hypothalamic activation during insulin-induced hypoglycaemia, as assessed from the beta-endorphin response, is independent of the insulin species being used, which supports earlier observations of an identical sympatho-adrenal response during HI- and PI-induced hypoglycaemia.

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EFFECTS OF ACUTE INSULIN-INDUCED HYPOGLYCAEMIA ON CARDIAC FILLING AND DIASTOLIC FUNCTION IN NORMAL SUBJECTS.

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Acute hypoglycaemia provokes an intense cardiovascular response, with increases in heart rate, pulse pressure and cardiac contractility. The effects of hypoglycaemia induced by intravenous insulin (0.15u/kg) on left ventricular diastolic function were examined in 6 normal males using pulsed Doppler echocardiography. During hypoglycaemia the peak velocity of early filling (PVE) increased from 0.64±0.08 to 0.98±0.09 m/sec, and the peak velocity of A (PVA) also increased, so that the E:A ratio was unchanged. The extent of early filling (TVIE) increased from 0.08±0.005 to 0.12±0.02 meters ($p<.005$), and the total time velocity integral (TTVI) which measures the total extent of filling per beat increased from 0.11±0.01 to 0.16±0.02 ($p<.005$). The early filling fraction (TVIE/TTVI) increased, while the atrial filling fraction decreased. These data demonstrate an increase in the rate and extent of relaxation of the left ventricle in response to hypoglycaemia, the major part of which occurs in early diastole before contraction of the atrium occurs, and indicate an increase in ventricular compliance in normal subjects. In diabetic patients, many of whom have resting abnormalities of diastolic filling, a reduced ventricular compliance could impair the cardiovascular response to hypoglycaemia.

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PLASMINOGEN ACTIVATOR INHIBITOR-1 DURING INSULIN AND ORAL GLUCOSE TOLERANCE TESTS

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In-vitro studies have shown that insulin increases PAI-1 synthesis and secretion in cells of hepatic origin. However, in-vivo studies have failed to demonstrate an acute or chronic effect of insulin on PAI-1 concentrations in man. To investigate the effects of exogenous insulin infusion compared to endogenous insulin secretion, eight obese subjects underwent an insulin tolerance test (ITT) and an oral glucose tolerance test (OGTT). During ITT, insulin levels rose from (median, range) 8 (5-18) μU/ml at time 0 to 139.5 (40-500) μU/ml after 30 min before falling to 7 (5-15) μU/ml at 2h. Plasma glucose fell from 3.9 (3.1-4.7) mmol/l at time 0 to 1.8 (1.1-2.6 mmol/l after 30 min. During OGTT plasma insulin rose from 6.5 (5-26) μU/ml at time 0 to 102 (9-300) μU/ml after 60 min and 78 (34-179) μU/ml after 90 min. Plasma glucose was 4.2 (3.6-4.7) mmol/l at time 0 and 8.1 (2.0-10.3) and 6.8 (3.2-9.2) mmol/l after 60 and 90 min respectively. During OGTT growth hormone levels were 1.8 mU/ml at time 0 and fell gradually to 0.8 mU/ml after 90 min. During ITT growth hormone rose from 1.0 mU/ml at time 0 to 50.5 mU/ml after 90 min. There were no changes in insulin-like growth factor-1 in either group. PAI-1 concentrations were 31.5 ng/ml at time 0 and showed a gradual fall to 22.3 and 18 ng/ml after 60 and 120 min of OGTT. During ITT PAI-1 was 15.3 ng/ml at time 0 and 14.8 and 18.4 ng/ml after 60 and 90 min. The results confirm that exogenous insulin does not acutely regulate PAI-1 concentrations. In addition, the high portal insulin concentrations seen during OGTT in these patients do not seem to have measurable effects on hepatic PAI-1 output.

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INCREASED NON-OXIDATIVE GLUCOSE METABOLISM AND ENERGY EXPENDITURE IN IDIOPATHIC REACTIVE HYPOGLYCEMIA

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Idiopathic reactive hypoglycemia (IRH) is characterized by plasma glucose levels less than 2.5 mM associated with neuroglycopenic symptoms, 3-5 hours after a meal during every day life. IRH patients display normal plasma insulin concentrations and an increased insulin mediated glucose uptake. In order to assess the intracellular pathways of glucose metabolism responsible for the increased insulin sensitivity in IRH patients, we performed indirect calorimetry in 6 patients and in 6 normal subjects (N) before and during the euglycemic hyperinsulinemic (1.0mU/kg·min) clamp. We evaluated the metabolized glucose, glucose and lipid oxidation and non-oxidative glucose metabolism. Total body glucose uptake during the steady state was increased in IRH (49.55±1.11 vs 38.72±0.55 μmol/kg·min; $p<0.002$). Glucose oxidation was similar in the basal state 7.88±0.55 vs 8±1.66 μmol/kg·min in IRH and N respectively; similarly increased during the clamp study, 16.77±1.11, 14.72±2.22 μmol/kg·min in IRH and N respectively. Contrariwise, non-oxidative glucose metabolism was significantly higher in IRH (33.61±1.11 vs N: 21.88±1.11 μmol/kg.min, $p<0.002$). Fat oxidation was slightly (p=ns) increased in IRH. Energy expenditure in the basal state was higher (+19% of predicted REE) in IRH patients, which could be considered hypermetabolic. In conclusion these data suggest that inceased insulin sensitivity in IRH is due to increased non-oxidative glucose metabolism.

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EFFECT OF TREATMENT ON INSULIN BIOAVAILABITY AND I-123 INSULIN BIODISTRIBUTION IN INSULIN IMMUNE HYPOGLYCEMIA

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A patient with autoimmune hypoglycaemia was identified with insulin autoantibodies (IAA) and late hypoglycaemia after carbohydrate ingestion. IAA determined by radio binding assay with I-125 monoiodo TyrA14 insulin and PEG precipitation at 1/2 serum dilution fluctuated between 72% and 60% over 12 months. Plasmapheresis and steroid treatment became necessary because of recurrent severe hypoglycemia. Hypoglycemia after i.v. injection of 0.1 U/kg of insulin was delayed at 120 min. After 4 plasmapheresis the delay was reduced to 60 min. Imaging after i.v. injection of 1 mCi of TyrA14 I-123 insulin showed persistence of tracer in the blood throughout the 60 min of study. Ratio between blood radioactivity measured at peak time and at 30 min was 73.8% before and 46.3% after plasmapheresis (n.v. 42±14.9%, mean±SD). Liver uptake reached 9% before and 12% after plasmapheresis (n.v. 21.1±1.7%) and remained almost constant throughout the study. Kidneys were barely visible in both occasions. After 3 months of treatment with 1 mg/Kg Prednisone IAA decreased to 26% and no more hypoglycemic episodes occurred.

In conclusion, treatment was effective in improving bioavailability and biodistribution of insulin by decreasing IAA.

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TRANSPLANTED BETA-CELL MASS DOUBLED AFTER 95% PANCREATECTOMY.

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To analyze the capacity of transplanted islets to respond to an increased metabolic demand, Lewis rats, (5-6 wk, blood glucose 95±3 mg/dl) were transplanted (Tx) with 500 syngeneic islets under the kidney capsule (day 0). Tx rats were randomly distributed in 2 groups. One group underwent 95% pancreatectomy (95%-Px) at day 14 (Tx+Px) and the grafts were harvested at day 28. In the non-Px group the grafts were harvested at day 28. In addition, a 95%-Px was performed in a group of non-Tx rats. Blood glucose was normal in both Tx groups during the follow-up. In contrast, after 95%-Px, non-Tx rats were severely hyperglycemic (249±28 mg/dl, p=0.002 vs Tx groups). Beta-cell mass of grafts was determined by point counting morphometry on immunostained sections. In Tx+Px group, beta-cell mass in the graft (1.75±0.5 mg) was twice the beta-cell mass in 500 isolated islets (0.92±0.06 mg, p=0.05), or in grafts of non-Px rats (0.65±0.29 mg). In summary, the originally transplanted beta-cell mass of 500 islets doubled after 95%-Px, preventing the development of hyperglycemia. Transplanted beta-cell mass can increase markedly when islets are challenged by greater metabolic demand.

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IMMOBILIZED HEMOGLOBIN IMPROVES ISLET FUNCTION AND VIABILITY IN THE BIOARTIFICIAL PANCREAS

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Hypoxia seems to be a major obstacle to islet survival and function in the bioartificial pancreas. In this study we investigated whether oxygen supply, survival and function might be improved by adding the oxygen carrier hemoglobin to the embedding matrix. Neonatal rat islets were encapsulated in hollow fibers (regenerated cellulose, 20 μm wall thickness, 400 μm, 3cm length) containing 1.5% alginate (Manugel GHB, Kelco) with or without hemoglobin (7.5 g/dl). With hemoglobin insulin secretion was 301±19 (mean ± SEM) mU/l at 5.5 mmol glucose and 827±190 at 16 mmol glucose after one week culturing and 790±155 at 5.5 mmol and 1181±242 at 16 mmol glucose after 5 weeks. Without hemoglobin the corresponding values were 69±22 and 126±48 after 1 week and 24±7 and 153±64 after 5 weeks. These secretory values were lower ($p<0.05$ in any case) than with hemoglobin. This was confirmed by histology showing more than 100 per cent higher viability with the use of hemoglobin. The better function and survival may indicate better oxygen supply by use of immobilized hemoglobin.

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OXYGEN DISTRIBUTION IN ISLET ORGANS: EFFECT OF CONVECTION AND BARIUM-ALGINATE ENCAPSULATION.

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We measured O_2-tension (PO_2) in isolated islet organs (Brockmann bodies=BBs) of Osphronemus gorami. PO_2-values were recorded at subsequent microelectrode positions on a radial track towards the center of the organ to study the effect of fluid convection (n=12) and encapsulation (n=12). The sigmoidal pO_2-profiles showed a O_2-depleted zone surrounding the surface, a steep decline inside the BBs corresponding to the O_2-consuming rim, and a plateau in the center without oxygen consumption. The pO_2-values decreased ($p<0.001$) when convection stopped. Similar low levels occurred in encapsulated BBs. Compared to starting values, pO_2-levels at the surface were 61±3% with and 41±4% without convection. We measured similar values for encapsulated and nonencapsulated BBs: 44±5% and 64±4% In the center of BBs, O_2 dropped to 27±5% with and to 6±3% without convection, and to 11±3% for encapsulated and to 22±4% for nonencapsulated BBs. The thickness of the outer O_2-depleted zone was 81±16μm with and 196±57μm without convection ($p<0.001$), and 188±16μm for encapsulated and 94±14μm for nonencapsulated BBs ($p<0.001$). The O_2-consuming rim was 295±22μm with and 235±36μm without convection (NS), and 216±15μm for encapsulated and 315±24μm for nonencapsulated BBs ($p<0.01$). These results indicate that 1. fluid convection is essential for the oxygenation of isolated islet organs and 2. alginate encapsulation may worsen oxygenation mainly by expanding the "unstirred water layer" surrounding the organ.

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ANATOMICAL AND PHYSIOLOGICAL EVIDENCE FOR SYMPATHETIC REINNERVATION AFTER ISLET TRANSPLANTATION IN DIABETIC RATS

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In the present study, neuroanatomical and physiological evidence was sought for possible sympathetic reinnervation of transplanted islets in diabetic rats. Streptozotocin diabetic AO-rats received 5 μl islet tissue into the portal vein. This resulted in normal baseline plasma glucose and insulin levels. Six to eight weeks later, transplanted (Tx) and control (C) animals were provided with a permanent heart catheter and subjected to strenuous swimming. Exercise was chosen since it leads to inhibition of insulin release by activation of the sympatho-adrenal system. During exercise, plasma insulin levels decreased from 49 ± 8 to 27 ± 5 mU/L (Tx) or from 44 ± 5 to 32 ± 5 mU/L (C). Blood glucose and plasma catecholamine responses were identical in both groups of animals. The exercise-induced increase of non-esterified fatty acids levels was exaggerated in the transplanted animals ($p<0.05$). Adrenodemedullation, performed to exclude a possible compensatory influence of adrenal epinephrine did not affect the exercise-induced decrease of insulin during exercise. Finally, immunocytochemical staining on Tyrosine Hydroxylase, Dopamine-ß-Hydroxylase and Neuron Specific Enolase was performed. All enzymes were present in the islet grafts. The results revealed noradrenergic reinnervation of transplanted islets of Langerhans, enabling suppression of insulin release during activation of the sympathetic nervous system.

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THE EFFECT OF SUCCESSFUL ISLET ALLOTRANSPLANTATION ON NEURONAL NGF LEVELS IN EXPERIMENTAL DIABETES OF THE RAT

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Evidence exists that alteration in nerve growth factor (NGF) levels is responsibel for some of the functional deficits occuring in sympathetic diabetic neuropathy.The aim of our study was to investigate changes in endogenous NGF levels during Streptozotocin (STZ) induced diabetes and to test the influence of successful islet allotransplantation on the NGF content in neuronal structures in early diabetes.We studied 3 groups of animals (BDE rats,MHC=Rt1^u,at the age of 9 months).Group 1: BDE rats without diabetes as normal controls.Group 2: Chemically induced diabetic BDE rats with constant hyperglycemia (>450 mg/dl) over 5 months (diabetes induction with STZ after 4 months of age).Group 3: Chemically induced diabetic BDE rats (like group 2) with successful islet allotransplantation (islets from Lewis rats,MHC=Rt1^l,cultured for 14 days at 22°C,no immunosuppression) 3-4 weeks after diabetes induction and resulting in indefinite postprandial normoglycemia (glucose homeostasis).All animals were sacrificed after 9 months of age and the iris,the submandibular gland and the sciatic nerve were harvested for detection of NGF levels (enzyme-immunoassay).In comparison to normal controls (NGF levels = 100%) untreated diabetic rats show a decrease of the NGF content to 35-45% ($p<.02$) in all investigated tissues, whereas after islet transplantation the endogenous NGF values increased up to 100% again (sciatic nerve) or even up to 150% (submandibular gland),respectively 200% (iris). In summary NGF levels are decreased in these investigated neuronal structures in our model of experimental diabetes. This decrease can be prevented by successful islet allotransplantation what could play an important role in the prevention of diabetic neuropathy.

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IN VITRO ACTIVATION OF HUMAN MACROPHAGES BY ALGINATE-POLYLYSINE MICROCAPSULES

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The biocompatibility of alginate-polylysine microcapsules used for islet microencapsulation can be debated since a foreign body reaction around implanted microcapsules were reported. We studied the capacity of alginate microcapsules to activate macrophages in vitro. Human monocytes were isolated from whole blood of healthy donors by a Ficoll density gradient and adherence to a plastic support. Monocytes were cultured for 24 hours with: 1) alginate-polylysine microcapsules, 2) lipopolysaccharide (LPS) (positive control group), 3) alone (negative control group). Monocyte activation was evaluated by measuring the secretion of interleukin-1(IL-1) ß, the production of intracellular interleukin-1 α and ß and by phenotype subtyping. Results are expressed as mean±SEM of 10 different experiments. The results of extracellular IL-1 ß secretion were : controls 19,7 ± 1,1fmol/ml; alginate microcapsules 34 ± 2,1 fmol/ml ($p<0,001$ vs control); LPS 282,1± 33,3 fmol/ml. Intracellular IL-1 ß production was : controls 20,7 ± 2,2 fmol/ml; alginate microcapsules 57,8 ± 18,5 fmol/ml (NS); LPS 138,4 ± 51,1 fmol/ml. IL-1 α production was : controls 31,3 ± 4,4 fmol/ml; alginate microcapsules 171,7 ± 52,9 fmol/ml ($p<0,05$ vs control); LPS 530,8 ± 153,6 fmol/ml. In additional experiments, we observed that insulin, up to a concentration of 4 U/ml had no effect on the macrophage activation. In conclusion, alginate-polylysine microcapsules activated human macrophages in vitro and this activation may play a role in tissular reaction observed in vivo. In addition, interleukin-1 can cross the microcapsule membrane and impair islet function.

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TOLERANCE INDUCTION IN MHC-IDENTICAL ALLOGENEIC ISLET GRAFT RECIPIENTS BY A SHORT-TERM ANTI-CD25 THERAPY

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Autoaggressive destruction of syngeneicly grafted islets into diabetic BB/OK rats can be successfully prevented by a short-term anti-CD25 therapy. MHC-identical allogeneic islets, which could be a source for islet transplantation of diabetics, were accepted in > 60% of the treated BB/OK rats.
In these experiments we proved the induction of tolerance in islet survivors. 2000 islets isolated from 8-12 d old LEW.1BB/OK rats were graftet into diabetic BB/OK. The recipients were treated for 10 days with 1 mg/kg b.w. ART18 (anti-CD25 mab) and 1.5 mg/kg b.w. CsA. In normoglycaemic animals the graft was removed 120 d later. After recurrence of hyperglycaemia a second islet graft was given without therapy. Furthermore, $2x10^7$ lymphocytes obtained from islet acceptors were transfused into diabetic BB/OK rats, which were metabolically compensated by transplantation. The lymphocytes transfused were phenotyped by FACS-analysis ($W3/25^+$-, $OX19^+$ -, $OX8^+$-, $ART18^+$-, $W3/13^+$-cells, B-, NK-cells).
The second allogeneic islet graft was accepted in 80% of the recipients. The lymphocytes transfused prevented graft rejection in 4 of 7 animals. Lymphocytes, which induced graft acceptance, were characterized by significantly enhanced numbers of $OX8^+$-cells ($12.3\pm1.9x10^5$ vs. $1.5\pm0.5x10^5$). Neither the absolute number of lymphocytes transfused nor the other phenotypes were different.
The results suggest the induction of tolerance in autoimmune diabetic animals to MHC-identical allogeneic islets, which seems to be related to $OX8^+$-cells.

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THE EFFECT OF CYCLOSPORIN A ON THE ESTABLISHMENT OF MOUSE PANCREAS ISOGRAFTS

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Perplexities about the use of cyclosporin A (CSA) in pancreatic islet transplantation have been raised after toxic effects on the endocrine pancreas have been reported, such as degranulation and hydropic degeneration and reduction in mRNA synthesis of islet ß-cells, impaired glucose tolerance and hyperglycemia. We therefore investigated its possible interference with the establishment of mouse pancreas isografts transplanted to streptozotocin diabetic mice. CSA was administered subcutaneously at 50 mg/kg/day, previously shown sufficient to immunosuppress the recipients and to allow xenograft survival and reversal of diabetes. When CSA was administered starting at day 0 post-transplantation, 7 of 19 mice attained normoglycemia, while 7 mice showed unstable blood glucose levels. In the oil treated control group, 16 of 20 mice became normoglycemic and none of them had unstable blood glucose levels. In a second set of experiments, we allowed the grafts to reverse diabetes, and 100-120 days after transplantation CSA or oil were administered for 8 weeks. There were no changes in blood glucose levels and glucose tolerance in both CSA treated and control group. We conclude that CSA interferes only with graft establishment, probably through inhibitory effects on neovascularization, without affecting the function of grafts that have already reversed diabetes.

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INFLUENCE OF PREGNANCY ON SURVIVAL OF ALLOGENEIC ISOLATED PANCREATIC ISLETS.

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Pregnancy represents specific case of allotransplant. Knowledge of fetal enhancement mechanisms may lead to direct application in transplant immunological processes. The present study was undertaken to determine the influence of iso- and allopregnancy on the survival of allogenic isolated pancreatic islets. The experiments were performed on 4 groups of female Lewis rats: I.-non-pregnant rats, II.-isopregnant Lewis rats /mated with male Lewis rats/, III.-allopregnant Lewis rats /mated with male BDII rats/ and IV.-allopregnant Lewis rats /mated with male BDII rats/ after injection of 100 ul serum from allopregnant rats and of 50000 lymphoids cells isolated from BDII rats. In all groups of rats the transplantation of 500 allogenic islets, isolated from male BDII rats, under the kidney capsule was performed. 5, 8, 10 and 20 days after transplantation the kidney was removed and histopathologicaly investigated, for detection of the transplant survival or rejection.
In the I group, 5 days after transplantation, 85.7% of transplanted allogenic islets survived but 8 days after transplantation no islets were detected under the kidney capsule - the transplant was rejected. In the II group 83.3% and no islets were detected, respectively. In the III group 10 and 20 days after transplantation [more than 10 days after the delivery of the fetuses] in 62.6% of the cases and in 33.3% the allogeneic islets under the kidney capsule were detected. In the IV group, 10-day survival was observed in 66.6% of the cases, and 20-day in 16.6% of the cases.
The mammalian fetoplacental unit bears a variety of embrional- and paternal- derived histocompatibility antigens against which the immune system of the mother can and does react and in this way, probably, the allogeneic isolated pancreatic islets survival was prolonged.

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SURVIVAL AND FUNCTION OF MEMBRANE ENCAPSULATED ISOLATED HUMAN PANCREATIC ISLETS

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In order to avoid immune suppressive therapy in clinical transplantation immune isolation by encapsulation of isolated human islets has become a tool of interest. We therefore investigated the survival of human islets enclosed in immune-protective devices, which protect rat islet allografts. Human islets were prepared at the "β Cell Transplant" in Brussels and after a 3-5-day culture period in Uppsala they were enclosed in Biopore 0.45 µm membrane (Millipore, Baxter) devices and implanted either in the epidydimal fat pad or freely under the kidney capsule of seven normoglyemic nude mice. Four weeks post-implantation the implants were examined histologically. In all animals there were many surviving epitheloid cells inside the membranes and most of them were insulin-positive. Other endocrine cells (α,δ, PP) were also present. The outside of the membranes was free of fibroblasts but displayed frequent close vascular structures. Qualitatively, there were no obvious structural differences between the freely transplanted and encapsulated islets. In order to monitor the functional activity of the encapsulated human islets eight nude mice were given encapsulated islets only. Preliminary results suggest that there are considerable amounts of human C-peptide reactivity in serum samples harvested 4-8 weeks post-transplantation. We conclude that isolated human islets survive and function when encapsulated.

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Improvement of the biocompatibility of microencapsulated pancreatic islets.

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Transplantation of microencapsulated islets can restore normoglycemia in diabetic rats, but islet function is limited by a cellular reaction around the capsules. The aim was to determine the cause of fibrosis and whether it could be prevented. Alginates with high -, medium -, or low guluronic acid content were used. Implantation of AO- or Lewis rat islets in medium G alginate capsules restored normoglycemia in diabetic AO rats as did AO islets in BB rats. After 2 weeks 72-95% of the capsules with islets and 64-81% of the empty capsules were retrieved. There were no differences between the percentages of overgrowth of the different strain combinations or between empty (3-8%) and islet containing capsules (4-13%). Thus overgrowth is induced by the capsule and not by the islets. To improve the biocompatibility of capsules, alginates were extensively filtered or extracted. After 4 weeks empty capsules prepared from filtered alginates sticked to the omentum and were surrounded by fibrosis. No free capsules were retrieved. Empty capsules prepared from extracted high G -, medium G -, and low G alginate were retrieved for 64, 66 and 91 %. Medium G alginate capsules were completely free of overgrowth. In conclusion, microencapsulated islets are not rejected and restore normoglycemia. With purified medium G alginate completely biocompatible alginate polylysine microcapsules can be prepared.

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BENEFICIAL EFFECT OF INSULIN PUMP THERAPY ON TRANSPLANT FUNCTION AFTER HUMAN FETAL ISLET TRANSPLANTATION

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This study was aimed to evaluate the effect of the strict metabolic control on the function of the transplanted human fetal islets.Therefore,we applied continuous subcutaneous insulin infusion(CSII) therapy in 5 patients with Type 1(insulin-dependent) diabetes during 2 months after transplantation and compared them with 4 patients with Type 1(insulin-dependent) diabetes remaining on conventional insulin therapy(CIT) after transplantation .In the CSII treated patients a strict metabolic control was achieved(MBG:5.3±1.7 mmol/l,HbA_{1c}:7.4±0.5%) together with rapid reduction in insulin daily dose(day 30:32±6U vs day 0:64±8U/day) contrary to the CIT patients(MBG:8.9±1.0 mmol/l,HbA_{1c}:8.8±1.2%)(day 30:44±7U vs day 0:67±9U/day). Moreover,the CSII treated patients exhibited significantly higher C-peptide levels detected by glucagon stimulation (1 mg iv,0/6 min;day 90:0.32±0.09/0.45±0.11 vs 0.21±0.09/0.25±0.06 mmol/l,$p<0.05$) and during C-peptide daily profile(before and 2 hrs after main meals, at midnight and at 03 h:mean values preprandially 0.29±0.09 vs 0.19±0.09,$p<0.05$, and postprandially 0.40±0.06 vs 0.24±0.06 nmol/l,$p<0.05$).Simultaneously,systemic free insulin levels in the CSII treated patients were not elevated (34.2±6.1 mU/l) and were similar to those in the CIT patients(37.5±5.2 mU/l,p=NS).Our results signify that CSII treatment exerts an important beneficial effect on the functional capacity of the human fetal islet transplant.

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A MODEL OF GRADED BETA-CELL FUNCTION FOR STUDY OF METABOLIC CONTROL IN SUBCLINICAL INSULIN DEFICIENCY

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The goal of this investigation was development of an animal model for study of the metabolic consequences of subclinical beta-cell deficiency. Male Wister-Furth rats were rendered diabetic with streptozotocin (55 mg/kg). Three weeks later islets of Langerhans were infused intraportally (500 - 3000 islets). Fasting glucose, free fatty acid and insulin levels were not different from controls in rats receiving > = 500 islets ($P > 0.15$). Fed plasma glucose was greater, (14.7 ± 1.8 vs. 6.3 ± 0.1 mM, $P < 0.05$) and body weight was lesser (233 ± 5 vs. 263 ± 7 g, $P < 0.05$) in rats receiving 500 islets as compared to controls. The acute insulin response to glucose (300 mg/kg) was well correlated with the number of transplanted islets ($r = 0.61$, $P = 0.0001$). Insulin sensitivity quantified during a 2-step glucose clamp, as the change in glucose infusion divided by the change in insulin was not correlated with islet number ($r = -0.30$, $P = 0.15$), although when quantified as maximal glucose infusion rate normalized to the insulin level, achieved a marginally significant inverse correlation ($r = -0.37$, $P = 0.068$). These studies demonstrate that the insulin secretory response to glucose is proportional to the number of transplanted islets, but decreased beta-cell mass does not impair insulin action when fasting normoglycemia is maintained. This model of graded beta-cell function may be useful in delineating the effects of metabolic perturbations in subjects with subclinical insulin deficiency.

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THE DETERMINANTS OF GLUCOSE TOLERANCE FOLLOWING DIVERSION OF PANCREATIC VENOUS DRAINAGE TO THE SYSTEMIC CIRCULATION

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To assess the metabolic impact of diverting pancreatic venous drainage to the systemic circulation, the gastroduodenal and splenic veins were transposed from the portal vein to the inferior vena cava in 5 dogs. Four animals underwent sham surgery. Glucose tolerance was estimated during a 3hr intravenous infusion of glucose (10mg/kg-min) 2 weeks postoperatively, following an 18hr fast. In dogs with venous transposition basal glucose was lower (5.2±0.3 vs 5.8±0.1mmol/l, $p<0.5$) but increases were equivalent (2.1±0.4 vs 3.1±0.6mmol/l in shams, n.s.). Insulin (IRI) concentrations however increased by 252±22 from 75±17pmol/l compared to shams (an increment of 109±39pmol/l [$p<0.05$] from 34±11pmol/l, $p<0.05$). Metabolic clearance of glucose (MCR) was measured using simultaneous constant infusion of H^3-3-glucose. Sensitivity to insulin (ΔMCR/ΔIRI) was 0.10±0.12 vs 0.34±0.02 in shams ($p<0.02$). Five additional hyperinsulinemic euglycemic clamps with insulin infusions of 33ng/kg-min, somatostatin (0.5μg/kg-min) and variable glucose corroborated the decrease in the sensitivity index: 0.3±0.05 vs 0.5±0.06 in shams ($p<0.05$). Glucose production was less suppressed (but n.s.) than in shams: 74±10% vs 92±4% with glucose infusion and 38±6% vs 60±15% during insulin clamp. Normal glucose tolerance is maintained following the diversion of pancreatic venous drainage systemically because peripheral insulin increases simultaneously with a decrease in insulin sensitivity.

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Bone and Connective Tissue

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INCIDENCE OF MICROVASCULAR COMPLICATIONS IN TYPE I DIABETIC SUBJECTS WITH LIMITED JOINT MOBILITY; A 10 YEAR PROSPECTIVE STUDY.

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Limited joint mobility (LJM) affecting the small joints of the hands is a common though often overlooked manifestation of Type I diabetes whose pathogenesis remains uncertain. Previous cross-sectional studies have shown a correlation between LJM and both the duration of diabetes and presence of microvascular complications. It is unknown whether LJM may precede and therefore be regarded as an early marker for complications. We followed 22 Type I diabetic patients [10 male/12 female; duration of diabetes 21.1±1.3yr (mean±SE)] with LJM and 22 subjects matched for age, sex and duration of diabetes without LJM over a 10 year period. Both groups were free of retinopathy and negative for dipstick proteinuria at baseline. After 10 years, 10 of 22 LJM patients had developed background and 3 proliferative retinopathy compared to 9 and 1 control subjects respectively. Microalbuminuria (20 ≤ albumin excretion rate < 200 mcg/min) was present in 5 LJM patients compared to 7 control subjects. Ankle and great toe vibration perception thesholds, HbA1c and arterial blood pressure did not differ between the two groups (p>0.05). At follow-up, 12 of the control subjects had developed LJM of whom 4 had retinopathy and 4 microalbuminuria. Thus, while LJM may be another 'chronic complication' of diabetes, its presence does not appear to predict those at increased risk of developing microvascular complications in contrast to previous reports.

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LIMITED JOINT MOBILITY IN TYPE 1 DIABETIC PATIENTS.RELATION TO OTHER DIABETIC COMPLICATIONS

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Limited joint mobility (LJM) was assessed in 285 type 1 diabetic patients and 288 healthy controls to determine the prevalence of LJM and to clarify whether the presence of LJM correlates to other diabetic complications independent of duration of diabetes. Limitation of different joints was recorded with a goniometer. The prevalence of LJM was 58.2 and 14.2 % in type 1 diabetic and control subjects respectively. The relative risk of diabetic patients with LJM to have proliferative retinopathy was 3.0 (95% confidence interval 1.1 - 3.7) and nephropathy 4.6 (95% confidence interval 1.9 - 11.5) compared to patients without LJM, when the confounding effect of the duration of diabetes was excluded. LJM was not related to microalbuminuria (nightly urine albumin excretion rate 20-200 µg/min). The association between LJM and peripheral symmetrical polyneuropathy was found to be only duration effect. No association was found between autonomic neuropathy or impotence and LJM. The correlation between LJM and serum total and LDL-cholesterol was duration effect of diabetes and LJM did not relate to serum HDL-cholesterol or triglyceride values. LJM is common in type 1 diabetic patients and is associated with an increased risk for the serious complications of nephropathy and proliferative retinopathy independent of duration of diabetes.

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DECREASED BONE MINERAL DENSITY IN MEN WITH TYPE 1, INSULIN-DEPENDENT DIABETIC PATIENTS

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There are some data that bone mineral density (BMD) might be lowered in men with type 1 diabetes. To determine the bone loss in men with type 1 diabetes, we measured BMD and bone mineral content (BMC) in 15 male diabetic patients with more than 5 years duration (Group A; age, 21.9 ∓ 4.2 years) and compared with those of 15 male newly diagnosed, BMI-and age-matched type 1 diabetic patients (Group B; 0-12 months duration; age, 21.8 ∓ 2.9 years) and also BMI- and age-matched 20 healthy men (age, 21.4 ∓ 3.4 years). BMD and BMC of the lumbar spine was measured by dual-photon absorptiometry (Norland Model 2600, Wisconsin). The BMD (0.88 ∓ 0.1 g/cm^2) and BMC (3.99 ∓ 0.5 g/cm) values of the Group A were significantly lower than BMD (1.01 ∓ 0.1 g/cm^2) and BMC (4.60 ∓ 0.8 g/cm) values of the Group B ($p < 0.05$, $p < 0.01$, respectively) or the BMD (1.03 ∓ 0.1 g/cm^2, $p < 0.05$) and BMC (4.55 ∓ 0.9 g/cm, $p < 0.01$) values of the control group. There was no significant differences in BMD and BMC values between Group B and control group. There were also no correlation between levels of serum and urine calcium and phosphorus, and BMD, BMC in both diabetic groups. We found a positive correlation between BMD, BMC and the age of onset of diabetes in all diabetic patients (r : 0.54, $p < 0.01$ and r : 0.49, $p < 0.01$, respectively). In conclusion, decreased BMD and BMC are more common in type 1, insulin-dependent diabetic male patients with duration of greater than five years and were related to duration of diabetes.

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BONE MASS IN TYPE I (INSULIN DEPENDENT) DIABETES MELLITUS

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Osteopenia has been demonstrated as a possible complication of Type I (insulin dependent). However, its pathogenic mechanisms and relatioship with others clinical features remains controversial. AIMS: To evaluate bone mass density and factors influencing it in a homogeneous group of Type I diabetic patients.

SUBJECTS AND METHODS: Forty males and twenty-nine females patients with type I (insulin dependent), aged 29,9±9,4 years (range 20-56), were studied. Their HbA1c (mean of last three) levels ranged between 4,6 and 13,8% (normal: 4.5-6.5%). Body Mass Index (BMI) was 23,9±3,5 Kgr/m^2. No patient had any antecedent of excesive consumption of tobacco or alcohol and all patients had an appropiate degree of physical activity and a daily calcium intake. Dual X-Ray absortiometry was carried out in order to stablish bone mineral density (BMD) in Lumbar spine (L2-L4= LS), femoral neck (FN) and Ward's trangle (WT) and compared with a healthy control group. The values were expressed as Z-score and student t test was applied for statistical studies.

RESULTS: Patients with type I (insulin dependent) showed a lower BMD than controls subjects in all LS (-1,12±0,97; p<0.001), FN (-0,90±1,23; p<0.001) and WT (-0,96±1,20; p<0.001) sites. There was not relation to disease duration (11,7±8,7 years), HbA1c levels (8,6±2,1%) or insulin (42±6,5 UI/24 h)/dosage. Furthermore, decrease of BMD was more apreciable in lower BMI subjects (< 25 Kgr/m^2) both LS (Z score= -1,47±1.04 vs -0,28±0,53; p<0.001) and FN (Z score= -1,20±1.20 vs -0,26±0,9; p<0.05).

CONCLUSION: Our results suggest that type I diabetic patient had a reduced bone mass both in trabecular and cortical bone. These findings seems to be independent of duration disease, metabolic control or insulin dosage. The decrease in bone mass is more significant in lower BMI subjects.

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LOW BONE TURNOVER IN DIABETES MELLITUS ACCOUNTS FOR PRESERVATION OF BONE MINERAL DENSITY.

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The aim of this study was twofold: first, to determine short-term and long-term changes in bone mineral density (BMD); and second, to compare changes in BMD with histomorphometric findings. Forearm BMD was measured twice in 41 patients at an interval of 2.5±1.7 years. BMD values were expressed as Z-scores (i.e. standard deviation units adjusted for age, sex, and race). BMD Z-score did not change in those with type I diabetes (-1.03±1.1 to -1.06±1.0; n=15) and increased in those with type II diabetes (-1.29±1.3 to -1.10±1.2; n=26; $p<0.001$). Eight patients (type I = 2; type II = 6) with low BMD or history of fracture had a transiliac bone biopsy following in-vivo double tetracycline labeling. In 7 of the 8 patients bone formation was low (3.4±2.9 $\mu m^3/\mu m^2$/year; reference range: 5-40). After 11.7±0.3 years of follow-up, BMD was obtained in 6 patients. In 4 of 6 patients, absolute BMD actually increased. Cancellous bone volume and the mineralization lag time (a measure which is inversely related to bone turnover) correlated significantly with changes in BMD (respectively, r=0.85, $p<0.05$; and r=0.83, $p<0.05$). Low bone turnover is associated with preservation of BMD in diabetes but would permit accumulation of microdamage in bone and predispose to stress fractures and delayed healing of fractures.

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PREDICTING RISK OF OSTEOPOROSIS SUBSEQUENT TO REDUCED BONE FORMATION IN FEMALE JUVENILE DIABETICS BY MEASUREMENT OF PEAK BONE MASS

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Postmenopausal osteoporosis is the result of accelerated bone loss following menopause. Peak bone mass, defined as maximum of bone achieved during growth and thus starting point of bone loss, is of importance for clinical manifestations of osteoporosis. In the present study we investigated the formation of peak bone mass in female juvenile diabetics.

Methods: Bone mineral density was measured by single-photon-absorption at the ultradistal site of radius and ulna in 42 subjects. A control group was obtained out of the population of Graz. Mean duration of diabetes was 14 years (range 1 - 34), mean age at manifestation was 16 years (range 3 - 33). Menstrual cycles.were normal

Results: Bone mineral density in our control group was 1.061 AU (arbitrary units, standard deviation 0.116, range 0.8 - 1.22) compared to 0.982 AU (standard deviation 0.161, range 0.61 - 1.21) in female juvenile diabetics, $p < 0.05$. Coefficients of correlation for duration of diabetes and age at manifestation to bone mineral density were 0.078 and 0.13, respectively.

Conclusions: Peak bone mass in female juvenile diabetics is significantly reduced compared to a control group out of the same region. Thus having a low starting point of bone loss, female juvenile diabetics are at high risk for postmenopausal osteoporosis. Contrasting other studies there was no significant correlation of bone mineral density to duration of diabetes or age at manifestation.

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NO EVIDENCE FOR DECREASED BONE MINERAL CONTENT OR ALTERED BONE METABOLISM IN DIABETIC PATIENTS.

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Studies about bone mineral content (BMC) in diabetic patients have shown contradictory results. The divergent findings might be explained by the use of insufficient methods (single photone densitometry) and/or the selection of low numbers and heterogenous patients. Therefore, we investigated BMC and parameters of bone metabolism in a large number (n=226) of carefully selected patients with type-1 or type-2 diabetes in comparison with age- and sex-matched control groups. BMC of the lumbar spine was measured with the dual photone absorptiometry (Lunar DPX) and parameters of bone metabolism including osteocalcin, calcium, phosphorus, and alkaline phosphatase were measured by standard methods. In addition, metabolic control parameters including HbA_{1c} (HPCL) and body mass index (BMI) were also evaluated. Patients with type-1 diabetes (53 males, mean age 31+8 yrs; 39 females, mean age 28+10 yrs) showed no significantly decreased lumbar BMC compared to the control groups. Interestingly, female patients with type-2 diabetes (n=80; mean age 61+7 yrs) showed a tendency to increased lumbar BMC compared to the control group. In male type-2 diabetics (n=54; mean age 56+9 yrs) BMC (kg/m^2) was significantly increased irrespective of type of treatment (oral hypoglycemic agents: 1,15+0,1; insulin treatment: 1,18 + 0,2) compared with controls (1,01+0,1; $p<0.01$). Osteocalcin levels (ng/ml) were not found to be different between diabetic (female type-1: 6.6+1.2; male type-1: 8.5+1.2; female type-2: 7.3+1.2; male type-2: 7.3+1.5) and control groups (females 8.0+1.2; males 7.5+1.3). No significant correlation could be observed between parameters of BMC, osteocalcin, HbA_{1c} and duration of disease.
In prospective studies, 20 newly diagnosed type-1 diabetic patients and 25 type-2 diabetic patients with secondary failure to sulfonylureas were studied before and 6 months after substitution with insulin therapy. Despite the significant improvement of glycemic control (HbA_1c: 7.9%+0.3 vs. 11.9% + 0.2; $p<0.0001$), no change of osteocalcin and BMC was observed. Our data indicate, that bone metabolism and bone mineral content is not disturbed in the majority of diabetic patients.

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New Insulins

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CONTROLLING INSULIN BIOAVAILABILITY BY CRYSTAL CONTACT ENGINEERING

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We describe a novel approach to the design of insulins with prolonged activity. The goal of our protein engineering studies is the stabilization of insulin microcrystals which are either injected directly as such or – in case of injection of an acidic solution – will form in the subcutaneous depot. The mutations which we introduced were designed on the basis of the X-ray structures of several of our insulin analogues, which we were able to crystallize in the R_6 form induced by the addition of phenol as a preservative (as used in most pharmaceutical insulin preparations). We find a good correlation between packing density and water content (43 – 49%) of the crystals of various insulin analogues on the one hand and both the clearance rate of the modified hormone from the subcutaneous depot and the duration of *in vivo* action on the other. We therefore conclude that the bioavailability of these insulins is determined by the properties of their crystals. On the basis of our X-ray structures, we are now able to modify individual contacts between neighbouring insulin hexamers in the crystal and influence the bioavailability of the hormone in a systematic way.

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GLYCATION AT B1 MAKES INSULIN FAST ACTING

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We recently reported that A1,B1 diglycated human insulin, i.e. A1,B1 di(1-deoxy-D-fructosyl) insulin, might form the basis for significantly faster acting insulin preparations than conventional Actrapid insulin. The observed properties are ascribed to the influence of the pendant carbohydrate residues in maintaining the insulin in the dimeric state at low zinc concentrations. We now find that glycation at B1 is sufficient to induce this effect. B1 (1-deoxy-D-fructosyl) insulin is obtained by terminating the reaction with glucose after 3-4 hours, and purifying by reversed phase HPLC. The absorption of the synthetic, s.c. injected B1 glycated insulin, and its blood glucose lowering effect in pigs are found to be similar to the previously described A1,B1 diglycated insulin, i.e. significantly faster than that of Actrapid insulin. Comparison of assays for potency and receptor binding, however, reveals that the values for B1 glycated insulin are much closer to those of human insulin than was the case for the diglycated insulin. B1 glycated human insulin, therefore, is more interesting as a candidate for development of fast acting insulin preparations for the treatment of diabetes.

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PERIPHERAL GLUCOSE UPTAKE AND HEPATIC GLUCOSE PRODUCTION DURING CLAMP WITH HUMAN INSULIN AND INSULIN ANALOGUE-GLU-A13,B10

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To address the question whether insulin analogue-GLU-A13, B10 (X37) is a muscle specific analogue, as suggested by in vitro data, we clamped 8 pigs on 2 separate days with 0.4 and 0.87 mU/kg/min. of human insulin (HI) and X37, using euglycemic clamp in combination with a 2 hour pre-infusion of 3-H-3-glucose. Muscle and liver biopsies were taken before and after clamp. Plasma insulin concentration was 10 µU/ml HI and 16 µU/ml X37,$p<0.05$ with the low infusion rate (0.4 mU/kg/min.), the amounts infused during the 3 hour clamp were 112 vs 43 g ($p<0.05$), respectively: total glucose infusion was greater with HI ($p<0.05$). C-peptide showed no difference in suppression of insulin secretion. Hepatic glucose production was suppressed: 58% (HI) vs 40% (X37) ($p<0.05$) during clamp. Plasma insulin was 27 µU/ml (HI) vs 48 µU/ml (X37) ($p<0.05$) with the high infusion rate; the total amount of glucose infused was 380 g vs 273 g ($p<0.05$), respectively. Muscle and liver glycogen formation were significantly higher after clamp with HI than with X37 ($p<0.05$). Conclusion: despite higher plasma concentration of X37 the effect on peripheral tissue and on hepatic glucose production was diminished, suggesting a lower potency of X37 compared with soluble human insulin. The results indicate that X37 is not an organ differential analogue. It does not have a relatively more pronounced effect on the periphery than on the liver.

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EFFECT OF INSULIN ANALOGUES ON LIVER GLYCOGENOLYSIS

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HAS BEEN WITHDRAWN

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ABSORPTION KINETICS OF NPH INSULIN: ^{125}I DISAPPEARANCE RATES COMPARED WITH INSULIN PROFILES DURING GLUCOSE CLAMPING

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Insulin pharmacokinetics are usually studied either by measuring the disappearance rate of ^{125}I labelled insulin from the subcutaneous tissue or by assessing the changes in plasma insulin levels and glucose requirements during glucose clamping. The objective of this study was evaluation of both techniques in 8 IDDM patients following the subcutaneous administration of NPH insulin (4/4 men/women, mean age 33.4 [21-55] years, duration of diabetes 21.0 [5-44] years). Continuous subcutaneous insulin infusion was discontinued and an i.v. infusion of short acting insulin was started (20mU/kg/h) the evening before the studies. Glucose clamping was performed at 4 mmol/l and disappearance rates of ^{125}I were measured for 24 hours following the s.c. administration of ^{125}I labelled NPH insulin (0.7 IU/kg body weight). The T_{50} % of ^{125}I was 19.2 hours ±5.6 (mean ± sd) and at 24 hours 55.5% ± 18.5 of the injected ^{125}I was absorbed. Mean insulin levels reached peakvalues at 6 hours and returned to baseline within 24 hours. The total incremental area under the insulin curve (232.8 ± 159.6 mU/l*h) as measure of insulin appearance showed no significant correlation with measures of insulin disappearance. In conclusion, the kinetic data obtained with the ^{125}I disappearance technique overestimate the duration of action of NPH insulin and do not correlate to changes in plasma free insulin dynamics during glucose clamping.

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EFFECT OF NASAL INSULIN IN HEALTHY VOLUNTEERS USING DEVICES WITH TWO DIFFERENT SPRAY ANGLES:

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Nasal administration of insulin as a needle-free alternative has evoked considerably interest. In attempt to optimize the delivery system the effect of a single dose of nasal insulin U500 applied with two different spray angles, 0° and 15°was studied in 8 healthy, lean volunteers after an overnight fast. The subjects were randomly allocated to 6 separate days with 3 replicates, and insulin dose was approximately 0.5 IU/kg bodyweight. After application plasma insulin rose immediately to mean 31±18/34±17 mU/l (0°/15°respectively) after 30±22/25±6 min and returned to baseline after 90 min. The plasma C-peptide diminished giving an estimated exogenous mean insulin peak of 28±19/31±17 mU/l. After 15 min blood glucose decreased with nadir at 63±39/53±11 min and returned to baseline after 120 min. The fall in blood glucose ranged 0.5-3.6 mmol/l. There was no significant difference in insulin absorption or glucose reducing effect between the two spray angles but 15° elicited a more rapid effect on blood glucose. Nasal application was well tolerated and no adverse reactions occurred. The dose accuracy was 5%, and intrasubject variation for the AUC-glucose ranged 2-21%. Thus, nasal insulin administration may be an alternative to injection therapy. The spray angle of the device has no significant importance.

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Insulin Therapy

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GLYCAEMIC CONTROL DETERIORATES DURING LONG-TERM FOLLOW-UP WITH MULTIPLE INSULIN INJECTIONS

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Short-term intensive treatment programs with insulin pumps or multi-injection techniques have achieved near normoglycaemic glucose control. Long-term results are lacking. We evaluated glycaemic control in our outpatient clinic four years after multiple injection program was started. All patients who began the program during 1983-1988 were included. Insulin regimen was changed from 2 injections to 4 (3 short-acting and 1 long-acting). HbA_{1c}, serum lipids, weight, blood pressure, insulin dosage, creatinine, urinary albumin secretion and complications were recorded. All data were pooled yearly after the start of the multi-injection program. There were total of 157 patients, 95 women and 62 men. The mean age was 25.0 ± 0.6 (SEM) years and the duration of diabetes 12.9 ± 0.6 years. HbA_{1c} values were 9.97±0.18% (n=157), 9.24±0.16% ($p<0.01$, n=154), 9.30±0.19% ($p<0.01$, n=147), 9.70±0.23% (p ns, n=123) and 9.78±0.30% (p ns, n=92) on years 0, 1, 2, 3 and 4, respectively. Between sexes there were no differences. Greatest reduction in HbA_{1c} values had patients with poor glucose control (HbA_{1c}>9.5%, whereas patients with HbA_1≤9.5% in the beginning had little or no benefit. Only 8% of the patients achieved good metabolic control over four years (HbA_{1c}<7.0%). Weight was gained during the first two years. Insulin dose per weight increased in men. Serum lipids and blood pressure did not change. Severe hypoglycaemic reactions were recorded 1/240 patient months. In conclusion the better glycaemic control achieved with multiple insulin injection program seems to disappear gradually after two years.

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AN INTERACTIVE, EDUCATIONAL MODEL FOR INSULIN DOSAGE ADJUSTMENT IN TYPE I DIABETES

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The aim of this study was to build a computer model of glucose-insulin interaction in insulin-dependent diabetes, based on physiological data from the literature. The model was used to simulate steady state carbohydrate metabolism in type I diabetic subjects. Preliminary validation of the model used retrospective data from 6 patients over a 4 day period. Data from the first day was used for parameterisation of the model for individual patients to allow patient specific glycaemic predictions to be made for subsequent days. Advice to improve the patient's blood glucose profile was also generated. The predictive capability of the model was assessed in terms of the root mean square (rms) deviation between observed and predicted glycaemic profiles. The rms values obtained, based on 105 pairs of blood glucose measurements over 18 days, ranged from 0.95 to 3.14mmol/l with a mean [±SD] of 1.89±0.62mmol/l. Using 100% to represent the dose of insulin actually injected the model suggested changes to the insulin regimen of 112±181% (mean±SD) [n=44 suggestions]. We believe this model, which offers an innovative approach for making glycaemic predictions and advising on insulin therapy, has potential application as an educational tool for patients and medical personnel regarding insulin dosage adjustment in diabetes.

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EFFECTS OF THE NOCTURNAL INCREASE IN CORTISOL ON MEAL CARBOHYDRATE METABOLISM.

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Supraphysiologic concentrations of glucocorticoids influence carbohydrate metabolism. To determine if the nocturnal variation in glucocorticoids influences meal metabolism, we studied 8 nondiabetic and 5 Type 1 diabetic volunteers once with a variable (V) hydrocortisone infusion (to mimic the normal nocturnal rise) and once with a continuous basal (B) hydrocortisone infusion (0.3 μg/kg·min) as a control study. On both days, endogenous cortisol production was inhibited by metyrapone. Diabetic volunteers were kept euglycemic prior to the test meal and were given an insulin infusion equal to half the normal nondiabetic systemic insulin delivery rate. Meal metabolism was studied using the dual isotope dilution method. Post-prandial glucose concentrations were higher ($p < 0.02$) with V than B in both nondiabetic (481 ± 65 vs. 287 ± 67 mmol/L·6 h) and diabetic (2885 ± 275 vs. 2480 ± 195 mmol/L·6 h) volunteers. The postprandial glucose appearance was equal to (non-diabetic) or higher (diabetic, $p < 0.05$) on V than B. $^{14}CO_2$ incorporation into glucose (an estimate of gluconeogenesis) was 60% higher ($p < 0.01$) with V than B in diabetic volunteers. Despite higher glucose concentrations tissue glucose uptake was equal to or lower with V than B following meal ingestion in both groups, indicating that glucose disposal was inappropriate for the prevailing glucose concentration. Thus the nocturnal rise in cortisol is an important regulator of hepatic and extrahepatic responses to carbohydrate ingestion in both diabetic and nondiabetic humans.

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RELATIVE CONTRIBUTION OF DAWN PHENOMENON AND INSULIN DEFICIENCY TO FASTING HYPERGLYCAEMIA IN TYPE 1 (INSULIN-DEPENDENT) DIABETES MELLITUS.

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To quantitate the contribution of *insulin deficiency* and insulin resistance between 0500 and 0800 h (*dawn phenomenon*) to development of fasting hyperglycaemia in type 1 (insulin-dependent) diabetes mellitus, 9 diabetic patients were studied overnight on 6 occasions (S1-S6). In S1, plasma glucose (PG) and insulin (FIRI) were monitored after the therapeutic evening s.c. NPH injection. Between 0400 and 0800 h, the increment in PG was 7.4±0.5 mM, whereas FIRI decreased by 25%. When in S2, insulin was infused i.v. at variable rate to maintain PG at 5 mM between 2400 and 0800 h, the insulin infusion rate (IIR) increased between 0500-0800 h by 22±1.1% vs. 0100-0300 h (dawn phenomenon). When in S3, a fixed IIR between 0300-0800 h was used, based on mean IIR between 0100-0300 h of S2, PG increased only 1.2±0.2 mM between 0400 and 0800 h (specific contribution of *dawn phenomenon* to fasting hyperglycaemia). S4 was as S2, but the IIR between 0300-0800 h was reduced by 10%, and PG increased by 4.1±0.4 mM (dawn phenomenon+modest insulin deficiency). S5 and S6 were as S3 and S4, but the studies were performed in the afternoon (1200-2000 h). In S6, PG increased only 0.9±0.1 mM between 1700-2000 h as compared to S5. Conclusions. The dawn phenomenon contributes marginally to fasting hyperglycaemia in type 1 (insulin-dependent) diabetes mellitus, whereas insulin deficiency plays the major role. However, even a modest insulin deficiency, causes important hyperglycaemia in the morning because of the insulin resistance of the dawn phenomenon.

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POORLY CONTROLLED OBESE PATIENTS WITH NON-INSULIN-DEPENDENT DIABETES, INSULIN OR DIET ?

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We compared evening injection of NPH insulin to the modified very-low-calory-diet (VLCD) in 30 obese and poorly controlled NIDDM patients (BMI 33.5±3.7 kg/m^2, age 56±8 yrs., basal C-peptide 1.10±0.33 nmol/l, fasting blood glucose 12.0±2.5 mmol/l, and HbA1c 10.0±1.6 %). After the run-in period of two weeks 10 patients were randomised to inject NPH insulin at 21.00 hrs in addition to previous oral therapy. The mean insulin dose was 22(8-30) U at the end of the study. Ten patients were randomised to substitute their dinner and evening snack with three packages (300kcal) of VLCD-formula. Ten patients continued their conventional hypocaloric diet. The clinical characteristics of the groups were similar. The metabolic control was evaluated at the metabolic ward before and after the 3 month trial. The mean weight was unchanged in insulin treated patients, but decreased on the average by 2.6 kg ($p<0.05$) both in those with intensified and conventional diet. Mean diurnal blood glucose decreased from 12.8±2.9 to 7.1±1.8 mmol/l ($p<0.001$) in insulin treated patients, whereas the change was not significant in patients with intensified (12.0± 3.8 mmol/l vs. 10.8±4.1 mmol/l) and conventional (10.3±3.2 mmol/l vs. 9.4±2.4 mmol/l) diet. In insulin treated patients fasting serum insulin concentration increased by 63 % ($p<0.001$) but the mean daytime insulin concentration remained unchanged. **In conclusion** it is justified to consider basal supplementation of insulin also in markedly obese NIDDM patients not responding to oral therapy.

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Insulin Delivery Systems

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INTRAPERITONEAL ABSORPTION OF INSULIN AFTER LONG-TERM INSULIN THERAPY IN TYPE 1 DIABETICS.

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Changes in permeability of the peritoneal membrane may limit intraperitoneal (IP) insulin therapy using implantable pumps. Aim of our study was to evaluate the absorption of insulin through the peritoneal membrane after long-term IP insulin therapy . Six type 1 diabetic patients were given 15 IU of insulin as a 20' square wave infusion using their implanted device and peripheral insulin levels were monitored for 180' . Hypoglycaemia was prevented by intravenous administration of 10% dextrose. Tests were performed twice in each patient after 3 and 30 months of IP insulin therapy. Basal plasma insulin concentrations were identical (13±8 μU/ml and 9±4 μU/ml, IP3 vs IP30, NS). Time for insulin to peak (46±12 min and 42±11 min, IP3 vs IP30, NS) and peak hight (105±100 μU/ml and 96±53 μU/ml, IP3 vs IP30, NS) were unchanged after 30 months of IP therapy. Areas under the insulin curves were not significantly different after long-term IP insulin therapy (7876±5695 μU/ml.min and 8394±2564 μU/ml.min, IP3 vs IP30, NS). We conclude that long-term intraperitoneal insulin therapy does not affect absorption of insulin through the peritoneal membrane in type 1 diabetic patients.

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A RANDOMIZED, CROSS-OVER COST-EFFECTIVENESS COMPARISON OF INTENSIVE SUBCUTANEOUS VS IMPLANTABLE PUMP INSULIN DELIVERY : PRELIMINARY RESULTS.

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Peritoneal insulin delivery with implantable programmable pumps (IP) has proven safe and feasible, but superiority over subcutaneous intensive insulin injections (SC) is unclear. We used a 6-month, randomized, prospective, cross-over design to assess cost-benefits of IP vs SC. Ten IDDM aged 41 ± SD 6 yrs, of 13 ± 9 yrs duration, were selected because of chronic hyperglycemia (Hb A1c > 8 %) and/or glycemic instability (standard deviation of blood glucose values [SD BG] > 65 mg/dl) under SC. All patients were equipped with an IP pump but insulin infusion was activated only during the 6-month IP period, whether during the 6 months SC period, the pumps were filled with saline. Diabetes control was assessed by monthly HbA1c and home BG. Costs and comfort were evaluated using a WHO-CSII Study - derived cost and the DCCT-Lifestyle questionnaires :

n = 5, m ± SEM	IP	SC	p
HbA1c (%)	6.7 ± 0.2	7.2 ± 0.2	< 0.05
Mild hypos (n/mo)	3.2 ± 0.4	8.4 ± 2.7	NS
SD BG (mg/dl)	52 ± 4	72 ± 3	< 0.05
Direct costs ($/yr)	5645	3300	< 0.01
Satisfaction (0-5)	1.9	2.5	< 0.05

Thus, IP may be superior to SC in terms of glycemic stability, hypoglycemic risk and satisfaction of therapy, but with a doubling of treatment costs, and only small differences in the HbA1c levels attained.

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LONG-TERM TREATMENT WITH IMPLANTED, PROGRAMMABLE PUMP IN TYPE I DIABETES.

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Nineteen type one diabetic subjects, previously treated with intensive subcutaneous (s.c.) insulin therapy, were implanted an Infusaid model 1000 pump connected to an intraperitoneal catheter. The pump is a positive pressure pump powered by freon gas. The flow rate is regulated by an external programmer. During the 24 months of follow-up clinic visit of the patients and refilling of the pump were performed monthly. None mechanical problems were observed. totally 610 refillings of the pump were performed. In 49 cases a slow-down of the flow rate was observed: 27 due to obstruction of the catheter, found with pump diagnostic procedure (PDP) able to measure the pressure into the catheter. 24 obstructions were solved flushing the catheter through the side-port; in three cases the catheter was substituted. 22 cases of slow-down were related to insulin precipitation into the pump and solved flushing the pump with a sodium hydroxide solution. Mean blood glucose was better during intraperitoneal than during subcutaneous treatment (208+43.01 s.c.; 169+25.9 mg/dL 24°month i.p.). Patients filled a quality of life questionnaire that showes an improvement during the intraperitoneal treatment.

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CONTINUOUS INTRAPERITONEAL INSULIN INFUSION (CIPII) WITH A NEW PORT SYSTEM (PERCUSEAL/R PORT SYSTEM), 30 MONTH EXPERIANCE

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We performed in 40 patients a new CIPII port system. It consists of an intracutan titanium frame with a silicon membrane. Attached to the frame is a double-core catheter which leads to the intraperitoneal cavity. A specially formed extern catheter is passed through the silicone membrane and connected to the external insulinpump. The system allows connection and disconnection of the insulinpump. It is possible to exchange or to short the intraperitoneal catheter ambulant. Patients: 21 female, 19 male; age: $\bar{x}$= 35,6 (19-60) years; diabetes duration: $\bar{x}$= 16,5 (2-36) years; diabetic complication: n= 29. Results: The system is still function after 12 month in 95%, after 24 month in 70% and after 30 month in 55%. We observed one ketoacidosis, one hypoglycaemia and no peritonitis (incidence < 0,014 per patient/year). There was one reimplantation and 16 explantations. Reasons: n= 10 catheter fibrine reaction, n= 2 local infection, n=3 psychological problems, n=2 skin perforation of the intracutaneous titanium plate. 4 patients change to CIPII without an intraperitoneal catheter (Intraseal/R Port System), 12 patients change to CSII. Metabolic results: HbA1c before implantation $\bar{x}$=7,81% (+/-1,26), after 12 month CIPII $\bar{x}$=7,41% (+/-0,94), after 24 month $\bar{x}$=7,48% (+/-0,80). (The data are demonstrated at the meeting). The Percuseal/R Port System seems to be suitable for longterm ip treatment.

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EFFICACY OF THE INTRAPERITONEAL INSULIN TREATMENT IN TYPE I DIABETICS: A CROSS OVER STUDY

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The multicentric clinical trial with the Infusaid model 1000 pump showed an improvement of glycaemic control and quality of life. After one year of intraperitoneal insulin treatment 17 patient were crossed over to intensive subcutaneous insulin therapy for at least 3 months in order to avoid the interference of carry over effect of intensive subcutaneous insulin therapy. The relative frequence of blood glucose determinations above 180 mg/dl was 52% during subcutaneous treatment preceding the implant, decreased to 40% after 12 months of intraperitoneal therapy but increased to 57% during the cross over period. No significant variations in hypoglycaemic episodes were observed. Mean blood glucose showed a significant decrease during intraperitoneal treatment and an increase during the cross over period (s.c. 188 ± 32.5; i.p. 168 ± 7.07; c.o. 198 ± 18.4 mg/dl). 5 patients interrupted the subcutaneous treatment before 3 months because of deteriorations of glycaemic control, in two cases with chetoacidosis episodes. A quality of the life questionnaire filled by the patients showed an improvement during the intraperitoneal treatment and a deterioration during the cross over period. Long term intraperitoneal insulin treatment achieves and maintains an improvement of metabolic control and quality of life in type I diabetics.

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A NEW INTRAPERITONEAL PORT SYSTEM WITHOUT INTRAPERITONEAL CATHETER: INTRASEAL/R PORT SYSTEM

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The Intraseal Port System has instead of an intraperitoneal catheter a second titanium part, which project into the intraperitoneal cavity. Between the intracutaneous and the inner titanium part, there is a double-core catheter. It is possible, to change the inner catheter ambulant. The reason for the development is the problem of fibrine reaction at intraperitoneal catheters. Since december 1990 until april 1992 we performed 29 implantations of that intraperitoneal system. Patients: 16 female, 13 male; age $\bar{x}$ =33,5 (19-56) years; diabetes duration: $\bar{x}$= 14,0 (2-33) years; diabetic complications: n=18. Results: During the study time, we observed no ketoacidosis, 2 hypoglycaemic reactions (wrong- handling by the patients, no technical complication) and no peritonitis. 24 patients with first ip- treatment have in 3 cases problems with a worse insulin distribution because of fibrine reaction at the intraperitoneal implantation part. One patient changed after 4 month to the Percuseal/R Port System, in two cases laparatomia was necessary to make the system function. 5 patients which have changed from Percuseal/R to Intraseal/R have in two cases fibrine reations at the same locations. The Intraseal/R Port seems to be an interesting system for ip-treatment. Changing from Percuseal/R to Intraseal/R Port with the same intraperitoneal entrance seems to be not favourable.

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A DOUBLE-BLIND STUDY OF INJECTION PAIN WHEN USING INSULIN PENS WITH 3 DIFFERENT NEEDLES.

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Recently 28G (0.36 mm diameter) needles were introduced as an alternative to standard 27G (0.4 mm) needles. We evaluated the pain when injecting with 3 different needles: B-D MicroFineIV-28G (B28), NovoFine-28G (N28) and Novo-27G (N27). 60 children and adolescents aged 9-21 years participated. Their HbA_{1c} was 7.1±1.4%. 11/60 used indwelling catheters (Insuflon, Viggo-Spectramed). They injected with each needle once in the abdomen, once in the thigh and once in Insuflon (if applicable) on 2 different occasions and scored injection pain on a 10 cm visual analog scale (VAS; 10=unbearable pain and 0=no pain). The study was double-blind and the injection order was randomized. The median VAS score was for abdominal injections 1.15 cm (B28), 1.03 cm (N28) and 1.23 cm (N27), for thigh injections 0.70 cm (B28), 1.10 cm (N28) and 1.18 cm (N27) (n.s. by Friedmans test). Insuflon injections scored 0.15 cm (B28), 0.13 cm (N28) and 0.15 cm (N27) ($p<0.007$ by Friedmans test when compared with abdominal and thigh injections). In 30% of injections B28 was preferred, in 24% N28 and in 19% N27 while 27% were without preference (n.s. by Kappa test). In conclusion we found no difference in injection pain or preference between the 3 needles while Insuflon injections scored significantly less pain.

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THE KINETICS OF INSULIN DELIVERY BY INSULIN PENS.

BH Ginsberg, Becton Dickinson and Co, Franklin Lakes, NJ, USA and Meylan, France.

Insulin pens deliver insulin more slowly than syringes due to the many compressible elements of the insulin cartridges, including air bubbles. The kinetics of insulin delivery with varying air content was determined with both the BD Pen® and the Novolin Pen® using A14-mono[^{125}I]iodoinsulin by two methods prior to radioactive counting 1) Timed injections into a closed vial and 2) Injection onto highly absorbent paper moving at constant velocity. The average time required for a pen to deliver 20 units of insulin increased with air. Thus, a patient injecting 20 Units of insulin and withdrawing the needle from the site after 4 seconds would miss 4 Units of insulin if there were 200 *ul* of air. Since we found that 42 of a sample of 50 commercially available insulin cartridges had air bubbles (average estimated to be 50 *ul*), additional air entering the cartridge would present a possible health hazard. Previous studies have demonstrated that air enters insulin cartridges when the pen needle is left on the pen between injections and thus, patients should be encouraged to remove the needle after each pen use as suggested by the manufacturer.

Percent of **Insulin Not Delivered** to Site

Time	Syringe	Pen				
(sec.)		No Air	50 *ul* Air	100 *ul* Air	150 *ul* Air	200 *ul* Air
2	0	4	**9**	**18**	**41**	**44**
4	0	1	4	**8**	**17**	**20**
6	0	0	1	4	**7**	**9**
≥10	0	0	0	1	3	**5**

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Oral Therapy

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A RANDOMIZED CLINICAL TRIAL OF GLYBURIDE VERSUS INSULIN USING STAGED DIABETES MANAGEMENT TO ACHIEVE EUGLYCEMIA IN NIDDM

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Following 8 weeks on diet, individuals with NIDDM were randomized to glyburide (G) (29) or insulin (I) (30) for 44 weeks. Blood glucose (BG) was monitored by HbA1c (4-6% normal) and SMBG (4 times /day) with a memory reflectometer. Ambulatory glucose profiles were produced to evaluate BG control. Staged Diabetes Management (SDM), a data-based approach to clinical decision-making was used. Due to poor BG, 7 G subjects were switched (S) to I. No differences were found between I and G at entry for age, sex, weight and duration of diabetes, HbA1c (8.8 ± 3%), fasting blood glucose (FBG) (13.4 ± 0.8 mmol), and stimulated C peptide (SCP) (4.6 ± 0.3 ng/ml) for I and G. S differed significantly ($p<.01$) from I and G at entry with higher FBG (16.9 ± 0.9 mmol), lower SCP (3.4 ± 0.3 ng/ml) and increased duration of diabetes. A fall ($p<.0001$) in HbA1c, I (6.6 ± 0.1%), G (6.5 ± 0.1%), S (6.5 ± 0.6%) and FBS, I (6.9 ± 0.3 mmol/l), G (7.7 ± 0.3 mmo/l), S (6.8 ± 0.1 mmol/l) and rise ($P<.0001$) in SCP, I (5.3 ± 0.5 ng/ml), G (5.4 ± 0.4 ng/ml), S (4.4 ± 0.3 ng/ml) were seen after 44 weeks. Following SDM, near normal levels of glycemia were achieved with either treatment. Improvement of BG enhanced SCP response. G failures may be predicted by the initial SCP, FBG and duration of diabetes.

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DEFECTIVE GLUCOSE UPTAKE AND STORAGE IN DIABETIC ERYTHROCYTES AND ITS CORRECTION BY METFORMIN.

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Red blood cells (RBCs) can store glucose (G) and may participate in blood glucose homeostasis. We investigated if a defect in this process exists in type 2 diabetes. Blood was obtained in fasting state from 10 normal and 10 newly diagnosed type 2 diabetic patients (before and after 1 month Metformin therapy). Washed RBCs were resuspended in media containing various G concentrations: 0.8/1.2/1.6/ or 2.4g/1. Total G uptake was calculated from measurements of lactate, free glucose before and after addition of amyloglucosidase to the pellet. RBCs from diabetics showed a marked reduction in G uptake, which was most pronounced in their capacity to store G as glycogen (reactive to amyloglucosidase). Metformin treatment almost completely normalized glycogen synthesis, whereas lactate production declined concomitantly. Our data demonstrate: 1) a defect in G uptake by diabetic RBCs, 2) that the defect concerns mainly G storage and 3) that it is reversible by Metformin, indicating that this drug is also capable of promoting glycogen synthesis in insulin-insensitive cells. Due to their total mass; RBCs may participate to a far-from negligible extent to the impaired glucose homeostasis in diabetes, particularly in periods of frank hyperglycemia such as during the postprandial period.

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IMPROVEMENT OF GLYCEMIC CONTROL AND METABOLIC RISK FACTORS BY METFORMIN IN OBESE INSULIN-TREATED DIABETICS

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Metformin was given to obese type-2 diabetic patients poorly controlled by insulin after a secondary failure to sulfonylureas. Fifty patients participated in this randomized, double-blind six-month trial. After a four-weeks run-in period during which all patients were given placebo (single-blind), patients were randomly assigned to continue to receive placebo (n=23) or to active treatment with metformin (1700 mg/day) At six months, significant improvement of glycemic control occurred in diabetics receiving insulin and metformin (glucose decrease: -4.1 (2.94) mmol/L, mean (SD); HbAlc decrease: -1.84 (1.7)%, $p<0.001$). No significant changes were seen in diabetics receiving insulin and placebo. There was a significant decrease in blood lipids (triglyceride and cholesterol), an increase in HDL-cholesterol and a reduction of blood pressure in diabetics taking metformin. These positive results were greatest in 14 diabetics who experienced a good response to treatment (glucose profile <10 mmol/L) and less marked in the remaining 13 diabetics whose response was not so good (glucose profile >10 mmol/L). The mean daily insulin dose was reduced by 21.6 (19) U/day ($p<0.001$) after six months of the combined treatment. Combining metformin to insulin may represent a safe strategy to achieve a better glycemic control with a reduction of some metabolic risk factors associated with the cardiovascular disease of diabetes.

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EFFECT OF METFORMIN TREATMENT ON INTERMEDIARY METABOLITE CONCENTRATIONS IN TYPE 2 DIABETES.

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Metformin increases lactate concentrations. However, most studies were performed either in the fasting state or by evaluating 24-hr metabolite profiles. Since metformin may also impair absorption of complex carbohydrates, its effects on circulating metabolites may be confounded by possible impairment of nutrient digestion. In this study, we have evaluated the effects of metformin (850 mg b.i.d. for four weeks) in diet-treated type 2 diabetic patients, using a double blind, placebo controlled design, both in the fasting state and following the continous administration of a synthetic mixed meal (≈11 Cal/kg of BW), composed of sucrose (50% of calories), cristalline amino acids (18%) and fat (32%). Postabsorptive (162±17 to 141±20 mg/dl) and post-meal plasma glucose (217±20 to 164±20 mg/dl) were decreased ($p<0.05$) by metformin. Fasting blood lactate was similar before (0.76±0.05 mmol/L) and after (0.66±0.06 mmol/L) treatment. However, post-meal lactate was one-fold greater ($p<0.002$) after metformin (2.04±0.18 vs 1.13±0.09 mmol/L). Blood alanine was also comparable in the fasting state (0.24±0.02 before, vs 0.24±0.01 mmol/L), but post-meal alanine was greater ($p<0.02$) after drug treatment (0.34±0.02 vs 0.42±0.02 mmol/L). The concentrations of other intermediary metabolites (pyruvate, 3-hydroxybutyrate, acetoacetate, and glycerol) were unchanged. These data show that, even after an elementary mixed meal, metformin can modestly increase post-prandial lactate and alanine levels, suggesting impaired utilization of these substrates for gluconeogenesis.

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Lessons from *in vivo* and *in vitro* studies of metformin concentrations in plasma and in erythrocytes.

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Using HPLC we measured the metformin concentrations in plasma (PM) and erythrocytes (EM) in metformin-treated diabetic patients either with (n = 10) or without (n = 58) lactic acidosis (LA). We also studied a model for cellular uptake of metformin in tissues involved in the initiation or aggravation of LA, measuring EM *in vitro* at 37°C (as a function of time and of blood concentrations: 5,10, 25, 50, and 100 $mg.l^{-1}$). Without LA, PM and EM were 0.59 ± 0.10 and 0.87 ± 0.10 $mg.l^{-1}$ respectively. PM and EM were low in 2 patients with LA (≤ 0.2 and ≤ 0.5) and high in 8 others (range : 2 - 68, mean : 25.0 ± 9.2 and 13.1 ± 3.7). The higher values were limited to three anuric patients. In one of the latter who was treated by dialysis, PM decreased after each session by 50% and EM by 10%. *In vitro*, EM increased slowly and linearly with time. After 48 hours' incubation, it had increased to half initial blood concentration, irrespective of what it has been. In conclusion these data suggest that metformin can accumulate in a deep-compartment. However, because EM increases slowly *in vitro*, the accumulation cannot become significant at normal dosages unless there is prolonged renal failure.

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METABOLIC EFFECTS OF METFORMIN ASSOCIATION TO GLIBENCLAMIDE TREATMENT IN TYPE 2 DIABETES

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In a randomized double-blind cross-over study the addition of metformin to glibenclamide treatment was assessed conventionally (blood glucose profile and HbA1c measurement) and with an euglycaemic-hyperinsulinemic glucose clamp in ten type 2 diabetics in poor metabolic control. All patients were non obese (body mass index 22.3±0.5(±SE)$kg\ m^{-2}$). Metformin (500 mg twice a day) or placebo were addeed for 6 weeks to the usual sulphanylurea treatment (glibenclamide 5 mg three times a day). On the last day of either metformin or placebo administration the euglycaemic (5.5±0.5 mmol l^{-1}), hyperinsulinemic (698.1±22.9 pmol l^{-1}) clamp was performed together with the study of insulin binding to circulating monocytes. Metformin reduced fasting (6.1±0.4 vs 6.4±0.4 mmol l^{-1}, P=0.036),and mean daily plasma glucose concentration (9.2±0.3 vs 11.4±0.4 mmol l^{-1}, P<0.001), and HbA1c (8.7±0.3 vs 9.3±0.2 %, P=0.027). No variations were registered in plasma insulin and in body weight. A significant reduction of hepatic glucose production (12.8±2.8 vs 33.9±4.4 umol $kg^{-1}\ min^{-1}$, P<0.001) together with an increase of glucose utilization (33.3±2.8 vs 26.1±1.1 umol $kg^{-1}\ min^{-1}$, P=0.033) was found after metformin while residual glucose production during insulin infusion did not significantly varied. Insulin binding to circulating monocytes was higher after metformin (4.8±0.9 vs 3.2±0.6 %, P=0.030). Lipaemic profile showed a reduction in triglycerides (1.2±0.1 vs 1.7±0.3 mmol l^{-1}, P=0.039) and an increase in HDL-cholesterol (1.3±0.1 vs 1.0±0.1 mmol l^{-1}, P=0.004) without variations in total-cholesterol after metformin. These findings confirm the metabolic improvement after combined treatment suggesting its possible mechanism of action.

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Miglitol and glibenclamide comparison in type 2 diabetes

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The efficacy of a 24-week treatment with miglitol , an a-amylase inhibitor, compared with glibenclamide was studied in type 2 diabetes treated with diet alone. 100 patients (age<65 y, BMI<30 kg/m^2) in fair metabolic control (HbA_{1c}>7.5%) despite diet were included in the study. After a run-in period patients were allocated randomly in a double-blind fashion to either miglitol (50 and 100 mg t.i.d.) or glibenclamide (2.5 and 5 mg b.i.d.) treatment. The dose was doubled after 4 weeks of treatment in absence of significant side effects. Metabolic control was assessed by HbA_{1c} before and during treatment. A standard mixed-meal (590kcal, 44% carbohydrates, 41% proteins, 15% lipids, 30 g fibres) was performed to evaluate glycaemic and insulinemic response at the beginning and at the end of treatment. HbA_{1c} values decreased from the baseline (8.1±0.2 and 7.8±0.1% for miglitol and glibenclamide group respectively) both after miglitol (-0.54±0.15, -0.70±0.18 and 0.86±0.22% at week 8,16 and 24, P<0.01 for all data) and after glibenclamide (-0.99±0.14,-1.21±0.17 and -1.16±0.21% respectively, P<0.01 for all data). In the mixed-meal test it was noted a decrease of glycaemic incremental area (402±40 vs 590±41 mmol/240min, P=0.0014) together with a decrease of total (64216±5506 vs 85695±5671 pmol/240min, P=0.0078) and incremental area (36746±4531 vs 49881±4667 pmol/240min, P=0.046) for insulin after miglitol vs glibenclamide. The tolerance of both treatments was good: only three patients withdrew the study and only one for adverse events during miglitol. Both preparations were found to have similar effects on blood glucose control as assessed by HbA_{1c}, even if with different mechanism of action as indicated by the decrease of insulin area during mixed-meal test after miglitol in comparison to glibenclamide.

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NO EFFECT OF BEDTIME MIGLITOL ON FASTING BLOOD GLUCOSE IN TYPE 2 DIABETIC PATIENTS.

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The fasting blood glucose value is partly determined by glucose production, i.e. glycogenolysis, in the process of which alpha-glucosidase activity is involved. To investigate whether the alpha-glucosidase inhibitor Miglitol (Bay m 1099) can influence this glycogenolysis and thus the fasting blood glucose value, 12 type 2 diabetic patients (M/F= 6/6) treated by diet and/or sulphonylurea were studied using a double blind, randomised, placebo controlled, cross-over study design during which subjects ingested 100 mg Miglitol or placebo at bedtime for 1 week each with a washout period in between of 2 weeks. Fasting blood glucose (FBG), insulin, C-peptide, glucagon, pyruvate and alanine were measured at start, days 0,1,2,3,6,7 of each study period. Statistical analysis was done with ANOVA. Results: FBG did not change after miglitol (initial FBG: 11.4±2.7 mmol/l; after 7 days of miglitol: 12.2±2.5 mmol/l; after 7 days of placebo: 12.4±2.6 mmol/l) nor any of the other parameters studied. Conclusions: 100 mg Miglitol given at bedtime has no effect on glycogenolysis and does not reduce the fasting blood glucose value in type 2 diabetic patients.

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A "POOR" FASTING BLOOD GLUCOSE CONCENTRATION DOES NOT PREDICT "POOR" POSTPRANDIAL VALUES IN TYPE 2 DIABETES.

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To evaluate whether a "poor" fasting blood glucose (BG) according to the European NIDDM Policy Group (>7.8 mmol/l) is predictive of "poor" postprandial values (>10 mmol/l) in Type 2 diabetes, we examined 420 BG profiles of patients on diet (Group A: M/F=0.95, age 57.5±0.5 years, diabetes duration 5.4±0.2 years, BMI 30.7±0.3 Kg/m²) and 620 BG profiles of patients on oral agents (Group B: M/F=1.03,age 61.6±0.4 years,diabetes duration 10.0±0.3 years, BMI 31.3±0.5 Kg/m²). BG (m±sem) after overnight fast, 2 hours after breakfast, 2 and 4 hours after lunch was 8.91±0.07, 8.68±0.10, 9.15±0.11 and 7.64±0.10 mmol/l in Group A, 9.52±0.06, 9.58±0.09, 9.74±0.10 and 8.39±0.10 mmol/l in Group B. 28% of Group A profiles and 20% of Group B profiles contained only "good" postprandial BG (4.4-8.9 mmol/l) (p=0.003); 23% and 19%, respectively, contained also "acceptable" postprandial BG (<10 mmol/l) (NS); 48% and 63% contained at least one "poor" BG (>10 mmol/l) (p=0.000). 31% of Group A profiles and 30% of Group B profiles contained their highest BG after the overnight fast (NS). HbA1c was 7.2±0.1% in Group A, 7.6±0.1% in Group B (p=0.02). In conclusion , a "poor" fasting BG does not predict "poor" postprandial concentrations in Type 2 diabetic patients. BG profiles should therefore be performed to check usefulness and safety of drug prescription in these patients.

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Chinese Tradional Antidiabetic Therapy Versus Sulfonylurea (Glibenclamide) in the People's Republic of China : Randomized Controlled Clinical Trial.

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A clinical trial was performed in non insulin-dependent diabetic patients in the People's Republic of China. The aim was to evaluate the efficacy of a traditional phytotherapy made from three plants, and its association with a sulfonylurea (2.5 mg x 3/d). The methodology for this multicentre randomized double-blind trial was a 2 x 2 factorial design : (4 groups : 1 = placebo (P) phytotherapy + P (glibenclamide ; 2 = P phytotherapy + verum glibenclamide ; 4 = verum phytotherapy + verum glibenclamide). To be included, patients had to be non insulin-dependent diabetic out-patients, aged 40-70 years, treated either by diet alone or by oral anti-diabetic drug, with a mean value of 3 fasting blood glucose levels between 8.9 and 12.2 mmol/l. Examinations were planned monthly, during the 3 months of treatment. The endpoint criteria to be evaluated were HbA1, blood glucose and plasma insulin (at fasting, 1hr and 2hr after a test meal). At each visit, a clinical examination was performed, and a questionnaire on the side effects and the associated symptoms was completed. The dose was reduced by half in the case of hypoglycemia. The two-hundred and sixteen patients recruited in the 5 centres (Shangaï (1) = 48 ; Shangaï (2) = 40 ; Beijing = 40 ; Canton = 42 ; Chendu = 46) were randomized in the 4 treatment groups (group 1 = 56 ; group 2 = 56 ; group 3 = 50 ; group 4 = 54). Eleven patients were withdrawn for administrative reasons. In patients treated with glibenclamide, a significant increase in weight and insulinemia were observed, together with a significant decrease in blood glucose values ; in those treated by phytotherapy, only blood glucose value 2 hr after the test meal decreased significantly. A synergetic effect on blood glucose was observed when both treatments were given. Hypoglycemia occured in nineteen patients (all in the 2 verum glibenclamide groups).

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Experimental Therapy

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BENFLUOREX IMPROVES HEPATIC AND PERIPHERAL INSULIN SENSITIVITY IN TYPE 2 DIABETIC PATIENTS.

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Benfluorex is a hypolipemic agent which improves blood glucose control in type 2 diabetic patients. To establish the mechanisms responsible for the hypoglycemic effect, 20 type 2 diabetic subjects underwent a double blind randomized treatment with benfluorex or placebo for 11 days. The two groups were balanced for number, sex, age, diabetes duration and body weight. The plasma concentrations of glucose, free fatty acids (FFA), insulin, glucose kinetics (^{3}H-glucose infusion), and insulin sensitivity (2 step hyperinsulinemic euglycemic clamp: 0.1 and 1 mU·kg^{-1}·min^{-1}) were determined before and after therapy. Statistical analysis was performed using ANOVA. Before treatment all parameters examined did not differ between the two groups. Benfluorex administration, when compared to placebo, reduced ($p<0.05$) plasma glucose (8.5±0.3 vs 8.9±0.1 mM), FFA (0.62±0.02 vs 0.72±0.03 mM), hepatic glucose production (1st step of glucose clamp: 35±3 vs 45±5 µmol·kg^{-1}.min^{-1}), increased ($p<0.05$) peripheral glucose utilization (2nd step of glucose clamp: 322±6 vs 272±19 µmol·kg.min), almost significantly ($p=0.06$) reduced (165±8 vs 177±6 pM) plasma insulin concentrations in the absence of changes in body weight. In conclusion: benfluorex ameliorates blood glucose control in type 2 diabetic patients by increasing both hepatic and peripheral insulin sensitivity. It is possible that the improvement of glucose kinetics was caused by the benfluorex induced reduction in plasma FFA concentrations.

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METABOLIC EFFECT OF A NEW ORAL HYPOGLYCAEMIC AGENT, AG-EE 623 ZW, IN SULPHONYLUREA (SU) TREATED TYPE 2 DIABETIC PATIENTS.

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AG-EE 623 ZW is a new, non-sulphonylurea oral hypoglycaemic agent with a stimulatory effect on insulin secretion. Its effect on metabolic control and lipids was assessed in comparison with glibenclamide (GLIB) in 44 SU-treated type 2 diabetic patients (age 63±7 yr, BMI 26.2±2.8 kg/m²). Patients received after randomisation either AG-EE 623 ZW (0.5-2 mg, n=29) or GLIB (5-10 mg, n=15) before breakfast and dinner. After 12 wks, median dose of AG-EE was 4.0 (range 1.5-4.0) mg, and GLIB 15 (1.25-15) mg. GLIB especially lowered fasting blood glucose (10.4±2.9 to 8.6±-2.4 mmol/l, $p<0.05$), while AG-EE 623 ZW lowered postprandial glucose (14.0±4.6 to 12.6±4.5 mmol/l, $p<0.01$). HbA_{1c} did not change in AG-EE patients previously treated with high doses SU, but fell from 8.6±2.2 to 7.9±2.1% ($p<0.01$) in patients previously using lower SU-doses. With GLIB, HbA_{1c} remained unchanged. Plasma cholesterol decreased with AG-EE from 6.9±1.4 to 6.6±1.3 mmol/l, and with GLIB from 6.5±1.1 to 6.1±0.9 mmol/l (both $p<0.05$). Two patients on AG-EE did not complete the study, one for personal reasons, one because of a rise in blood glucose. No abnormal findings attributable to AG-EE 623 ZW were observed in clinical and laboratory examinations; no hypoglycaemic symptoms were observed. AG-EE 623 ZW is an effective and safe drug for the treatment of patients with type 2 diabetes.

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Clinical evaluation of a new oral hypoglycemic agent CS-045 in Type 2 diabetes

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CS-045 is a new oral hypoglycemic agent for the treatment of Type 2 diabetes by improving insulin resistance. We investigated the efficacy and safety by dose increasing method in 216 type 2 diabetic patients whose glycemic control was insufficient(fasting plasma glucose(FPG)≧ 8.3mM) with surfonylurea. CS-045(400mg/day) was administered for 8 weeks and the dose was increased to 800mg in patients whose FPG failed to decrease below 7.8mM at 8weeks and the drug was continued for additional 8weeks. In responded patients at 8weeks evaluation, 400mg was administered for 8weeks further. 159 patients were evaluated for efficacy. They were divided into 3 groups; A(n=47):400mg for 16weeks(FPG≦7.8mM at 8weeks). B(n=62):400mg for 8weeks then to 800mg for 8weeks(FPG>7.8mM at 8weeks). C(n=43):400mg for 16weeks(FPG>7.8mM at 8weeks). In A, FPG fell from 9.8mM to 6.7mM(8weeks) and 7.4mM(16weeks). In B, FPG slightly decreased after increasing the dose (0weeks;11.2mM, 8weeks;10.4mM, 16weeks;9.9mM),but the hypoglycemic effect was insufficient. In C, FPG at 8 and 16weeks was almost similar (0week;10.8mM, 8weeks;9.3mM, 16weeks;9.6mM). Thus increased dose failed to further potentiate the hypoglycemic effect of CS-045. Adverse events occurred in 9 out of 216 patients, and were mainly gastrointestinal symptom and swelling sensation of face and legs. In conclusion, CS-045 was an effective and safe hypoglycemic agent and the recommended dosage was 400mg in Japanese type 2 diabetes.

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THE NEW ALPHA-2 ADRENOCEPTOR BLOCKING COMPOUND (SL 84.0418) INHIBITS BLOOD GLUCOSE INCREASE FOLLOWING ORAL GLUCOSE TOLERANCE TEST IN HEALTHY SUBJECTS.

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Alpha-2 adrenoceptor blocking drugs may represent a new therapeutic tool in the treatment of non insulin dependent diabetes mellitus. The effects of 10, 50, 100 mg SL 84.0418 (SL) on blood glucose (BG), plasma insulin, C-peptide, glucagon, adrenaline and noradrenaline were investigated in comparison with placebo and glipizide 5 mg in a double blind crossover study in 15 healthy subjects. Glucose (75 g) was given 1 h after a single dose of each treatment at one week interval. Area under the BG curve and peak BG were dose-dependently reduced by SL, the extent of this reduction was the same with 100 mg SL than with 5 mg glipizide. Glipizide but not SL decreased nadir BG compared with placebo. Plasma insulin and C-peptide were increased by glipizide.SL slightly increased plasma insulin prior to glucose load but did not enhance glucose-induced elevation of plasma insulin. Treatments did not modify plasma glucagon. Plasma adrenaline rose significantly on glipizide and plasma noradrenaline on SL 50 and 100 mg. Heart rate, systolic and diastolic blood pressure were dose dependently increased by SL (mean maximal increase with 100 mg SL: 5 beats/min, 16 and 14 mmHg, respectively). Adverse effects reflecting alpha-2 adrenoceptor blockade (tremor, pilo-erection, hot flushes) occurred more frequently with 100 mg SL. The adverse effect profile of 50 mg SL was not different from that observed with glipizide. Conclusion: The alpha-2 adrenoceptor blocking compound SL dose-dependently inhibits BG increase after OGTT in healthy subjects without modification of glucose-induced increase in plasma insulin and C-peptide.This antihyperglycemic effect is accompanied by a dose-dependent increase in plasma noradrenaline (corresponding to a pre-synaptic alpha-2 blockade), heart rate and blood pressure. The lack of action of SL on plasma insulin may suggest an extrapancreatic effect.

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GLUTATHIONE-ISOPROPYL-ESTER PREVENT ALLOXAN DIABETES IN RAT

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It has been suggested that alloxan, which destroys islets by free radical formation, produces diabetes in rats. Though reduced glutathione can decrease free radicals, it can prevent alloxan toxicity only just before alloxan injection, because exogenous glutathione cannot penetrate into tissue. In this study, we use glutathione isopropyl ester, which can increase intracellular glutathione, and discuss its possibility of prevention of alloxan toxicity. Glutathione isopropyl ester (YM737) was supplied from Yamanouchi Ltd (Japan). 3h after intraperitoneal injection of 3mmol/kg YM737 (n=8) into fasted Wistar rats, alloxan (40mg/kg) was injected from tail vein . We were measured its postprandial plasma glucose, serum insulin lebel, and pancreas insulin content in rats treated with YM737, reduced glutation (3mmol/kg, n=9) and control (154mmol/l Nacl, n=9) 1 week later. In rats treated with YM737, the plasma glucose was significantly lower compared with glutathione and control (288±25 vs 465 ±33, 500 ±65mg/dl, P<0.05), while panceras insulin content was significantly higher (3.5±0.9 vs 0.5±0.3, 0.9±0.8 mg/mg-protein, P<0.05). But, there was no significant difference in serum insulin lebel in the three groups (3.65 ±0.25 vs 2.65±0.5, 4.55 ±0.85 ng/ml). These results suggest that glutathione isopropyl ester might prevent Alloxan toxicity compared with glutathione and control.

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THERAPEUTIC EFFECTS OF VITAMINS E AND C ON THE SERUM LIPID PEROXIDATION AND GLYCAEMIA IN DIABETIC SUBJECTS

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Toxic oxigen radicals have been linked to β cell damage brought about by some chemicals and might conceivably also be of importance in the pathogenesis of spontaneous Insulin dependent Diabetes Mellitus. We have investigated the serum lipid peroxidation and blood glucose concentration 5 and 12 days after the treatment of diabetic subjects by vitamin E and C. The level of lipid peroxidation products was examined in serum of IDDM subjects by measuring their thiobarbituric acid (TBA) reactivity. Hyperglycaemia was assesed by measuring of blood glucose. The treatment by vitamins E and C caused significant decrease in serum thiobarbituric acid value and blood glucose concentration. The antioxidative capacity of serum diabetic subjects, after the treatment, was tested in phospholipid suspension in the presence of traces of netal ions. These vitamins caused the increase of antioxidative capacity of the serum of IDDM patients. This study suggests a significantly altered lipid composition and an acumulation of lipid peroxidation products in serum of IDDM subjects. We have documented that the treatment by vitamins E and C, the substances possesing antioxidative properties, acted prophylactically against lipid peroxidation and hyperglycaemia in IDDM subjects.

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DAILY VITAMIN E SUPPLEMENTS IMPROVE INSULIN ACTION IN TYPE 2 (NON-INSULIN) DEPENDENT DIABETIC PATIENTS.

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To study the possible effects of Vitamin E (Vit.E) on insulin action 15 non-insulin dependent diabetic (fasting plasma glucose:7.3+0.2 mmol/l) patients treated by oral hypoglycemic agents were submitted to an euglycemic hyperinsulinemic (1 mU/kg x min) glucose clamp in a double-blind,randomized,cross-over procedure after 4 mounths treatment with either Vit.E (900 mg/day) or placebo (PBO) at 4 weeks intervals.In fasting conditions and during insulin infusion D-3-H glucose and indirect calorimetry allowed determination of glucose turnover parameters and substrate oxidation.After Vit.E there was a significant reduction in HbA1 (7.8+0.2 vs 7.1+0.3% p<0.05) while body mass index (26.1+0.1 vs 26.1+0.2 kg/m2 p=NS) and lean body mass (63.4+0.7 vs 62.8+0.4 kg p=NS)were unchanged.During glucose clamp , plasma glucose and insulin levels were similarly clamped at 6 mmol/l and 650 pmol/l in both occasions respectively. In the last 60 min of the experiment, Vit.E vs PBO strongly increased glucose disappearance rate (Rd:27.1+0.5 vs 18.4+0.4 umol/kg x min p<0.02),total body glucose diposal (TBGD:28.1+0.4 vs 19.0+0.7 umol/kg x min p<0.01) and non-oxidative glucose metabolism (NOGM:13.9+ 0.3 vs 8.5+0.3 umol/kg x min p<0.02). Fasting and insulin mediated changes in hepatic glucose output,oxidative glucose and lipid metabolism were similarly affected in both experimental conditions. In conclusions Vit.E administration contributes to improve insulin action and metabolic control in non-insulin dependent diabetic patients.

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THE EFFECT OF VITAMIN C AND DESFERRIOXAMINE ON OXIDATIVE STRESS IN DIABETES

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Increased oxidative stress may contribute to the development of complications in diabetes mellitus. We have therefore assessed the effect of treatment with ascorbate and desferrioxamine on oxidative stress in the streptozotocin diabetic rat. Untreated diabetes was characterised by increased lipid peroxidation as shown by increased levels of plasma malondialdehyde (MDA) [diabetic 1.92±0.21 μmol/l (n=11, mean±SEM) vs control 1.03±0.23 μmol/l (n=9), p<0.01] and conjugated dienes (CD) [0.595±0.007 U vs 0.540±0.010 U, p<0.01]. In addition, there were reduced levels of the antioxidant vitamins ascorbate (40.6±5.6 vs 67.2±5.7 μmol/l, p<0.01), retinol (0.98±0.12 vs 1.39±0.14 μmol/l, p<0.01) and tocopherol (18.0±1.5 vs 28.4±1.6 μmol/mmol cholesterol, p<0.01). Insulin treatment returned these parameters to normal. Ascorbate supplementation normalised vitamin C status but failed to reduce lipid peroxidation [MDA 2.10±0.25 μmol/l, CD 0.620±0.008 U (n=9), p=NS vs untreated diabetes], probably due to pro-oxidant effects of ascorbate mediated by interactions with transition metals. In support of this, the addition of daily subcutaneous desferrioxamine to oral ascorbate treatment significantly reduced lipid peroxidation [MDA 1.27±0.21 μmol/l, CD 0.559±0.008 U (n=11), p<0.05 vs untreated diabetes] although desferrioxamine alone was without effect [MDA 1.67±0.21 μmol/l, CD 0.595±0.007 U (n=12), p=NS vs untreated diabetes]. Due to interactions with transition metals, the use of ascorbate supplementation as an antioxidant treatment in diabetes may be of no benefit.

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DEFEROXIAMINE AND NICOTINAMIDE IN NEWLY DIAGNOSED TYPE 1 DIABETIC PATIENTS: A RANDOMIZED DOUBLE-BLIND PLACEBO CONTROLLED TRIAL.

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Deferoxiamine (DN) is an iron chelator that could be beneficial on remissions in newly diagnosed type 1 diabetics; effects of nicotinamide (NC) in these patients remains controversial. To test effects of DN and NC, 36 newly diagnosed type 1 diabetics (symptoms < 90 days; age: 22.8±6.9 years; sex: 18 M, 18 F), 5 days after beginning insulin therapy, were randomized in a double-blind fashion to one of the following groups: 1.- DN (4.5 gr/day, s.c. infusion pump; 10 days) + oral placebo (3 months); 2.- S.c. placebo (10 days) + oral placebo (3 months); 3.- S.c. placebo (10 days) + NC (1.5 gr/day; oral; 3 months); 4.- DN + NC (same doses and routes). At entry, differences between groups in relation to age, sex, islet cell antibodies, insulin auto-antibodies, HbA1c and basal and stimulated C-peptide levels were NS. At 15, 45, 90 and 180 days evolution significant differences (Kruskal-Wallis test) in relation to insulin doses, HbA1c, mean capillary glycemia and basal and stimulated C-peptide were not found; at 180 days evolution, differences related to complete and partial remission rates and times were NS (X^2 and Lee-Desu tests, respectively). Conclusion: neither DN nor NC alone or associated seemed to have additional effect on natural history of newly diagnosed type 1 diabetics.

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ORAL NICOTINAMIDE IMPAIRS GROWTH IN YOUNG RATS

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Nicotinamide (NCT) is reported to protect against the development of type 1 diabetes. Although widely believed to have no significant toxic effects, NCT has been associated with weight loss in prepubertal rats when given intraperitoneally. Trials with oral NCT are currently under way in children, and we felt it important to establish the influence of oral NCT on weight gain. NCT was given to newly weaned Wistar rats (n = 5, weights 39-50g) at a dose of 0.62g/Kg/day body weight in the drinking water. The weight increase was compared with that of controls. A divergence in weight gain was apparent from the start, and after 17 days on NCT the difference was statistically significant (control weight = 201.9 ± 21.8; NCT-treated weight = 168.6 ± 11.4 p < 0.05). After 50 days on NCT the mean weight of NCT-treated rats was 72% that of controls (p < 0.01). In contrast, the same dose of NCT given to adult rats (weight 200-220g) had no effect on weight over an observation period of 4 months (control weight = 289.6 ± 30.4, n = 5; NCT-treated weight = 293.2 ± 45, n = 5). This study suggests that nicotinamide has no effect on the maintenance of weight in adult rats, but significantly impairs weight gain in young rats. Although the dose used was higher than that currently used in human studies, caution should be exercised in the treatment of children with nicotinamide.

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NICOTINAMIDE TREATMENT IN CHILDREN WITH NEWLY DIAGNOSED TYPE 1 DIABETES MELLITUS.

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The effect of nicotinamide (NA) treatment in addition to insulin therapy on diabetes control as well as on onset and duration of remission was investigated in the group of 34 children with newly diagnosed Type 1 (insulin-dependent) diabetes mellitus. The control group included 14 children with Type 1 diabetes mellitus treated only with insulin. NA was administered in the dose of 200-600 mg/day since the day of diagnosis of diabetes and the therapy with NA continued for 12-18 months. Initial mean postprandial blood glucose and HbAlc levels and insulin requirments were not significantly different in both groups. After 12 months the patients on NA therapy had significantly lower mean postprandial glucose (7.5±2.7 vs. 11.5±7.1 mmol/l, p/0.01) and HbAlc (6.8±2.3 vs. 8.9±3.3 umol fructose/g Hb, p/0.025) levels as well as lower insulin dose requirment (0.51±0.19 vs. 1.01±0.25 IU/kg/day, p/0.01) in comparison with controls. No significant difference was found when comparing mean time of onset of diabetes remission in both groups while the duration of remission was significantly longer in NA treated patients. The results of the present study suggest that NA may have beneficial effects on diabetes control and duration of remission phase in children with newly diagnosed Type 1 diabetes mellitus.

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COMPARISON OF THE PHARMACOLOGY OF TWO ALDOSE REDUCTASE INHIBITORS - PONALRESTAT AND (4-AMINO-2-6-DIMETHYLPHENYL-SULPHONYL)NITROMETHANE.

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A new inhibitor of aldose reductase (ALR2), (4-amino-2,6-dimethylphenylsulphonyl)nitromethane (SMN) may offer the potential to clarify the role of ALR2 in diabetic neuropathy. Despite being twenty fold less potent as an inhibitor of ALR2 in vitro (IC_{50} 1.8 X 10^{-7}M vs 8.0 X 10^{-9}M for SNM and Ponalrestat (P) respectively) SNM is >8 fold more potent in vivo. In rat, P and SNM inhibited ALR2 in sciatic nerve with an ED_{50} and ED_{95} of 7.6 and 103, and 1 and 13.9 mg.Kg^{-1} day-1 respectively. Incubation of rat erythrocytes with P or SNM, either in buffer or plasma containing galactose, provided a measure of protein binding. Results showed 6% of P was free in plasma compared to 50% of SNM. Incubation of rat nerve in vitro showed that P was a less potent inhibitor of ALR2 than SNM (IC_{50} 2.1 X 10^{-5}M vs 1.73 X 10^{-6}M). These data provide a rational basis for differences observed between the in vitro and in vivo efficacy of P and SNM. SNM is a potent and well tolerated inhibitor of ALR2 in vivo and provides the sustained and high level of ALR2 inhibition required to block polyol flux and to reverse motor and sensory nerve conduction deficits in diabetic rats.

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EFFECT OF CISAPRIDE ON BLOOD GLUCOSE CONTROL IN PATIENTS WITH UNSTABLE DIABETES

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We studied 12 long-standing (mean duration of diabetes 22 years, range 9-48), insulin-taking patients with unstable diabetes confirmed under Metabolic Unit conditions. Gastric emptying of solids (radio-isotope method) was significantly impaired in all patients. 11/12 had impaired or absent awareness of hypoglycaemia. 9/12 had abnormal cardiovascular autonomic function (on-line computerised analysis of R-R interval in 5 test situations). Cisapride 10mg TDS significantly improved blood glucose stability in 11/11 patients while in hospital. One patient could not tolerate the drug. At out-patient follow-up (mean duration of treatment 5.5 months, range 1-25) improved blood glucose stability was maintained in only 5/11 patients. Re-examination of those who relapsed as out-patients (so far 2/6 re-examined) shows that the defect in gastric emptying remains corrected by cisapride. We conclude that impaired gastric emptying of solids is common in patients presenting with unstable diabetes. Such patients do not always have symptoms of gastroparesis and may have normal cardiovascular autonomic function tests. Cisapride corrects the defect in gastric emptying effectively and this is invariably associated with improved glucose regulation in the in-patient situation. However on discharge from hospital blood glucose control deteriorates again in some patients. Such relapse seems to be due to factors other than impaired gastric emptying of solids.

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Glucose Sensors

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Blood-glucose determinations using bacterial cellulose based membranes.

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The application of glucose sensors for whole blood online glucose measurements is still lacking because of biological interactions of sensor surfaces and blood, e.g. surface fouling and encapsulation. Therefore, the aim of this study was to build up a sensor-system, containing a long-term stable glucose sensor, suitable for blood glucose measurements. We used a well known amperometric sensor system and examined the long-term stability of a sensor with a bacterial cellulose based membrane (BC) as outer membrane, and a commercial dialysis membrane (Cuprophan®). Both systems were tested in contact with diluted and undiluted human blood (reference measurements were done with a Beckman Glucose Analyser 2).
We choose bacterial cellulose as membrane material, because of its high purity, good storage stability and mechanical properties. Furthermore, it shows hydrogel character and allows steam sterilisation.
The present study gave the following results:
- in 1:10 diluted blood the BC membrane showed a long-term stability of about 200 h. The Cuprophan® membrane was stable for only 30 h.
- in undiluted blood, a similar trend in long-term stability was found. Cuprophan® was stable for 3-4 h and the BC membrane for more than 24 h.
- To improve the linearity range, the BC membrane was covered with a polyamide and we found a linearity range up to 2,5 g% glucose.
- Investigations of blood glucose in rats showed a good correlation of our sensor-system with the commercial available Exactech®-sensor.

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SUBCUTANEOUS IMPLANTED GLUCOSE SENSORS: SELF-TUNING QUALITY ASSURANCE IN VIVO

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Application of s.c. glucose sensors in metabolic care is hampered by unpredictable changes of in vivo function. Thus, repeated in vivo calibrations are required. This study presents a model-based procedure where s.c. glucose concentration is simulated as a state variable $x_{sc(s)}$ as a reference of measured $x_{sc(m)}$, i.e. to the current of implanted glucose electrode. In the related state equation $\dot{x}_{sc(s)}=-b_{sc}/T_{sc}\cdot x_{sc(s)}+b_{sc}\cdot x$, T_{sc} is the time constant and b_{sc} the gain factor of system, s.c. glucose compartment plus sensor in situ. Self-tuning is initiated by automated in situ assessment of T_{sc} and b_{sc} (dogs: 10 - 60 min and 0.45 - 0.95 respectively), which together with individual model constants, and inputs of insulin, food and exercise are applied to computer. It predicts patterns of $x_{sc(s)}$ which are compared to $x_{sc(m)}$ according to the criteria $PV=(x_{sc(m)}/x_{sc(s)})\cdot 100$ (predictive value) and drift $RD=((x_{sc(s)}-x_{sc(m)})/x_{sc(s)})\cdot 100$ both actually and at intervals of 6, 12 and 24 hours by regression analysis. When significant deviations occur, sensor signal is adapted automatically to $x_{sc(s)}$ or external alarm initiate intervention. This system has been validated on GOD-H_2O_2 sensors in 9 normal dogs (5-hours i.v. glucose infusion) and in 3 diabetic animals (daily monitoring on insulin injection treatment). Conclusion: Self-tuning in situ calibration is feasible to guarantee reliable sensor function for metabolic monitoring.

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SATELLITE G AND COMPANION 2 - ADVANCED BIOSENSOR TECHNOLOGY FOR SELF MONITORING OF BLOOD GLUCOSE

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In 1989, MediSense introduced first generation biosensor technology for self-monitoring of blood glucose with ExacTech. The aim of this study was to evaluate the advanced second generation, satellite G and Companion 2 (MediSense, Mainz), featuring "autostart"; shorter test time (20 seconds); lower sample volume (15µl); and compensation for interferences and temperature fluctuation. Satellite G, with capillary or venous whole blood options, is designed for professional decentralised blood glucose monitoring; Companion 2 is the equivalent sensor for patient use. The accuracy of Satellite G was determined with 98 venous blood samples in comparison with Glucoquant BM (HK-method). Results: mean bias = -0.06 mg/dl; interval of confidence (± 2SD) = ± 19.9 mg/dl; r=0.99. All values were within zone A of the "Error Grid," characterizing this system as clinically reliable. Precision (% coefficient of variation) within 20 replicates for venous blood samples, was as follows: 1) 46 mg/dl - 8.7%; 2) 91 mg/dl - 4.2%; 3) 243 mg/dl - 3.3%; 4) 428 mg/dl - 2.8%. Evaluation of satellite G and two Companion 2 sensors with 71 capillary samples gave similar results: 96% of values within ± 20% of the reference; 90% within 15%; r = 0.98. The confidence intervals (± 2SD) were between -20 mg/dl and +32 mg/dl; mean bias within 8 mg/dl. The determined performance and the user friendliness and portability of satellite G and Companion 2 demonstrate their respective benefit in decentralised testing and patient self-monitoring.

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COMPLETELY NONINVASIVE MEASUREMENTS OF HUMAN BLOOD GLUCOSE IN VIVO USING NEAR INFRARED WAVES

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To avoid the pain and discomfort of self monitoring of blood glucose, noninvasive measurements have long been desired. We have previously reported (Jap J Diab Assoc 34: Suppl. 1 p. 274 1991, Med Biol Eng Comp 29: Suppl. p. 1138 1991) a possibility to measure blood glucose levels by near infrared (NIR) waves; the wave length which we chose was vulnerable to the temperature. To decrease sensitivity to temperature and increase accuracy, higher resonance had been chosen for the measurement, and "normalized optical density" technique was used for calculation. After preliminary studies to confirm our technique, the method was applied to the in vivo measurements. Absorption spectra was measured by a spectrometer for NIR light passed through a whole finger. The obtained (calculated) values were compared to real plasma glucose (glucose oxidase method) values. During oral glucose tolerance tests, each correlation coefficient between calculated and real glucose values was not less than 0.90. This method of blood glucose monitoring is not also noninvasive but both real-time and can be continuous. Applications of this method for each patient will contribute to realize the ideal metabolic control and prevent various diabetic complications.

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AMPEROMETRIC 25-GAUGE-NEEDLE GLUCOSE SENSOR TESTED IN BLOOD.

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An amperometric 25-gauge-needle glucose sensor has been developed for subcutaneous use. However, subcutaneous tissue may not rapidly equilibrate with blood so this device has now been tested in a flexible plastic chamber through which heparinized venous blood was drawn from human subjects in whom the glucose was varied up to 12mmol/l over 5h. The response rate of 4 glucose-sensing needles was assessed by monitoring current output while the glucose from the chamber was measured by YSI. The response to glucose change occurred within 30sec of glucose infusion in the contralateral arm and was maximal within 2min – a time indistinguishable to within 15sec of the change monitored by YSI. The current output from the devices was median 210nA (range 70–300nA) at euglycaemia and median 560nA (range 410–700nA) at 12mmol/l glucose hyperglycaemic clamp. Devices with basal current <200nA performed less well, with correlations between glucose and current <0.7. The two devices with basal currents >200 had current correlations with glucose of 0.96 and 0.97 respectively and negligible hysteresis ($p<<0.001$). The needles needed *in vivo* calibration. In conclusion glucose-sensing needles have the capacity for fast reaction (<15sec) and high correlation with glucose change and these sensors can function for at least 5h.

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IMPROVEMENT OF BIOSENSORS FOR GLUCOSE MEASUREMENT IN VIVO

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Glucose measurement in vivo needs biostable sensors. This study was aimed at exclusion of potentially function limiting problems. Methods: (1) To overcome alterations of the sensor surrounding tissue, caused by sensors' size (outer-diameter (o.d.): 1.5-2 mm, length: 20 mm), miniaturized dual sensors (o.d.: < .5 mm) with single electrodes were polarographically investigated. (2) To exclude influences resulting from nonsterility, the combination of gamma irradiation (gi) and H_2O_2 was developed. (3) Improving biostability of the covering Polycarbonate (Pc) has been used as a diffusion barrier on the sensing surface. Results: Independent of the distance between the two single electrodes, there were excellent polarographic characteristics (current < .01 nA/50 mV at voltage = 550-750 mV) measuring both pO_2 and glucose. Employing Pseudomonas aeruginosa as a test germ, the combination of gi (< 1.5 kGy) and H_2O_2 (44 mmol/l) could be proved as a feasible and reproducible protocol for a germicidal effect. Comparing sensor signals as generated by both glucose-related H_2O_2 and H_2O_2 as a substrate, the 30 mmol/l glucose concentration related current equals that of .2 mmol/l H_2O_2, without any disturbing influence on enzyme activity in both oxidized and reduced form. Pore size and density of Pc can be influenced by special radiation to realize the needed ratio of substrate and cosubstrate. Conclusion: Thus, from the results of these in vitro studies the upgraded functional biostability of implanted sensors can be presented.

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IN VIVO CALIBRATION OF A SUBCUTANEOUS MINIATURIZED GLUCOSE SENSOR UNDER CLINICAL CONDITIONS IN DOGS

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The feasibility of calibrating a glucose sensor by using a wearable glucose meter for blood glucose determination and moderate variations of blood glucose concentration was assessed. Six miniaturized glucose sensors were implanted in the subcutaneous tissue of dogs, and the parameters used for the *in vivo* calibration of the sensor (sensitivity coefficient and extrapolated background current) were determined from values of blood glucose and sensor response obtained during glucose infusion. 1) Venous plasma (x) and total blood glucose (y) levels were measured simultaneously on the same sample, using a Beckman Analyzer and a Glucometer II ®, respectively. The regression equation was $y = 1.12 x - 0.08$ mmol/l ($n = 114$, $r = 0.96$, $p = 0.0001$). The Error Grid Analysis indicated that the use of a Glucometer II ® was appropriate in dogs. 2) The *in vivo* sensitivity coefficients were 0.57 ± 0.11 nA/mmol/l when determined from plasma glucose, and 0.51 ± 0.07 nA/mmol/l when determined from whole blood glucose ($p = 0.18$, NS). The background currents were 0.88 ± 0,57 nA when determined from plasma glucose, and 0.63 ± 0.77 nA when determined from whole blood glucose ($p = 0.45$, NS). 3) The regression equation of the estimation of the subcutaneous glucose level obtained from the two methods was $y = 1.04 x + 0.56$ mmol/l ($n = 171$, $r = 0.98$, $p = 0.0001$). Thus, the calibration of a glucose sensor by using a glucose meter is feasible, and is still valuable when using moderate variations of the glycaemia.

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LONG-TERM BLOOD GLUCOSE REGULATIONS IN DIABETIC PATIENTS WITH A WEARABLE ARTIFICIAL ENDOCRINE PANCREAS

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We have developed a microdialysis sampling method for continuous subcutaneous tissue glucose monitoring and applied it to wearable artificial endocrine pancreas as sensing system. The system consists of needle-type microdialysis probe (regenerated cellulose (Cuprophan) hollow fiber, 15 mm length, 0.22 mm O.D.), needle-type glucose sensor for extracorporeal sensing and microperfusion pump system. The characteristics of this monitoring system were as follows : percentage recovery of glucose (30 %), time delay for measurement (3 min) and linearlity to glucose (0 to 500 mg/100ml). The microdialysis probe was implanted in the abdominal subcutaneous adipose tissue and perfused (120 µl/hr) via microperfusion pump. With this monitoring system, the subcutaneous tissue glucose concentrations in healthy subjects and diabetic patients could nicely follow to the changes in blood glucose excursions without any calibrations for up to 4th day, followed by 7th day with in situ calibrations. Then, by applying a wearable artificial endocrine pancreas with this monitoring system, perfect glycemic control could be obtained in diabetic patients for longer-period by exchanging the microdialysis probe every 5th day.

In conclusion, this monitoring system based on subcutaneous tissue dialysis method is stable and reliable for at least 4 days, and thus can be applied for continuous glucose monitoring and for glycemic control with wearable artificial endocrine pancreas in diabetic patients for longer periods.

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Quick HbA_{1c} Assay

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HOMOGENEOUS IMMUNOTURBIDIMETRIC DETERMINATION OF HAEMOGLOBIN A1c

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Haemoglobin A1c (HbA1c) is determined by TINIA-technology in EDTA-, heparin- or capillary blood. An immunocomplex is formed by reaction of a HbA1c-specific antibody with either HbA1c or a polyhapten consisting of a dextran carrier with several bound N-terminus-glycosylated peptides of the haemoglobin ß-chain (HbA1c-specific epitope). Binding of the antibody to the polyhapten produces aggregates, which can be measured by the increased turbidity at 340 nm. Binding of the antibody to the HbA1c molecule, which has only one specific epitope, gives no agglutination. Thus a calibration curve typical for a competitive assay is obtained, which allows the determination of HbA1c. The determination of total haemoglobin (Hb) is carried out simultaneously and the HbA1c content is expressed as % of total Hb. For the determination of total Hb a CN-free method utilising a new haemolysis reagent was developed. This method is extremely customer convenient: the sample (EDTA-, heparin- or capillary blood) is haemolysed with the new haemolysis reagent and placed in a common clinical analyser, e.g. BM/Hitachi. The Hb and HbA1c determination is accomplished automatically within 10 min. The result is printed out as % HbA1c within 15 min. The high antibody specifity allows a correct determination of the HbA1c content without interference from other glycosylated haemoglobins (HbA1a, HbA1b or side glycations) or carbamoylated, acetylated and foetal Hb. This new method agrees very well with the established Diamat method from BioRad (r=0,98).

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CLINICAL VALUE, PRECISION, ACCURACY AND FEASIBILITY OF USE OF THE DCA-2000, A RAPID HBA1C ASSAY.

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The Bayer DCA-2000 has been introduced as a rapid, simple to use desktop HbA1c meter to provide within-clinic results. We assessed the value of having HbA1c results during clinic, and the performance and feasibility of using the DCA-2000 in a Diabetes Centre by laboratory (biochemist) and non-laboratory (nurse) staff. The DCA-2000 and current laboratory method (Glycomat; mini-column chromatography, Drew) were compared with the Diamat (HPLC, Bio-Rad- CV <4%) as reference. Since assay time is 9min (6 samples/hr), to satisfy the 15min appointment system of 3 parallel consultations (12/hr), 2 meters were employed. Compared with reference, correlations coefficients were; Glycomat- 0.94; meter A- 0.98; meter B- 0.96. 20/117 (17%) of Glycomat results differed from reference by >10%, but only 5/131(4%) of DCA-2000 results; p<0.002. For DCA-2000 meters, within and between batch CVs were <4.5%. Precision was similar for nurse (CV 2.8%) and biochemist (3.1%). Without knowledge of the HbA1c, drug treatment would have been altered in 14% of consultations, whereas having the result influenced treatment in 37%. Knowing the HbA1c was considered helpful or very helpful in 71% of consultations and unhelpful in 29%. In conclusion, the DCA-2000 was simple to use, required minimum training and produced accurate precise results even when used by non-technical staff. Knowledge of the HbA1c result was of considerable value, but if made available to all clinic attenders, this would require 2 or more meters and impose a small increase in waiting time, even in small clinics.

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Autoantibodies

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DETECTION OF GLUTAMIC ACID DECARBOXYLASE ANTIBODIES ON ELECTROPHORETICALLY SEPARATED BRAIN EXTRACT

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One of the most prominent autoantigens in the autoimmune disease insulin-dependent diabetes mellitus is the 64 KD protein identified as glutamic acid decarboxylase (GAD). The aim of this study was first to generate and to identify monoclonal antibodies (mab) against GAD and second to detect autoantibodies against GAD in sera by immunoblotting. Hybridoma supernatants were screened on two ELISA systems, first on a GAD fraction purified by Sepharose 6B chromatography from a 100.000 g supernatant of rat brain extract and second in a more specific sandwich assay with polyclonal antibodies against GAD from a stiff-man-syndrom (SMS) serum as capture antibody. The monoclonals positive in the second assay were tested by Western blotting of SDS-PAGE separated GAD fraction. Some of our mab were reactive with a 67 KD band in contrast with the SMS serum, which reacted with an antigen of 64 KD. Sera of diabetic and nondiabetic but diabetes-prone BB/OK rats and of Type 1 (insulin-dependent) diabetic patients tested by Western blotting were found to bind the same bands in some cases, indicating, that we were able to detect autoantibodies against GAD in sera by immunoblotting. GAD bands were also detected by autoradiography after immunoprecipitation of purified 125-I labeled GAD.

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ANTIBODY FORMATION AGAINST COMPONENTS OF GLUCOSE SENSOR WITHOUT TOXICITY TESTED IN CELL CULTURE

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In contrast to in vitro validation of electrochemical glucose sensors (amperometric GOD/H_2O_2-electrodes) one of the unsolved problems is the loss of monitoring glucose within a short time in vivo. Aim of this study was to examine the immune response against the sensor materials by measurement of antibody formation. We have implanted microsensors into LEW.1W rats and immunized controls with glucose oxydase (GOD) used in the sensor. In contrast to rats immunized with GOD no antibodies to GOD could be detected in the sensor group by ELISA ($O.D._{490nm}$ + SD: 0.86 + 0.52 vs. 0.073 + 0.016). However antibodies against the outher membrane (cellulose acetate) but not the inner membrane (polyethylene) could be detected in rats with implanted sensors. Furthermore kinetic of antibody response was investigated using different membranes (polyurethane, regenerated cellulose, cellulose acetate) as immunogens in rats and as targets in ELISA. The highest antibody formation has been detected against regenerated cellulose. In contrast to antibody formation in vivo no cytotoxicity in vitro (cell growth; morphology; viability > 95 %) could be observed with monolayer cultures of the cell lines L929 (mouse fibroblast) and BHK (baby hamster kidney). However, as known, various polymer materials used in artificial membranes have been shown to activate macrophages involved in foreign body reactions in vivo.

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STUDY OF BETA-CELL MARKERS AND ANTIGEN EXPRESSION BY A HUMAN INSULINOMA CELL LINE

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The human insulinoma cell line CM was isolated in 1987 from a patient suffering from severe hypoglycaemia. This cell line grows spontaneously in RPMI1640 supplemented with 5% FCS and keeps in culture after approximately 130 passages. Aim of the present study was to evaluate the positivity of the CM cell line for different beta-cell markers and antigens as well as for the reactivity with sera from type 1 diabetes patients and prediabetic subjects positive for islet cell antibodies (ICA) to determine whether this cell line could be used for diagnosis of type 1 diabetes. Using indirect immunofluorescence we evaluated the reactivity with monoclonal antibodies A2B5 and 3G5 detecting islet gangliosides and HISL19, for islet cell proteins. We also studied the positivity with ICA+ve sera and found all these antibodies reacted with the insulinoma cell line, suggesting a strong similarity of these cells with native beta cells. We conclude that the use of the human insulinoma cell line CM could be an extremely useful diagnostic tool, in particular for the detection of islet cell antibodies in type 1 diabetes, since the cells are stable after numerous passages and in many respects are representative of native beta cells.

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MOST OF THE MONOCLONAL ISLET CELL SURFACE ANTIBODIES FROM THE DIABETIC BB/OK RAT ARE NOT SPECIFIC FOR BETA CELLS

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Insulitis and diabetes-associated autoantibodies against surface and cytoplasmatic islet cell antigens are present in the spontaneously diabetic BB rat. Using this animal model the aim of present study was to dissociate the heterogenous polyreactive antibodies by generation of monoclonal islet cell reactive antibodies (mc-ICRA):by fusion of splenocytes from a 30 day old BB/OK rat (R18) and by fusion of spleen cells from a newly diagnosed BB/OK rat (R17) . After initial screening with a CELISA and following indirect immunofluorescence using rat insulinoma cells (RIN-5AH) 49 mc-ICRA were established. Using viable rat islet cells as target the surface binding was confirmed for 35 antibodies (R18: n=19 mc-ICSA; percentage binding on islet cells 86.3 + 13.97 / R17: n=16 mc-ICSA; 80.5 + 14.84 % binding). For determination of Beta cell specificity the islet cell surface binding was combined with intracellular immunostaining of Beta cells. Five out of 35 tested mc-ICSA showed a preferential Beta cell binding. The mc-ICSA from the two fusions showed no differences in their Beta cell specific binding. The results suggest that non-Beta cells and Beta cells share many common epitopes occurrencing probably on different antigens, thus, the specificity of mc-ICSA is limited by molecular mimicry.

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ELISA OF AUTOANTIBODIES IN THE DIABETIC BB/OK RAT BY THE USE OF GLUTAMIC ACID DECARBOXYLASE ENRICHED BRAIN EXTRACT

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The diabetic syndrome of the BB rat resembles human Type 1 diabetes including the prevalence of Beta cell reactive autoantibodies against the 64 KD autoantigen. The aim of this study was to develop an immunoassay to detect predominantly autoantibodies/antibodies against glutamic acid decarboxylase (GAD) in sera from diabetes-prone BB/OK rats and in hybridoma supernatants of immortalized splenocytes from the BB/OK rat and Balb/c mouse immunized against Beta cell antigens. At the serum dilution of 1/25 up to 1/200 there was a highly significant autoantibody binding of sera from diabetic BB/OK rats (O.D. 0.56 + 0.23; n=19) and non-diabetic age-matched BB/OK rats (O.D. 0.61 + 0.31; n=18) vs. control LEW.1U rats (O.D. 0.11 + 0.01; n=15). The GAD-specificity of the antibody immunoassay was improved by the use of immunoglobulin of a patient suffering from the stiff-man-syndrome with a high GAD autoantibody titre as the capture antibody in a sandwich assay modification. The sandwich assay is particulary suitable for screening of monoclonal GAD antibodies. We have been successful in generation of monoclonals against GAD from mice immunized and from diabetes-prone BB/OK rats.

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ANTI-COMPLEMENT ACTIVITY IN NOD MOUSE: PROTECTIVE ROLE IN BETA-CELL AUTOIMMUNITY

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In order to investigate the role of complement on diabetes development in NOD mice, we evaluated the anti-complement activity (ACA) to detect whether an abnormality of ACA is related to the different incidence of the disease between the two sexes. Sera from 154 NOD mice (80F, 74M) were collected at different ages in non-diabetic mice (4, 8, 12, 16, 20, 28 week old) and in 28 week old diabetic animals. ACA was measured by incubating serum in progressive dilutions with guinea pig complement, sheep red blood cells and haemolysine. As controls, 60 normal Balb/c mice (30F, 30M) were also studied. Positive sera were considered those with ACA at dilution greater than 1:4. ACA was significantly higher in male compared to female mice at all ages starting from 12 weeks ($p<0.001$, Mc Nemar tes:). We also observed a significant difference in ACA titer between diabetic and non-diabetic animals of the same age (non diabetic M 343±227; diabetic M 22.6±5.8; non diabetic F 483±227; diabetic F 15.4 ±6.7). These data suggest that increased ACA could induce resistance to beta-cell damage mediated by the immune system.

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ASSOCIATIONS BETWEEN GENETIC RISK MARKERS AND AUTOANTIBODIES IN FIRST DEGREE RELATIVES OF PATIENTS WITH TYPE 1 DIABETES.

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The HLA DQA1*0501-DQB1*0201/DQA1*0301-DQB1*0302 genotype and circulating autoantibodies against islet cells (ICA) or insulin (IAA) are often associated with development of Type 1 diabetes. It was investigated to which extent both antibody markers coexist with the high risk genotype in 268 first degree relatives of patients with Type 1 diabetes. The high risk genotype was present in 30/268 (11%) subjects. IAA were detected by radiobinding assay in 8/30 (27%) relatives at high genetic risk, but only in 14/238 (6%) subjects lacking this risk genotype ($p<0.001$). ICA-positivity (≥6 JDFU), was comparable in both groups, occurring in 6/30 (20%) subjects with the high risk genotype vs. 49/238 (21%) without it. Irrespective of genotype, ICA-positive relatives presented higher prevalence of IAA (10/55 or 18%) than ICA-negative relatives (12/213 or 6%; $p<0.01$). This difference was still present in relatives without the high risk genotype where IAA were detected in 8/49 (16%) ICA-positive and in 6/189 (3%) ICA-negative subjects ($p<0.001$). IAA thus occur preferentially in relatives at increased risk for Type 1 diabetes as defined by genetic markers or ICA-positivity.

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ASSOCIATION OF AUTOIMMUNE RESPONSIVENESS TO ADVANCED GLYCATION ENDPRODUCTS OF LOW DENSITY LIPOPROTEINS WITH HLA-DR4 ALSO IN TYPE II DIABETES

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Autoantibodies to advanced glycation endproducts of low density liporoteins (AGE-LDL) were demonstrated by competitive radioimmunoassay in about 30% of type 1 and type 2 diabetic patients. In type 1 diabetics this autoimmune response is associated significantly with HLA-DR4, which occurred in 29% of autoantibody positive and in 69% of autoantibody negative patients ($p=0.01$). In order to verify the immunogenetical relevance of this finding, 100 type 2 diabetic patients with and without autoantibodies to AGE-LDL were typed for HLA-A,B,C,DR,DQ antigens. As could be expected from the lack of an association between type 2 diabetes and HLA, no significant differences were found between HLA frequencies of all 100 diabetics and healthy controls. Among 67 autoantibody negative patients, however, HLA-DR4 occurred in 35,8% and in 33 autoantibody positive diabetics in 15.2% ($p=0.03$). Accordingly autoimmune responsiveness to AGE-LDL is associated with HLA-DR4 in type 1 as well as in type 2 diabetes. Suppression of autoimmune response to AGE-LDL seems to be most effectively caused by HLA-DR4 and/or DR4 linked genes of the HLA-DP and DQ loci.

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I-45: A NOVEL ISLET / NEUROENDOCRINE PROTEIN DISTINCT FROM GLUTAMIC ACID DECARBOXYLASE (GAD).

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A series of monoclonal islet cell antibodies (I-45, I-51, I-52 and I-39) were generated using human insulinoma as the immunogen. (Diabetes, 40, 227A, 1991). MAb I-45 reacts with both the islet beta and non-beta cells. I-45 antigen is also expressed by related neuroendocrine cells (anterior pituitary, adrenal medulla, and gut endocrine). A 68KD protein was isolated and purified by a single step MAb I-45 immunoaffinity chromatography (antigen source: pheochromocytoma). This 68KD protein was not reactive with monoclonal islet cell antibodies I-51 or 4F2. Anti-68KD autoantibodies (immunoprecipitation of affinity purified 125-I 68KD protein) were identified in human sera from 6/7 islet cell autoantibody (ICAb) positive IDDM patients, and 2/7 ICAb negative healthy controls. MAb GAD6 - anti glutamic acid decarboxylase - (J Neuroscience, 8, 2123, 1988) selectively reacts with islet beta cells (immunohistochemistry), but fails to immunoprecipitate the 68KD I-45 protein.
CONCLUSION: I-45 is a novel islet/ neuroendocrine protein distinct from glutamic acid decarboxylase.

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FOLLOW-UP OF ANTI-ß-LACTOGLOBULIN ANTIBODIES IN CHILDREN WITH INSULIN-DEPENDENT DIABETES MELLITUS

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It has been hypothesized that cow milk may contain a triger factor for the development of insulin-dependent diabetes mellitus. The aim of our study was to evaluate IgA and IgG antibodies to ß-lactoglobulin (ß-LG), determined by ELISA, in 20 insulin-dependent diabetic patients (9 males and 11 females, aged 1.5-13.5 years) at diagnosis and during a 2.5 year follow-up. Total serum IgA, IgG and IgM were normal in all patients. Fifty-nine healthy, age- and sex-matched subjects served as controls. At diagnosis 12 patients (60%) had elevated IgA ß-LG antibodies (>+2SD) and 4 (20%) elevated IgG ß-LG antibodies (>+2SD). During follow-up we observed a transient increase of IgA ß-LG antibodies in another 2 patients. At the end of follow-up the IgA ß-LG antibodies decreased to normal in all but 2 patients and the IgG ß-LG antibodies in all but one girl. No correlation was found between IgA and IgG ß-LG antibodies and chronologic age, anti-islet cell, anti-insulin and other organ or non-organ specific antibodies and HLA types. Our results suggest that anti ß-LG antibodies could reflect an abnormal immunologic response in the early stage of insulin-dependent diabetes mellitus.

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Presence of anti-ganglioside antibodies in the sera of new-onset type 1 diabetics.
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There is evidence that gangliosides are somehow involved in a number of autoimmune disorders ranging from neurologic pathologies to type 1 diabetes : a pancreatic monosialo-ganglioside has been implicated as an ICA antigen, and antibodies against the trisialoganglioside GT3 have been reported in type 1 diabetics. Aim of this work was to study in both new-onset type 1 diabetic and normal sera the presence of antibodies against an acidic glycolipid extract of rat whole pancreata or single standard-gangliosides (GM3, GM2, GM1, GD3, GD1a, GD1b, GT1b). After extraction and purification of rat pancreata, gangliosides were qualitatively and quantitatively analyzed by HPTLC, HPLC and lipid-bound sialic acid determination. The binding to gangliosides of 27 (age 3-14) new-onset type 1 diabetic sera and of 19 (3-14) normal controls was analyzed by an ELISA assay. Binding to GD3 ($p<0.005$), GD1a ($p<0.05$), GD1b ($p<0.05$), GT1b ($p<0.05$) was significantly elevated in new-onset type 1 diabetics vs normal controls. All the gangliosides identified as antigens have a sialic-acid molecule in terminal position. No differences between normal and diabetic sera were found in binding to rat pancreatic glycolipid extracts and to standard monosialo-gangliosides. These findings identify a panel of polysialogangliosides as targets of autoantibodies present in the sera of new-onset type 1 diabetics.

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HUMAN ISLET GLUTAMIC ACID DECARBOXYLASE AUTOANTIBODY LEVELS PERSIST UP TO 5 YEARS AFTER ONSET AND ARE UNAFFECTED BY PLASMAPHERESIS IN TYPE I DIABETES.
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We quantitated GAD-autoantibody ($p64^{GAD2}Ab$) levels through 5 years after onset and evaluated plasmapheresis effects. $p64^{GAD2}Ab$ quantitation used recombinant antigen immunoprecitiation; densitometric fluorogram scanning compared to standards allowed $p64^{GAD2}Ab$ index calculation. 1/49 healthy controls had nonzero $p64^{GAD2}Ab$ index. 19 new onset diabetics (mean{range} age 22{15-32} years, C-peptide 0.50{0.19-1.00} nmol/l; ICA 31{0-170} JDFU) underwent true (n=9) or sham (n=10) plasmapheresis; sera taken before treatment and after 3, 6, 12, 24, and 60 months were analysed (mean 5 samplings/patient). Of 9/19 positives for $p64^{GAD2}Ab$ (index range 0.03-3.44) at onset, 8/9 maintained index within 50% of onset value, remarkably preserved throughout the study. Of negatives at onset, 7/10 remained so. Index remained unchanged at 3, 6, and 12 months in 8/9 plasmapheresis-treated patients. One plasmapheresis and two sham-treated patients developed detectable $p64^{GAD2}Ab$ 24 months post treatment, one with high index. Onset $p64^{GAD2}Ab$ index was not correlated with onset ICA titer ($p>0.05$). Thus $p64^{GAD2}Ab$ levels remained very stable years up to 5 years after diagnosis, plasmapheresis removing pathogenic autoantibodies did not affect later $p64^{GAD2}Ab$ index, and $p64^{GAD2}Ab$ index was unrelated to ICA titre.

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AUTOANTIBODIES TO P-64-69 KDA ANTIGEN AND TO NON-ISLET ENDOCRINE CELLS AND FIBROBLASTS IN NEWLY DIAGNOSED TYPE I DIABETIC PATIENTS.
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Clinical onset of Type I diabetes(IDDM) is preceded by the appearance in circulation of autoantibodies to 64 KDA betta cells antigen. There are many contradictory opinions about variations of immune system in onset of disease. We investigated 88 newly diagnosed IDDM patients. We have been studing circulating antibodies to beta cells antigen 64-69 KDA by time-resolved fluororescence assay (TRFIA), autoantibodies to microsomal fraction of thyreocytes (MsT), thyreoglobulin (Tg), pituitary cells (Pit), ICSA and fibroblasts (Fib) by ELISA-method. 8 patients were negative (9%), to all type of antibodies. Part of patients had different autoantibodies: 17 were positive only for p64-69 antigen or ICSA (51,5%) and 13 subjects had also autoantibodies either to MsT, Tg, Pit and Fib (39,4%).
It is important to mark that autoantibodies to Fib. were discovered only in patients, who were positive for ICSA or p64-69 antigen. According our data there are polyclonal activation of immune system in some newly diagnosed insulin-dependent diabetics.

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AUTOANTIBODIES AGAINST ISLET AMYLOID POLYPEPTIDE IN HUMAN SERUM. LACK OF ASSOCIATION WITH TYPE 1 OR TYPE 2 DIABETES OR WITH AGEING.

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A radiobinding assay (RBA) for the detection of autoantibodies against islet amyloid polypeptide (IAPP-AA) was developed and, together with a well established RBA for insulin autoantibodies (IAA), applied to sera sampled at onset of Type 1 diabetes and to sera from an equal number of age- and sex-matched controls. There was no difference in IAPP-AA titers between patient groups and matched controls, nor within subject groups according to age. At onset of Type 1 diabetes elevated IAPP-AA levels (> percentile 97 of controls) were detected in 1/30 patients aged 0-19 yr and in 2/35 aged 20-39 yr. By contrast, IAA were frequently detected in the same patient groups, especially if onset of diabetes occurred before 20 yr (0-19yr : 18/30; 20-39 yr : 10/35; $p < 0.01$ vs matched controls). IAPP-AA were not detectable in 3 insulinoma patients nor in 37 patients (33-70 yr) with Type 2 diabetes (vs 1/40 in controls). In positive serum, IAPP binding activity was absorbed onto protein A-Sepharose, thereby confirming its antibody nature. In conclusion, Type 1 diabetes elicits an age-dependent autoimmune reaction involving insulin but not IAPP. Moreover, conditions associated with a tendency towards amyloid deposition in islets (Type 2 diabetes, insulinoma and ageing) do not favor the emergence of autoantibodies directed against IAPP.

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SLOWLY PROGRESSIVE TYPE 1 DIABETES: A RETROSPECTIVE ANALYSIS OF CLINICAL AND LABORATORY FINDINGS

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Although the criteria of slowly progressive Type 1 diabetes has not yet been clearly defined, this form is unique for its clinical presentation, natural course and therapy which is to be differentiated from Type 2 diabetes, and the chance of immunoprotection is higher at this stage. In this study we evaluated a group of patients from retrospective analysis of 2000 patients seen regularly at our Diabetes Outpatient Clinic. All the patients in this group presented as Type 2 diabetes at the beginning (but not having MODY criteria) and were divided into two groups on the basis of ICA positivity and insülin reguirements. **Group I** included 14 patients
(3 female, 11 male) with ICA≥ 40 JDF units and had no insulin requirement for at least 3 months after clinical onset (mean age at onset 29.5±7.4 years) and **Group II** included 19 patients (7 female, 12 male) who had some insulin requirement not earlier than 1 year after clinical onset and ICA were either positive or negative (mean age at onset 29.4±6.1 years). Initial laboratory findings for **Group I**; were as follows: HbA1C 7.2±2.0 %, fasting C-peptide 1.33±0,72 ng. ml^{-1}, and AIR glucose (n:5) 1+3 min. 23.6±3.4 uU. ml^{-1} Of the 14 patients 9 developed insulin requirement (0.28±0.14 IU. kg^{-1}. day^{-1}) after 27.7±23.8 months of clinical onset. In **Group II**, initial HbA1C was 7.6±2.3 % and fasting C-peptide was 1.08±0.62 ng.ml^{-1}. Some insulin requirement had appeared after 36.9±20.4 months of clinical onset in all patients in this group. 5 of 19 patients were still ICA positive for at least 24 months after clinical onset. Current insulin requirement was 0.24±0.11 IU kg^{-1}. day^{-1}.
To conclude, slowly progressive natural history in this clinical form of Type 1 diabetes, explains the presence of beta cell reserve with no prominent metabolic derangement besides the loss of first phase insulin secretion.

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PREDICTION OF INSULIN DEPENDENCE BY ICA AND HLA-DR-TYPE IN NEWLY DIAGNOSED DIABETIC PATIENTS

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At diagnosis of diabetes the clinical classification of Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetes is difficult. About 10% of the initially non-insulin treated patients are later treated with insulin. In this study we have elucidated if ICA and HLA-DR typing could improve classification already at diagnosis. Samples from all consequtively diagnosed diabetic patients (n=244) during two years in all ages (3-88 yrs) in Malmö were collected. HLA-DR/DQ-typing with RFLP and ICA with an immunofluoresence assay, were performed in 11/42 initially insulin treated and in 90/202 non-insulin treated patients. After three years, 22/101 patients were on insulin treatment and 79/101 on diet or oral agents. Of 14 ICA positive diabetic patients all were on insulin (predictability; 14/14, 100%), while 14/22 (67%) of the insulin-treated patients at follow-up were ICA positive at diagnosis. Of 63 patients with HLA-DR3 and/or 4, 19 were on insulin (predictability; 19/63 30%), and in all patients later treated with insulin, DR 3 and/or 4 was present in 19/22 (86%). Typing for HLA-DQ did not improve the results. In conclusion, ICA-positivity seems to be a better predictor for insulin dependence in diabetic patients, than the more sensitive but less specific genetic markers.

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FACTORS PREDICTING BETA CELL FUNCTION AND METABOLIC CONTROL IN TYPE 1 DIABETES MELLITUS.

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To analyze the factors that can predict evolution of beta-cell function and metabolic control after diagnosis, we prospectively studied 86 consecutive newly diagnosed type 1 diabetic patients (age 18+9.4, 54 men).Age, sex, weight loss, duration of sypmtoms, glycemia, pH, bicarbonate, ketonuria, HbA1, ICA (JDF units), basal and post-glucagon C-peptide and insulin dose were determined at diagnosis. Patients were followed at least one year.Variables that correlated with good control (HbA1 < 9%) or significant beta-cell function (post-glucagon c-peptide > 0.6 nmol/L) at one or two years of diagnosis were included in a stepwise logistic regression model. Good metabolic control at one year was predicted by female sex (Odds ratio 5.9), ICA < 10 JDF (OR 4.8), basal C-peptide > 0.3 nmol/L (OR 4.5) and HBA1 < 14.2% (OR 4). Beta-cell function at one year was predicted by ICA < 10 JDF (OR 5.5) and post-glucagon C-peptide > 0.6 nmol/L (OR 3.3). ICA and C-peptide at diagnosis also predicted good metabolic control (OR 5 and 14.5 respectively) and significant beta-cell function(OR 9.1 and 7.5) at two years in the patients followed until that time. We conclude that sex, HbA1 , ICA and C-peptide at diagnosis predict the ulterior degree of metabolic control and beta-cell function in type 1 diabetes .

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ISLET CELL ANTIBODIES IN TYPE II DIABETIC PATIENTS INDICATE PROGRESSIVE BETA-CELL DYSFUNCTION.

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To evaluate the association between islet cell antibodies (ICA) and Beta-cell function in patients diagnosed as type II diabetic, fasting C-peptide (fCp), C-peptide 1+3 min after 0.5 mg/kg intravenous glucose (1+3Cp), and Δ C-peptide after 1 mg glucagon (ΔCp) were followed prospectively for 3 years in type II patients with (n=11, age 50±5 years) and without (n=10, age 52±4 years) ICA and in type I patients (n=17, age 37±5 years).Type II patients with ICA showed as impaired ΔCp as type I patients at diagnosis (0.38±0.06 nmol/l and 0.35±0.11 nmol/l) that did not decrease while ΔCp was higher (1.10±0.18 nmol/l; $p<0.05$) and remained unchanged in type II patients without ICA. Type II patients with ICA (0.92±0.17 nmol/l) showed higher ($p<0.05$) 1+3Cp than type I patients (0.53±0.11 nmol/l) at diagnosis, but after 1 year 1+3Cp had decreased (0.18±0.11 nmol/l; $p<0.05$) reaching the levels of type I patients. 1+3Cp did not decrease further in type II patients with ICA (0.18±0.10 nmol/l) and was unchanged in type II patients without ICA (2.31±0.50 nmol/l) after 3 years. Although similar in type II patients with ICA and type I patients at diagnosis (0.30±0.03 nmol/l and 0.24±0.03 nmol/l), fCp decreased ($p<0.05$) in both groups (to 0.09±0.04 nmol/l and 0.13±0.04 nmol/l) but was higher ($p<0.001$) and unchanged in type II patients without ICA (0.97±0.17 nmol/l) after 3 years. In conclusion, ICA in type II patients indicate progressive Beta-cell dysfunction; ΔCp is severely disturbed at diagnosis, 1+3Cp deteriorates within 1 year, and fCp deteriorates within 3 years.

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COELIAC DISEASE IN ADULTS WITH TYPE I DIABETES.
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The prevalence of coeliac disease (CD) in adults with type I diabetes has been reported to be about 4.1%, when screened by anti-reticulin antibody(ARA). Moreover CD is thought to be underdiagnosed in adults. The aim of our work was to evaluate the prevalence of CD in an adult unselected type I diabetic population screened by anti-gliadin antibody (AGA) measured by ELISA (Eurospital Pharma, Trieste Italy). Fiftyseven consecutive patients were studied; 15 out of 17 AGA positive patients(29.8%) underwent an endoscopic biopsy(Fujon EVE) of descending duodenum(2 out of 17 refused the biopsy). Cardiovascular autonomic test (CAT) were performed to evaluate the autonomic neuropathy. The histological pattern, according to Perera grading, was abnormal in 11 patients (19,3%): 2 with total atrophy and 9 with subtotal atrophy. In coeliac patients we found : diarrhea(2), hypochromic hyposideremic anemia (1), IgA deficiency(2), abdominal disconfort(2). 4 were symptoms free. All patients had negative CAT. The prevalence of CD in our patients was higher than that reported in previous studies possibly due to the higher sensitivity of our test. According to our experience we recommend the AGA test as a useful tool in type I patients, to detect CD in simptom-free patients .and in those with mild signs of abdominal disconfort.

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LACK OF ASSOCIATION OF ISLET-CELL AND OTHER ORGAN SPECIFIC AUTOANTIBODIES IN GESTATIONAL DIABETES.
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Presence of islet-cell antibodies (ICA) in sera of gestational diabetic (GD) women may identify a subset of subjects prone to develop type I diabetes in the future. We have previously reported that 12% of our population of GD women show ICA in their sera. As an increased frequency of thyroid and gastric autoantibodies has been reported in type I diabetic subjects, we aimed to investigate the frequency of thyroid autoantibodies (ATA) (microsomal and thyroglobulin) and parietal gastric cell autoantibodies (PGA) during gestational diabetes and their relation to ICA. ATA were assayed by passive hemagglutination, and PGA and ICA (ICA Proficiency Program) by indirect immunofluorescence. Chi-square test was used for comparison between groups. Sera from 65 pregnant women with known normal glucose tolerance, 133 ICA- GD women and 53 ICA+ GD women were assayed for these antibodies. In these 3 groups, ATA were found in 3, 11 and 6, and PGA in 5, 8 and 7 women, respectively. No difference among groups was found. We conclude that there is no difference in the frequency of humoral thyroid and gastric autoimmunity between ICA+ and ICA- gestational diabetic women during pregnancy.

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PS 44
Interleukin-1

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PCR ANALYSIS OF INTERLEUKIN-1β mRNA CONTENT IN PANCREATIC ISLETS OF PREDIABETIC NOD MICE.
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Interleukin-1β (IL-1β) has been suggested to mediate β-cell destruction in insulin-dependent diabetes mellitus. However, data on the production of IL-1 in pancreatic islet during the process of insulitis are scarce. In this study we have isolated pancreatic islets with accompanying insulitis from 5 and 16 weeks old female and male NOD mice and from nondibetes-prone male NMRI mice. mRNA was isolated from 1000-2000 islets and cDNA was synthesized. The expression of the IL-1β gene was estimated by PCR (polymerase chain reaction) amplification, using specific primers for murine IL-1β sequences. The optical density (OD) of the autoradiographs was normalised by parallel PCR-amplification of the samples for the enzyme glyceraldehyde-3-phosphate dehydrogenase. NMRI mouse islets contained very low levels of IL-1β mRNA (0.01 ± 0.006 OD). On the other hand, in female NOD mouse islets the values were 3.92 ± 0.34 and 4.64 ± 1.13 at 5 and 16 weeks and corresponding values in males was 1.03 ± 0.75 and 2.56 ± 0.16. Culture of 16 weeks female NOD islets, which depletes the mononuclear cell infiltration, reduced the IL-1β mRNA to 47 ± 6 % ($P<0.05$) of the values of freshly isolated islets. It is concluded that expression of the IL-1β gene in cells of islets isolated from NOD mice is much more marked than in nondiabetes-prone mice.

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B-CELL CYTOTOXICITY STUDIED BY NON-RADIOACTIVE IN SITU HYBRIDIZATION - A FEASIBILITY STUDY.
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Cytotoxic interleukin 1 (IL-1) concentrations reproducibly regulate the expression of many (46) rat islet proteins. Their kinetics, identity and cell specificity remain unknown. Synthetic non-radioactive, biotinylated oligo nucleotide probes (SNRBOP) capable of identifying low copy numbers were constructed and used to study mRNA expression by in situ hybridization in islets exposed to IL-1 (150 pg/ml) for varying periods of time (0-48 h). This capability of detecting low copy numbers is crucial since IL-1 induces protein changes including upregulation as well as down regulation.
So far, specific hybridization signals for the oxidative stress proteins manganese superoxide dismutase (MnSOD) and heme oxygenase (HO) 1 and 2 were found to be induced by IL-1, while a control probe (malarial probe) showed no or very weak signal. Signal quantification by computerized image analysis revealed characteristic time-dependent variations in MnSOD and possibly HO expression following IL-1 exposure. Although quantitatively different positive signals were seen in core (B-cells) and mantel (A-cells) areas.
Conclusion: The use of SNRBOP is a sensitive precise and cell specific method to study details of variation in B-cell gene expression during IL-1 cytotoxicity.

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INTERLEUKIN 1 (IL-1) INDUCED DIABETES IS DEPENDENT UPON IL-1 CONCENTRATION AND FOOD INTAKE.

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The effect of route of administration and dose of IL-1, food intake and addition of 10% glucose to the drinking water on IL-1 induced diabetes was studied in normal rats. After last injection the rats were fasted or fed for 10 h, and then blood was analysed for glucose (BG) and insulin.

INJECTIONS	FOOD	BLOOD GLUCOSE (mmol/l)	INSULIN (pmol/l)
IL-1 s.c.	+	22,5 ± 3,0*	231 ± 25▾
4,0 µg/kg	-	7,4 ± 1,7	173 ± 20
IL-1 i.p.	+	22,1 ± 2,4*	248 ± 36▾
4,0 µg/kg	-	8,1 ± 1,8	208 ± 61
IL-1 s.c.	+	11,3 ± 1,9*	848 ± 210
0,4 µg/kg	-	5,9 ± 1,1	303 ± 67
IL-1 i.p.	+	8,8 ± 1,9	650 ± 305
0,4 µg/kg	-	6,6 ± 1,1	408 ± 183
Vehicle	+	6,4 ± 0,7	783 ± 399
i.p.	-	5,3 ± 0,4	207 ± 104

Mean ± SD, (n = 6)
Access to food + IL-1 4,0 µg/kg induced higher BG and lower insulin concentrations compared to 0,4 µg/kg of IL-1 or vehicle (all $p < 0,005$). BG was significantly higher in fed compared to nonfed rats ($p < 0,002$) except for the low-dose i.p. injected. Insulin concentrations were reduced in the 4,0 (▾ $p < 0,005$), but not in the 0,4 µg/kg IL-1 treated compared to vehicle treated rats. Route of administration made no difference in the 4,0 µg/kg injected rats. 10% glucose did not alter the BG or insulin concentrations. We conclude that IL-1 induces a diabetes like state dependent upon IL-1 concentration and access to food.

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2-D Protein Map of Islets of Langerhans: Effects of Interleukin-1ß (IL-1ß) and Nicotinamide (NA)

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We studied whether NA mediated prevention of IL-1ß-induced inhibition of islet insulin release was associated with prevention of NO-production and of alterations in protein synthesis. The molecular basis for IL-1ß cytotoxicity is unknown. IL-1ß generates intracellular production of nitric oxide free radicals (FR) (NO_2^-, NO). Inhibitors of NO_2^--production counteract the effect of IL-1ß and TNFα on isolated rat islets. IL-1ß (150 pg/ml, 24h) significantly inhibited islet accumulated insulin release ($p < 0.02$, $n = 7$). This effect was abolished by NA (25mM). Measured by the Griess reagent, IL-1ß induced NO_2^--production was significantly higher than in control cultures (9.16 vs. 0 pmol/islet/24h, $n = 7$, $p < 0.02$). The FR-scavenger nicotinamide (NA) alone had no effect on NO_2^--synthesis. NA inhibited IL-1ß induced NO_2^--synthesis by 48% ($n = 7$, $p < 0.02$). Utilizing high-resolution 2-D gels ($n = 9$), we have produced a precise map of more than 3,000 proteins in neonatal rat islets. IL-1ß induced the up-regulation of 23 proteins and the down-regulation of 23 proteins. NA inhibited the up-regulation of 11 proteins and the down-regulation of 7 proteins otherwise induced by IL-1ß. NA by itself changed only 2 proteins. Hypothesis: Characterization of these proteins provides the molecular basis of initial ß-cell destruction, i.e. the pathogenesis of Type 1 (insulin-dependent) diabetes.

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PREVENTION OF THE CYTOTOXIC EFFECT OF IL-1 BY HUMAN LYSOZYME ON CULTURED RAT ISLETS.

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Macrophages are involved in the autoimmune destruction of the pancreas in IDDM via the secretion IL-1 and TNF- but macrophages secrete also lysozyme which is known to inhibit the production of superoxide anions. We investigated the protective role of human lysozyme against the cytotoxic effect of IL-1 on isolated rat islet. Precultured newborn rat islets were pretreated or not during 24 h with human lysozyme (50.000 U/ml) or BSA before addition of IL-1 and a continued 48 h incubation in the same three conditions. Human lysozyme abolished the lowering of labeling index induced by IL-1 in islet cells (IL-1 : 0,91 ± 0,14% vs control : 1,98 ± 0,21%, $p<0,05$) (IL-1 + lysozyme : 1,72 ± 0,16%). Picnotic nuclei were rather abundant in islets treated with IL-1 (5,09 ± 1,12% vs control : 0,63 ± 0,13%; $p<0,01$). Basal values were observed when human lysozyme was applied (IL-1 + lysozyme : 1,04 ± 0,16% vs IL-1; $p<0,01$) but not BSA (IL-1 + BSA : 26,14 ± 4,5%). Chicken lysozyme had no protective effect in the same protocol, but both lysozymes (chicken and human milk) stimulated thymidine incorporation in neonatal rat mesenchymal cells.
In conclusion, only the macrophagic lysozyme seems to have a protective effect against IL-1 on islet cells. Moreover, to be protected, the islet cells have to be pretreated with lysozyme before IL-1 application.

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THE ISOELECTRIC POINT OF A PROTEASE IS RAPIDLY ALTERED BY INTERLEUKIN-1β IN ISOLATED RAT ISLETS.

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We previously reported that interleukin-1β (IL-1β) induced action on β-cells can be prevented by the protease inhibitor Nα-tosyl-L-lysine chloromethyl ketone (TLCK). To further characterize this putative IL-1β activated protease, we have presently affinity labelled isolated rat pancreatic islets with [^{3}H]TLCK. The labelling pattern of islets treated with or without IL-1β was studied by one- (1-d) and two-dimensional (2-d) gel electrophoresis. It was found that when intact islets of both groups were analyzed by 1-d gel electrophoresis, four proteins with molecular weights of 25, 27, 30 and 34 kD were specifically labelled. These proteins were found by a subcellular fractionation procedure to be localized to the cytosol. IL-1β exposure (5 ng/ml) for 5, 15 or 60 min did not induce any alteration in the intensity of the labelling of the proteins on 1-d gels. When analyzed on 2-d gels, the 25 kD and the 27 kD proteins were separated into two isoforms with different isoelectric points. Furthermore, incubation of islets with IL-1β for 15 min induced a shift of the relative distribution from the form with a lower pI to the form with a higher pI. This suggests that IL-1β induced signal transduction in islet cells may involve a post-translational modification of a cytosolic protease.

PS 45
Cellular Autoimmunity

822

MONONUCLEAR CELLS AND ENDOTHELIAL CELL ACTIVATION IN THE PANCREAS AT THE ONSET OF IDDM

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Current knowledge of the phenotype of mononuclear cells accumulating in pancreatic islets in IDDM and factors determining their homing into the pancreas is limited. We studied the pancreas of a child who died from brain edema at the onset of IDDM. Cryostat sections, stained with monoclonal antibodies, were studied by immunofluorescence microscopy, and peripheral blood mononuclear cells were phenotyped using flow cytometry. Monocytes/macrophages (lysozyme or CD14- reactive cells) were identified among other mononuclear cell types in islet infiltrates. Vβ8-positive T-cells were overrepresented, but T-cells with other Vβs studied (Vβ5, Vβ5.1, Vβ6, Vβ12) were also found. The vascular endothelium of the islets and many small vessels nearby islets strongly expressed intercellular adhesion molecule-1. A few non-endothelial cells expressed vascular cell adhesion molecule-1, while E-selectin was not expressed at all. We conclude, that increased expression of ICAM-1 on vascular endothelium may increase endothelial adhesion of mononuclear cells and enhance their accumulation in the pancreas during diabetic insulitis; that T cells with certain T cell receptors can be enriched in the pancreas at the clinical onset of IDDM; and that macrophages and antigen-specific CD8 positive T lymphocytes are especially involved in pancreatic beta cell destruction at the onset of IDDM.

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NEWLY DIAGNOSED TYPE I DIABETIC T-CELLS ARE CHARACTERIZED BY A TCR/CD3 SIGNAL TRANSDUCTION DEFECT.

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As in most autoimmune diseases, T cells are activated in newly type I diabetic patients, but they release low amounts of IL2 and soluble-IL2 receptor when stimulated with PHA in vitro. To investigate whether there was an alteration in the activation pathway through the TCR/CD3 complex, we studied the functional requirements during activation in T cells from 12 newly type I patients compared with 8 healthy controls (HC) The proliferative response induced by anti-CD3 coupled beads was heavily impaired in diabetic PBMC (96 hr= 30.8±5.6 vs 76.5±10.2, p< 0.001), but it could be overcome by IL-2 addition (20 U/ml) (124.8±13.4 vs 134.9±11.6,p NS), indicating that TCR/CD3 signal transduction is defective in these patiens and fails to activate IL2 gene expression. Cytoplasmic free calcium, determined by FACS analysis using Fluo-3, increased equally in T cells from diabetics and HC, when stimulated by anti-CD3 antibodies. Interestingly, when PKC was independently stimulated by PMA, PBMC from newly type I patiens proliferate normally in response to anti-CD3 stimulation (96 hr= 88.4 ±16.3 vs 79.7±7.6,p NS). Moreover, the anti-CD3 induced proliferation was strongly increased in PBMC from these patients after depletion of monocytes and B lymphocytes. This study demonstrates the presence, in type I diabetes, of a defect in TCR-mediated activation. Apparently, this defect occurs downstream of calcium mobilization and seems to involve protein kinase C activation, since it can be overcome by PMA addition. Finally, the strong increase in proliferation, observed in PBMC from type I patients after depletion of monocytes and B lymphocytes, suggests that accessory cells may play a regulatory role in this defect.

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DETECTION OF β-CELL AUTOREACTIVE CELLS FROM BB RATS AFTER SENSIBILISATION OF LYMPHOCYTES BY SYNGENEIC PANCREATIC ISLETS

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During the development of diabetes pancreatic ß-cells decreases as a result of a cell-mediated autoaggression. To characterize the presence respectively the reactivity of cytotoxic immunocytes mononuclear spleen cells (MNC) obtained from dp BB/OK rats were both phenotyped and incubated for 6 h with ^{51}Cr-prelabelled syngeneic pancreatic islets to ascertain cell-mediated anti-islet cytotoxicity (CMC). Furthermore, $2x10^6$ MNC were cultured for 6 d with increasing numbers of syngeneic islets as antigen (effector/target cell ratio between 1:0,25 and 1:2). Afterwards, cell proliferation, CMC and phenotypes ($CD5^+$, $CD4^+$, $CD8^+$ T-lymphocytes, B-lympocytes, NK-cells) of sensitized MNC were estimated.
MNC obtained from diabetes-prone, newly diagnosed diabetic or long-term normoglycaemic rats do not differ markedly in its phenotypes. Only MNC from newly diagnosed diabetic BB/OK rats exerted in 20% a cytotoxic effect against syngeneic islets. A 6 d sensibilisation period with syngeneic islets caused a marked cell proliferation and the induction of CMC in all diabetic animals. In cultivated splenocytes from long-term normoglycaemic animals the induction of cytotoxicity was missed in the majority of rats. When MNC from 90 d old normoglycaemic BB/OK rats were cultured with syngenic islets 37% of the investigated animals failed to develop a cytotoxic reactivity, whereas 63% developed a CMC with a different threshold.
The results underline that cytotoxic immunocytes can be activated by syngeneic pancreatic islets in vitro, but the reactivation is probably depending on the in vivo situation.

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Anti-ß cell autoimmunity in ß cell deprived NOD mice

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Triggering events which initiate the primary activation of most forms of autoimmune diseases remain elusive. The defect could reside within the immune system or in the target cells. In order to evaluate the role of islet ß cells in the primary activation of autoreactive clones in autoimmune diabetes, we studied NOD mice rendered devoid of ß cells by a massive dose of 150 mg/kg alloxan before the onset of insulitis. First, spleen cells from spontaneous diabetic mice were not maintained in an activated state when transfered into pre-irradiated, alloxan-treated mice, as evidenced by the loss of capacity of the latter animals to transfer diabetes into 8 week-old male recipients. Alloxan, hyperglycemia and insulin treatment were not involved in this loss of transfer, as demonstrated by control experiments. Second, 6 month old NOD mice, which received alloxan at 3 weeks did not transfer diabetes into 8 week-old NOD males, contrary to control animals. Development of extra-pancreatic autoimmunity (sialitis), or induction of autoimmune hemolytic anemia, or antibody response to exogenous insulin were not modified in alloxan-treated mice. We conclude that the presence of islet B-cells is a prerequisite to the activation of autoreactive effector T-cells in the NOD mouse.

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CLASS I MHC EXPRESSION ON LYMPHOCYTES IN TYPE 1 DIABETES

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A recent report suggests that the level of expression of class I MHC molecules on lymphocytes is reduced in patients with long-standing Type 1 diabetes and individuals at high risk of developing the disease. Using cryopreserved lymphocytes, FITC-labelled antibodies and flow cytometry we measured lymphocyte surface expression of class I MHC molecules and β_2-microglobulin in 12 new-onset and 10 long-standing patients and 11 healthy controls. In addition, we studied 5 identical twin pairs more than 6 years discordant for the disease and 5 twins during the pre-diabetic period. In all groups, >98% of gated lymphocytes expressed class I MHC and β_2-microglobulin and the percentages of positive cells in the diabetic groups and controls were similar. There was no difference in the median fluorescence intensity (MFI) of the staining (ie the number of molecules/cell) for either class I or β_2-microglobulin in the disease groups compared with controls. Four out of 5 co-twins with diabetes had slightly lower levels of MFI than their non-diabetic twin, but this difference was not significant. These results suggest that analysis of cryopreserved lymphocytes does not provide evidence in support of a reduction in class I expression in Type 1 diabetes or in the prediabetic period.

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THE TIME KINETICS OF CELLULAR AND HUMORAL IMMUNE REACTIONS AGAINST ISOLATED RAT PANCREATIC ISLET CELLS IN TYPE I DIABETIC PATIENTS

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24 patients with newly diagnosed type I diabetes mellitus were investigated three times after the onset of disease in order to study the time kinetics of immune reactions. Direct lymphocyte-mediated cytotoxicity (LMC) of separated T-lymphocytes was studied against 51 Cr-labeled,isolated rat pancreatic islet cells, and islet cell surface antibodies (ICSA)and islet cell antibodies (ICA) were measured in sera of patients with indirect immunofluorescence. At the first investigation in almost all (22/24) patients a significant lymphocyte-mediated cytotoxicity (mean±SD of killed terget cells: $5.6 \times 10^6 \pm 1.8$ vs $1.3 \times 10^6 \pm 0.4$) was found in vitro. ICSA and ICA were simultaneously detected in 75 % and in 50 % of patients respectively. After six and 12 months the frequency and the intensity of lymphocyte-mediated cytotoxicity and ICSA were decreased, while the frequency and intensity of ICA positivity was not changed. Lymphocyte mediated cytotoxicity seems to be more sensitive and specific than humoral immune reactions. LMC and ICSA indicate earlier the cellular and humoral sensitization against pancreatic islet cells than ICA positivity.

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DETECTION OF PANCREATIC INSULITIS IN TYPE 1 DIABETES BY USING 123I-LABELLED INTERLEUKIN-2.

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We have shown that Interleukin-2 (IL2) labelled with ^{123}I binds in vitro and in vivo to activated T lymphocytes, allowing to image insulitis in diabetes susceptible animals. To date we have used this technique in 4 Type 1 diabetic patients at diagnosis, 2 long standing diabetics and 4 volunteers. Human IL2 was labelled with ^{123}I, purified by HPLC and injected into patients (1mCi). Dynamic gamma camera images were acquired for 60 mins and a static image of the abdominal region was also acquired 4 hours later. Regions of interest were drawn over the pancreas and spleen and time-activity curves generated. We also performed an NMR study before and after bolus of Gd-DTPA using a 1.5 Tesla magnet. Gamma camera images showed that IL2 is rapidly cleared from the blood being mainly up-taken by kidneys. After 20 mins liver and blood activity was undetectable. Only in newly diagnosed patients we observed IL2 accumulation in the spleen and in the pancreatic region. In 2/4 cases we found a concordant positive NMR result with high pancreatic signal intensity enhancement after Gd-DTPA, a sign of inflammation. ^{123}I-IL2 is highly specific and it use can be valuable for diabetes prediction in susceptible subjects.

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GLIPIZIDE SIGNIFICANTLY INHIBITS PROLIFERATION OF T-LYMPHOCYTES INDUCED BY POLYCLONAL ACTIVATORS.

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Sulfonylurea compounds stimulate insulin secretion by blocking ubiquitous ATP-sensitive K^+ channels. The present study was undertaken to determine if glipizide in vitro could influence human T-lymphocyte mitogenesis induced by lectins or by a mitogenic anti-CD3 monoclonal antibody (BMA030). DNA synthesis was estimated by ^3H-thymidine incorporation assay and activation markers were analysed by flow cytometry. In all donors tested, glipizide at 100 µg/ml ($2.4\ 10^{-4}$ M) strongly inhibited ^3H-thymidine incorporation in response to concanavalin A (ConA : 5 µg/ml) (inhibition : 51.3 ± 9.7 % ; n = 4). Responses to phytohemagglutinin A (PHA 1 %) or BMA030 (100 ng/ml) were also significantly inhibited although more pronounced interindividual variations were noted. The inhibition was dependent on the concentration of glipizide and was still present at 1 µg/ml (16.4 ± 9 % ; n = 4) for ConA response. Interestingly, when glipizide (100 µg/ml) was secondarily added to cultures previously activated for 3 days in presence of ConA (5 µg/ml), proliferation was also significantly reduced (33 ± 19 %). No difference could be found between the expression of activation markers HLA-DR, transferrin receptor and low affinity interleukin-2 receptor (CD25) in 7-day cultures with ConA alone or ConA + glipizide (100 µg/ml). In conclusion, these experiments demonstrated that glipizide significantly inhibits human T-cell responses to polyclonal mitogens without preventing the expression of low affinity IL-2 receptors.

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CD4+CD25+ T CELLS INCREASE IN PATIENTS WITH NEWLY DIAGNOSED TYPE 1 (INSULIN-DEPENDENT) DIABETES MELLITUS

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Recent investigations showed involvement of certain types of T cells in autoimmune destruction of B cells in experimental animals. We analyzed the percentage of activated T cells from peripheral blood from 18 newly diagnosed type 1 diabetics and 38 healthy persons. The cells were analyzed by two--colour flow cytometry for CD4, CD8 and CD25. The results were compared with appearance of islet cell antibodies (ICA) and stimulated c-peptide level (6 minutes after i.v. glucagon application). The percentage of CD4+ cells was 44.0 ± 7.2% in diabetics and did not differ from that obtained in the controls (42.0 ± 2.5%). Number of activated CD4+ cells (CD4+CD25+) was significantly increased in diabetic patients (10.7 ± 1.4%) in comparison with healthy persons (2.0 ± 0.4%, $p < 0.001$). Percentage of CD8+ and CD8+CD25+ cells did not differ between two groups. 11 patients were ICA(+) (61%). The appearance and the ICA levels did not correlate with the activation intensity of CD4+ cells. Stimulated c-peptide level did not correlate with ICA levels and stimulated CD4+CD25+ percentage. The high percentage of activated T cells at the time of clinical manifestation of type 1 diabetes suggests their importance in disease pathogenesis. Their determination could be of value in the prediction of natural course of preclinical and early phases of the disease.

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ANALYSIS OF ISLET CELL DEATH INDUCED BY REACTIVE OXYGEN RADICALS

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Pancreatic islet cells were found to be highly susceptible to the toxic effects of reactive oxygen intermediates (ROI) which may be released from activated macrophages and endothelial cells during early phases of islet destruction. In our studies we examined the effects of ROI, generated by xanthine oxidase (25mU/ml) and hypoxanthine (0.5mM) on rat islet cells in vitro. Exposure to ROI for 18h induced membrane damage (trypan blue exclusion) in 85±7% and DNA-damage (nick translation) in 82±4% of the islet cells, and decreased their respiratory activity (conversion of tetrazolium salt) to 15±3%. Kinetic studies revealed considerable DNA-damage in 41±4% of the cells and a reduction of mitochondrial activity to 21±4% already after 2h whereas membrane damage was detectable only after 8 to 10h of exposure to ROI. Protection of the islet cells from ROI-mediated damage was achieved by preincubation (2h) with lipoic acid (0.15mg/ml), a dithiol compound with radical scavenger activity, and by the presence of the poly(ADP-ribose)synthetase-inhibitors nicotinamide (10mM) or 3-aminobenzamide (20mM) during the incubation period. We conclude, that DNA-damage and mitochondrial dysfunction are the initial events in ROI-induced islet cell lysis.

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THE IMMUNE DYSREGULATION IS MORE PRONOUNCED IN DIABETIC THAN IN NONDIABETIC BB/OK RATS: EVALUATION BY ALLOGENEIC SKIN TRANSPLANTATION

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Hyperglycaemic BB/OK rats are characterized by a delayed rejection of allogeneic skin. This experiments were performed to compare the effect of hyperglycaemia (streptozotocin(STZ) versus autoaggression) prior to or after the transplantation and the prevention of hyperglycaemia by insulin pellet implantation on allogeneic graft rejection.

When STZ-diabetic BB/OK rats (MHC: RT.1^u) were grafted with allogeneic skin (BB.1A/OK, MHC: RT.1^a) a delay of rejection could be observed in 2w diabetic animals, which is not further prolonged in 13w hyperglycaemic rats (22.9 ± 3,0 d). However, when spontaneously-diabetic rats were grafted, the rejection occured at 27.7 ± 3,6 d after 2w and at >58,5 ± 15,5 d after 13w of hyperglycaemia. When the hyperglycaemia prior to transplantation was avoided by grafting newly-diagnosed diabetic BB/OK rats, the graft rejection was still delayed. Also the prevention of hyperglycaemia after transplantation by insulin pellet implantation did not alter the graft survival in newly-diagnosed diabetic BB/OK rats.

The results demonstrated an"immunosuppressive" effect of hyperglycaemia, which is more pronounced in spontaneously-diabetic animals. Despite normalization of plasma glucose to avoid the effect of hyperglycaemia skin graft rejection was still delayed, indicating a marked immune dysregualation in animals having developed an insulin-dependent diabetes.

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QUANTITATIVE ANALYSES OF IN SITU ISLET T-CELL SUBSETS IN THE PREDIABETIC AND DIABETIC BB RAT.

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We recently reported markedly increased NK cells (50%) in inflammed islets of the prediabetic and diabetic BB rat. Using semiquantitative methods, T cell subsets are also suggested to be important. We now report more quantitative methods for T cell subsets including islet isolation, dispersion into single cells and FACS analyses. Total T cells are similar to NK (50%). $CD4^+$ T cells are most prominent (60%) of the total TCR alpha/beta$^+$ population. Double negative (DN) T cells ($CD4^-$ $CD8^-$) are the second most prominent (30%), and $CD8^+$ T cells the least (10%) in early and late stages of prediabetes. Each subset (n=3/subset) is different from one another ($p<.05$). Contrary to prior semiquantitative reports, activated T cells assessed by class II and IL-2R McAb were virtually undetectable as was the RT 6.1 subset. Similar results for all subsets are observed in diabetic rats. These results are markedly different from quantitative spleen subset data where activated T cells comprise 20-50% of all T cells. These data indicate that a)quantitative methods reveal major differences from semiquantitative methods, b)quantitative spleen data differs markedly from in situ islet results, c)the previously undescribed DN T cell in islets is the second most predominant subset, and d)functional studies should now focus on both the DN as well as $CD4^+$ and $CD8^+$ T cells to comprehensively understand autoimmune diabetes.

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PASSIVE TRANSFER OF DIABETES IN LETL RAT.
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Long Evans Tokushima Lean (LETL) rat is a newly discovered animal model for human IDDM in which mononuclear cell infiltration into the islets was observed and was not accompanied with T-lymphocytopenia. In the present study to investigate the pathogenesis of diabetes in LETL rat the adoptive transfer of spleen cells from LETL rat was performed.
The spleen cells were obtained from LETL rat aged 8 weeks and cultured with concanavalin A (5ug/ml) for 72 hours. Non-diabetic LETL rat aged 8 weeks (n=12) were used as recipient and spleen cells ($5-20x10^6$ cell) were injected intravenously. Urine glucose was measured twice a week and 4 weeks after the transfer the pancreas were examined histologically. The intensity of insulits was graded 0 to 3. 10 out of 12(83.3%) LETL rats showed insulitis with a mean intensity of 0.67 ± 0.88. And 2 out of 12 (16.7%) LETL rats developed diabetes. In contrast, none of the saline-injected rat (n=6) demonstrated insulitis and diabetes. These results indicate cell-mediated immunity play an important role in the development of insulitis and diabetes in LETL rats.

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NITRIC OXIDE, A HITHERTO OVERLOOKED PATHOGENIC FACTOR IN TYPE I DIABETES

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We have recently identified nitric oxide (NO) as the most potent islet-toxic product of inflammatory macrophages. Islet cell lysis caused by interleukin 1 also was found to be mediated via NO formation. Finally, treatment of mice with inhibitors of NO synthase partially suppressed diabetes development in the low-dose streptozotocin model. We have now found that isolated rat islet cells exposed to chemically generated nitric oxide (0.5 mM nitroprusside or 0.36 mg/ml S-nitroso-N-acetyl-penicillamine) lyse within 8 h at 37 °C. Islet cells could be protected from NO toxicity by inhibitors of mono- and poly-ADP-ribosylation (10 mM nicotinamide or 3-amino-benzamide) but not by several potent radical scavengers (5 mM N-acetylcysteine, dimethylurea or citiolone). The known benefical effect of nicotinamide in some animal models of type I diabetes thus may be due to protection of islet cells from NO. Both, beta cells and non beta islet cells were lysed by NO in vitro. Islet cells were found unusually susceptible towards NO toxicity when compared to macrophages, endothelial cells or hepatocytes. We conclude that all islet cell types are highly vulnerable to nitric oxide in vitro and that islet cell death requires mono- and/or poly-ADP-ribosylation.

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T LYMPHOCYTE VACCINATION IN NON OBESE DIABETIC MICE.
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The aim of this study was to investigate whether lymphocyte vaccination can prevent diabetes occuring in the non obese diabetic (NOD) mouse, the animal model of human type 1 diabetes. The lymphocyte vaccine was composed of concanavalin A activated lymphocytes isolated from the spleens of diabetic mice and rendered immunogenic using gluteraldehyde treatment. These cells ($5x10^6$) were used to vaccinate mice at 6 weeks with further boosters at weeks 10, 14 and 18. The animals were monitored for signs of diabetes until week 30. 14 randomly selected NOD mice (8 male, 6 female) have been T cell vaccinated whilst 20 littermates (10 male, 10 female) have been sham vaccinated with saline. Diabetes was detected in significantly more sham vaccinated (17/20) than T cell vaccinated mice (7/14, $p<0.05$). When females and males are analysed separately diabetes was detected in all female sham vaccinated mice (10/10) and 4/6 T cell vaccinated females ($p=0.051$), whilst in males there was no significant difference between groups. These results suggest that lymphocyte vaccination can prevent diabetes in NOD mice, and has its greatest effect on females. The therapy is safe and its efficacy indicates that it will be of value in human diabetes.

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PREVENTIVE AND REVERSIBLE INFLUENCE OF MONOCLONAL ANTIBODIES REACTIVE WITH ISLET CELL ANTIBODIES ON INDUCEMENT AND COURSE OF DIABETES

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We have generated monoclonal antibodies (MABs) reactive with islet cell antibodies (ICA) by fusing mouse myeloma cells with spleen cells from NOD mice immunized with ICA. Hybridomas producing MABs reactive with ICA were detected by enzyme-linked immunosorbent assay. NOD mice were being treated by MABs in dilution of 1:1000 for 4wk. Only in 2 out of 10 mice treated by MABs developed diabetes in comparison with untreated control group where development of diabetes was in 8 out of 10 cases. Preventive effect of MABs on development of diabetes was being lasted for 6-9mo. Also, a MABs were used in treatment of streptozotocin-induced diabetes in 10 Wistar rats who had a ICA and residual beta-cells. After 4wk treatment, 6 out of 10 rats given MABs from the day of the first occurrence of marked gyperglycemia (>15mmol/l) displayed a near normoglycemia, a increase of C-peptide levels, disappearance of glucosuria. In control group of rats, treated by subdiabetogenic dosage of streptozotocin without administration of MABs, severe diabetes have been developed in all rodents. These data suggest that MABs reactive with ICA may have preventive and reversible influence on inducement and course of diabetes.

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$1,25(OH)_2$ VITAMIN D PREVENTS INSULITIS IN NOD MICE

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Receptors for the active form of Vitamin D, $1,25(OH)_2D_3$, are present in immune cells (monocytes, lymphocytes) and *in vitro* this substance has important immunosuppressive activities. In *in vivo* models for experimentally induced autoimmunity, such as allergic encephalitis, autoimmunity can be prevented by treatment with $1,25(OH)_2D_3$ at the time of disease induction. No data exist in spontaneously occurring autoimmune diseases, such as type I diabetes. The aim of the present study was to investigate the influence of $1,25(OH)_2D_3$ on insulitis, the histological lesion preceeding diabetes. Forty three NOD mice, which spontaneously develop type I diabetes, were treated with 5 µg/kg $1,25(OH)_2D_3$ intraperitoneally every other day from the age of 21 days on, when no insulitis is present. At day 100, 16 control mice receiving the treatment vehicle (arachis oil), had an incidence of insulitis of 75%, whereas only 41% of the $1,25(OH)_2D_3$ treated animals developed insulitis ($p<0.025$). Calcemia, determined 24 hours after the last $1,25(OH)_2D_3$ injection was 9.9 mg/dl ± 1.3, which is higher than in controls (mean : 9.1 mg/dl ± 0.4), but is well tolerated (mice showed normal weight gain). This study demonstrates that treatment with $1,25(OH)_2D_3$ at high doses is well tolerated and is able to decrease the incidence of insulitis in autoimmune diabetes.

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BCG VACCINE REVERSES NEW-ONSET DIABETES IN NONOBESE DIABETIC MICE.

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We reported that one-time injection of BCG *(Bacillus Calmette Guerin)* vaccine reverses insulitis and prevents the onset of autoimmune diabetes life-long in nonobese diabetic (NOD) mice. The efficacy of BCG to reverse diabetes was tested in 30 female NOD mice, without or with nicotinamide (a stimulant of islet-cell replication). On second day of onset of diabetes, random blood glucose (RBG) was 19.5±3.4 mmol/l (mean±SD); the mice received injections of [1] BCG intraperitoneally + into a footpad, one-time (B), [2] B + nicotinamide subcutaneously daily x 40 days (B+N), [3] N alone, or [4] saline (S). Insulin was not administered. Persistent normoglycemia (RBG 7.8±2.1) and aglucosuria were induced within 14±17 days in 4/7 B and 4/10 B+N, but not in N (0/6) or S (0/7); (B & B+N 47% *vs* N & S 0%, $p<0.001$). Survival (days after onset): B & B+N, 8/17 dead, median survival >122 days; N & S, 13/13 dead, median survival 55 days ($p<0.001$). Conclusion: Efficacy of one-time injection of BCG to prevent or reverse diabetes, apparently life-long, justifies studies to prevent or reverse human autoimmune diabetes.

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Nicotinamide and insulin secretion in normal subjects

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Nicotinamide has been administered both before and after clinical onset of Type 1 (insulin-dependent) diabetes in the attempt to prolong beta cell survival. Nicotinic acid, structurally similar to nicotinamide, induces insulin resistance and hence increases insulin secretion in normal subjects. Since the acute insulin response (AIR) to intravenous glucose is commonly used in prediction of diabetes and to monitor interventions, the effect of nicotinamide upon insulin secretion in healthy subjects must be known before changes in prediabetic or diabetic individuals can be interpreted. Intravenous glucose tolerance tests (IVGTTS) were performed according to the ICARUS standard protocol in 10 healthy, adult subjects (age 32 ± 5.7 years, mean ± S.D.) before and after 14 days of treatment with nicotinamide 0.25mg/kg/day. The AIR after nicotinamide treatment did not differ from control whether measured as incremental 0-10 minute insulin area (278 ± 142 vs 298 ± 130 mU/l/10 minutes) or 1+3 minute insulin level (78 ± 39 vs 81 ± 44 mU/l). The late insulin response (incremental 10-60 minute area: 1256 ± 414 vs 1075 ± 510 mU/l/50 min) was equally unaffected. Basal insulin (5.2 ± 1.6 vs 5.6 ± 2.1 mU/l) and glucose (5.0 ± 0.4 vs 4.9 ± 0.2 mmol/l) levels and glucose disposal rates (1.98 ± 0.88 vs 2.04 ± 0.68 %/min) were also similar. Nicotinamide does not appear to affect insulin secretion or glucose kinetics in normal subjects, confirming its suitability for trials designed to delay or prevent the onset of insulin-dependent diabetes.

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COMPARISON BETWEEN NICOTINAMIDE AND NICOTINAMIDE + DEFLAZACORT IN RECENT ONSET TYPE 1 DIABETES

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The aim of this study was to compare the effect of nicotinamide (NCT) alone or in combination with a cortisone-like substance (Deflazacort-DFL) on clinical remission and the integrated parameters of metabolic control in patients with recent onset type 1 diabetes (< 4 weeks diagnosis). Twenty two patients entered a randomized double blind one year prospective study: **group A** received for 12 months NCT 25mg/Kg/die; **group B** received NCT (dose and length of treatment as above) + DFL for 3 months (0.6mg/Kg/die in the 1st month, 0.3mg/Kg/die in the other 2 months). All patients received intensified insulin therapy. Patients in the two groups did not differ at entry for insulin requirement, glycosilated haemoglobin (HbA1) and basal or stimulated (1mg glucagon i.v.) C-peptide. At 3 months, as expected, the insulin dose was significantly higher in group B vs group A (0.6±0.1ng/ml vs 0.3±0.2 ng/ml P<0.02, respectively) with similar HbA1 levels (group A: 6%±0.3; group B: 6.3%±1.8). However, stimulated C peptide in group B was significantly higher (p<0.04) compared to group A. At 12 months basal and stimulated C peptide were significantly raised in patients of group B vs group A but insulin requirement was not different between the two groups. In conclusion addition of DFL to nicotinamide can further increase C peptide secretion in the course of 1st year of treatment.

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TREATMENT OF TYPE 1 DIABETES MELLITUS WITH DAB486-IL-2, A TOXIN CONJUGATE WHICH TARGETS ACTIVATED T-LYMPHOCYTES

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Evidence point to autoreactive T-lymphocytes in the pathogenesis of insulin-dependent diabetes mellitus (IDDM). Treatment with Cyclosporin A (7.5-10 mg/kg.d^{-1}) of IDDM patients induced remission and partial recovery of insulin secretion, but nephrotoxicity and relapses occured. Therapeutics with no major side effect and a more selective action on T-lymphocytes commited to beta cell destruction are thus required. In a phase I/II trial 18 IDDM patients were treated with DAB486-IL-2 (0.025, 0.05, and 0.075 mg/kg.d^{-1}, for 7 days, 6 patients in each group) which selectively targets activated T-lymphocytes expressing high affinity IL-2 receptors, then with Cyclosporin A (5 mg/kg.d^{-1}) 5 weeks later. Insulin needs decreased from 0.79 ± 0.24 to 0.30 ± 0.15 UI/kg.d^{-1} within 6 weeks. Among 8 patients followed for 8 months 4 required less than 0.1 UI/kg.d^{-1} (HbA1c 5.9 ± 0.6%) within 3 months of Cyclosporin A. Fasting C peptide levels increased from 0.20 ± 0.12 nM/L at start to 0.36 ± 0.20 (n=18) at week 6, and to 0.48 ± 0.31 (n=9) after 3 months of Cyclosporin A. Corresponding glucagon-stimulated C peptide values increased from 0.37 ± 0.22 to 0.62 ± 0.34, and to 0.93 ± 0.63, respectively. Treatment with DAB486-IL-2 had minimal and transient side effects (transaminases > 2N in 7, mild fever in 7). Plasma creatinine increased by less than 8% at 8 months. This pilot study opens new directions in immunotherapy and prompts to a randomized trial.

Abstract was not included on page A129 under Poster Section 15 Epidemiology in Type 1 Diabetes

RELATION BETWEEN FREQUENCY OF ICA-POSITIVITY IN HEALTHY CHILDREN AND INCIDENCE OF TYPE 1 DIABETES: AN ESTONIAN-FINNISH COMPARISON

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The incidence of Type 1 diabetes (DM) among children younger than 15 years in Estonia is about 1/3 of that in Finland. To study whether the frequency of ICA-positivity in unaffected healthy children is related to the incidence of DM we collected and analyzed samples from 621 Estonian children and adolescents aged 3-18 years for conventional (IF-ICA) and complement-fixing islet cell antibodies (CF-ICA). The results were compared with those obtained in 1,212 Finnish subjects of corresponding age. Ten Estonian (1.6 %) and 50 Finnish children (4.1 %) had IF-ICA ($p < 0.01$). CF-ICA were detected in two Estonian (0.3 %) and twelve Finnish subjects (0.9 %; NS). There were no significant differences in the levels of IF-ICA between positive Estonian (median 34 JDF-U, range 3-97 JDF-U) and Finnish subjects (median 24 JDF- U, range 2-128 JDF-U). Six Estonian (1.0 %) and 35 Finnish children (2.9 %; $p < 0.05$) had IF-ICA levels of 20 JDF-U or more. When combining data on ICA-frequency in healthy children and incidence of DM in five countries (Finland, Estonia, Sweden, England and France) a strong correlation was found ($r = 0.93$; $p < 0.01$) indicating that 86 % of the variation in the incidence of DM can be explained by differences in the frequency of ICA-positivity among healthy children. Given that about 0.7 % of Finnish children will contract DM before the age of 20 maximally one out of six ICA-positive children in the general population will present with DM and about one out of four with an ICA-level of 20 JDF-U or more.